Chemical characterization of an aqueous extract and the essential oil of *Tithonia diversifolia* and their biocontrol activity against seed-borne pathogens of rice

Albert Nanfack Dongmo1 · Julienne Nguefack1 · Joseph Blaise Lekagne Dongmo1 · François Romain Fouelefack2 · Rene Udom Azah1 · Ephrem Augustin Nkengfack3 · Emilio Stefani4,5

Received: 18 January 2021 / Accepted: 27 January 2021 / Published online: 15 February 2021

**Abstract**

The high cost of chemical pesticides and their negative impact on the environment prompted the search for natural pesticides from plants. The objective of our study was to control rice seed pathogenic fungi and bacteria using aqueous extract and essential oil from *Tithonia diversifolia* leaves. We obtained aqueous extract and essential oil, respectively, by maceration and hydrodistillation; the antimicrobial activities were determined in vitro on a solid medium by the food poisoning method. The secondary metabolites were determined by qualitative and quantitative assays; the chemical composition of the essential oil obtained from *Titonia diversifolia* was studied using gas-chromatography coupled with mass spectrometry. The results showed that phenols, tannins, flavonoids, alkaloids, terpenoids, sugars and saponins were present in the aqueous extract. The essential oil contained mainly hydrocarbonated, oxygenated monoterpenes, terpenoids and sesquiterpenes. α-terpineol (20.3%), eucalyptol (14.6%), camphor (14.3%) and α-pinene (13.5%) as the main compounds. Regarding the antimicrobial activity, all tested bacteria were sensitive to aqueous extract and essential oil. The activity of the aqueous extract on the tested fungi showed an inhibitory concentration 50 (IC50) of 50 mg/mL against *Bipolaris oryzae* and *Fusarium moniliforme*. The activity of the essential oil on bacteria and fungi showed MIC of 125 μg/mL (*Xanthomonas oryzae pv. oryzae* and *Pseudomonas fuscovaginae*) and MFC of 5000 μg/mL (*Bipolaris oryzae* and *Fusarium moniliforme*). These results allow us to consider *Tithonia diversifolia* as a potential source of natural biopesticides against rice seed-borne pathogens.

**Keywords** *Tithonia diversifolia* · Seed-borne pathogens · Biopesticides · Secondary metabolites

**Introduction**

The rice demand in Cameroon has more than doubled over the last decade; milled rice imports rose from 469,450 to 728,433 tons, while the paddy yield fell from 2.74 to 1.33 tons/ha from 2009 to 2017, respectively. (FAOSTAT 2019).

Yield reductions are mainly due to the increasing impact of pests and diseases and their limited control, especially in the case of seed-borne pathogens (Oerke 2006).

Disease surveys of rice grown in Cameroon revealed the existence of brown spot (*Bipolaris oryzae*) and bakanae disease (*Fusarium moniliforme*), which can, respectively, lead to a yield reduction of about 67% and 20% (Barnwal et al. 2013; Nguefack et al. 2013). Bacterial leaf blight (*Xanthomonas oryzae pv. oryzae*) is present in Cameroon, as in several rice-growing areas worldwide and can lead to a yield loss of 30–35% (Jones et al. 1993; Sere et al. 2005) or even rise to 50% or more, depending on variety, growth stage and...
climatic conditions (Kala et al. 2015). *Pseudomonas fuscovaginae*, the causal agent of sheath brown rot, although not yet officially reported in Cameroon is an emerging threat for rice cultivation (CABI 2019). Like *X. oryzae pv. oryzae*, *P. fuscovaginae* is a seed-borne and seed-transmitted pathogen of rice and contribute to the reduction in the photosynthetic capacity of plants (Lamichhane et al. 2015; Slomnicka et al. 2018), thus causing severe yield losses, estimated from 30 to 60%, depending on the species susceptibility (Olczak-Woltman et al. 2008).

In Cameroon, as in other developing countries, synthetic pesticides used in plant disease management are frequently unavailable, expensive for poor farmers and often have negative effects on the ecosystem, including their action on non-target organisms and the development of pathogen resistance (Wasim 2009). Nowadays, the development and implementation of innovative and sustainable pest management strategies, based on the use of plant extracts as an alternative to synthetic agrochemicals, is becoming more and more explored. Plant extracts are important sources of new agrochemicals with satisfying antimicrobial properties for the control of plant diseases (Fouelefack et al. 2018; Mekam et al. 2019). Plant extracts are usually broad-spectrum antimicrobials, eco-friendly and with minor effects as environmental pollutants; sometimes they are beneficial to soil organisms (Sharma et al. 2015).

*Tithonia diversifolia* (Hemsl.) A. Gray (*T. diversifolia*) is a pan-tropical plant species belonging to the *Asteraceae* family; it is commonly known as Mexican sunflower and is traditionally used for medicinal purposes in tropical and subtropical regions. In traditional agricultural systems, *T. diversifolia* is used by farmers as biofertilizer for soil amendment (Kaho et al. 2009; Nguefack et al. 2020). Linthoingambi et al. (2013) reported an excellent antimicrobial activity of *T. diversifolia* leaves against several phytopathogenic fungi. This work aims to describe the biochemical characteristics and evaluate the activity of the aqueous extracts and the essential oil from *T. diversifolia* against the most challenging seed-borne fungi and bacteria (*B. oryzae*, *F. moniliforme*, *X. oryzae pv. oryzae* and *P. fuscovaginae*) that dramatically reduce rice production in Cameroon.

**Materials and methods**

**Plant material and media**

Plant material consisted of leaves of *T. diversifolia* (Hemsl.) A. Gray (*Asteraceae*) harvested in June 2018, in Cameroon, in the council of Yaoundé 3 and identified at the Cameroon National Herbarium by comparison of official samples of the botanical species from the herbarium collection number 57410 HNC. Plants were grown until the flowering stage, harvested and shade dried for two weeks. Dried leaves were then milled into a powder, which was stored in small bags at room temperature until use.

Culture media: Potato dextrose agar, Nutrient sucrose agar and the reference antibiotic (gentamycin) for biocontrol activities were purchased from Sigma-Aldrich (Milan, Italy). Deionized water was obtained from a Milli-Q System (Bedford, MA, USA). Reference fungicide Banko plus® was purchased from ADER, Douala, Cameroon.

**Preparation of aqueous extract and essential oil from *T. diversifolia***

The aqueous plant extract was obtained by maceration in distilled water. One hundred grams (100 g) of powder of *T. diversifolia* leaves were weighed and macerated into 600 mL (1:6, w/v) of distilled water under a magnetic stirrer at 120 rpm for 24 h, at a temperature of 25 ºC. After filtration through a Whatman No.1 paper, the filtrate was centrifuged at 5000 rpm for 5 min and the supernatant was collected and dried in an oven at 48 ± 2 ºC overnight. The extraction yield was calculated by weighing the dried extract per total mass of powder used and extracts were stored at 4 ºC until use.

Besides, the collected fresh *T. diversifolia* leaves were subjected to steam distillation using a Clevenger-type apparatus; 2.5 kg of fresh leaves in 5 L of water were boiled for 4 h. The extracted essential oil was then dried over anhydrous sodium sulphate and stored in a dark amber glass vial at 4 ºC until its use. The yield was calculated.

**Phytochemical screening**

The standard modified methods of qualitative analysis described by Harbone (1998) and Edeoga et al. (2005) were used for the determination of phenols, tannins, saponins, flavonoids, alkaloids, glycosides, triterpenes, steroids and anthocyanins in the aqueous extract.

**Quantitative assay of phenols and flavonoids in aqueous extracts of *T. diversifolia***

The determination of phenols and flavonoids was chosen since the majority of the biological properties of the plant are attributed to them (Boizot et al. 2006). *T. diversifolia* leaf powder was weighed and dissolved in the corresponding volume of distilled water to obtain different concentrations (1%, 3%, 5% and 10%). After 24 h, the mixture was decanted and filtered. The filtrate was kept at 4 ºC for the determination of the phenol and flavonoid content.

The Folin–Ciocalteu’s assay was used for the quantification of total soluble phenols, using the method described by Siddhuraju et al. (2007) and gallic acid as a standard. Briefly, 15 µL extract at 1%, 3%, 5% and 10% concentrations...
were each mixed with 3 mL of distilled water, 250 µL of Folin–Ciocalteu’s reagent, 750 µL of 70% Na₂CO₃ and vortexed thoroughly. The mixture was then incubated at room temperature (18–25 °C) for 10 min and allowed to stand for 2 h at room temperature, after adding 950 µL of distilled water. The optical density was measured at λ = 765 nm. The experiments were performed in triplicate, and the total phenol content was expressed as gallic acid equivalents (mg of GAE/g of dry powder) through the calibration curve [OD = f (weight of gallic acid)].

The total flavonoid content was evaluated using the aluminium chloride protocol as described by Enujiugha (2010). Briefly, 0.25 mL of aqueous extract (as prepared above) was mixed with 1.25 mL distilled of water (1:5, v/v) and 50% NaNO₃ (75 µL) and allowed to stay for 6 min at room temperature. Then, 150 µL of a 10% aluminium chloride solution was added in the mixture and incubated for 5 min at room temperature. The reaction solution was brought to 5 mL with distilled water and 0.5 mL of 1 M sodium hydroxide was added. After homogenization, the absorbance was measured at λ = 510 nm using a spectrophotometer (UV 160, Shimadzu, Japan). The total flavonoid content of each treatment was expressed as catechin equivalents (mg of CE/g of dry powder) using a calibration curve [OD = f (weight of catechin)].

**Determination of the chemical composition of essential oil**

The composition of the essential oil from *T. diversifolia* leaves was determined by using an analytical gas-chromatography (GC-FID) and gas chromatography coupled with mass spectrometry (GC/MS) techniques. The column used and experimental conditions were both the same in GC and GC/MS. An Agilent 6890 N Network GC system for gas chromatography was equipped with an HP-5MS column [30 m × 0.25 mm (5%-phenyl)-methylpolysiloxane capillary column, film thickness 0.25 µm], a splitless injector heated at 250 °C and a flame ionization detector (FID) at 240 °C. The oven temperature was programmed as follows: initial temperature 50 °C for 1.50 min, increase 10°C C/min up to 180 °C, 2 min at 180 °C and then increase by 6°C C/min up to 280 °C, 10 min at 280 °C. Helium (99.999%) was used as a carrier gas at a flow rate of 1.0 mL/min. The injection volume was 1.0 µL (split ratio 1:20). GC/MS analyses were performed using an Agilent 6890 N Network GC system with an Agilent 5973 Network mass selective detector, mass spectrometer in EI mode at 70 eV in m/e range 10–550 amu. The essential oil components were identified by comparison of their mass spectra with NIST 2002 library data of the GC–MS system. The retention index was calculated according to the formula set by Kováts (1958).

**Antimicrobial activity in vitro**

**Bacterial and fungal strains**

The antibacterial activity of the aqueous extract and the essential oil of *T. diversifolia* were evaluated against two rice seed-borne bacteria: *Xanthomonas oryzae pv. oryzae* and *Pseudomonas fuscovaginae*, isolated from Cameroon and Italy, respectively. Furthermore, the extracts were tested on two pathogenic fungi of rice seed: *Bipolaris oryzae* (teleomorph: *Cochliobolus miyabeanus* (S. Ito & Kurib.), strain DLS 1586, isolated from rice in Italy and *Fusarium moniliforme* (teleomorph: *Gibberella fujikuroi*) belonging to the *F. fujikuroi* species complex provided by the Institute of Agricultural Research for Development (IRAD), PO. BOX. 2123 Messa-Yaoundé, Cameroon.

**Antibacterial activity**

To check the possible antibacterial activity of both aqueous extract and essential oil, assays were done using the modified disc diffusion method (CLSI 2007). This method is based on the diffusion of extracts from filter paper discs in contact with the solid culture medium (NSA) into Petri dishes, previously inoculated with a bacterial inoculum (10⁶ CFU/mL). Essential oil was diluted in 5% Tween 20 (v/v) to obtain a concentration of 100 mg/mL; the same concentration was used for the aqueous extract (100 mg/mL). Then, 10 µL of essential oil and 30 µL of extract were spotted on different sterilized paper discs, before plating them on the agar surface with the two phytopathogenic bacteria. Gentamicin (1 mg/mL) was used as a control (5 µL were spotted on sterilized paper discs, before plating them on the agar surface). The inoculated Petri dishes were then incubated at 27 °C, and the antibacterial inhibition was assessed after 48 h by measuring the inhibition haloes. The microbial sensitivity was classified according to the diameter of the zones of inhibition as follows: not sensitive for diameters less than 8 mm; sensitive for diameters between 9–14 mm; very sensitive for diameters between 15–19 mm; and extremely sensitive for diameters ≥ 20 mm (Moreira et al. 2005).

**Antifungal activity**

The antifungal activity of the aqueous extract and the essential oil from *T. diversifolia* were checked in vitro by measuring the inhibition of the mycelial growth on PDA, supplemented with increasing concentrations of the extract and essential oil. Three increasing concentrations of the aqueous extract were used: 10, 50 and 100 mg/mL; five increasing concentrations were considered for the essential oil: 625;
1250; 2500; 5000; and 10,000 µg/mL. The positive control used was Banko Plus®, the most common fungicide indicated for rice (Chlorothalonil 550 g/L—Carbendazim 100 g/L as active substances) at following concentrations: 62.5; 125; 250; 500; and 1000 µg/mL; the negative control was represented by PDA plates supplemented with sterile distilled water. Each agar plate was then inoculated with a 5-mm mycelium plug taken from the margin of a 6-day-old culture of each fungus and kept in an incubator at 27 °C. Growth inhibition was assessed after seven days: this was done by measuring the two perpendicular diameters of the fungal colony (Nyegue et al. 2014). The mycelium growth inhibition relative to the controls was then calculated according to the following equation:

\[ \text{Mycelium growth inhibition (\%) = \frac{(D-d)}{D} \times 100} \]

where \( D \) = mycelium diameter in the control PDA plate, \( d \) = mycelium diameter in the amended PDA plate. The tests were carried out in triplicate and the experiments were independently repeated three times.

The concentration of plant extracts required to inhibit by 50% the fungal growth (IC\(_{50}\)) was determined by plotting the growth inhibition percentage as a function of final plant extract concentration (base-10 logarithm). IC\(_{50}\) values were expressed as mg of extract/mL. The antifungal activity of the aqueous extract and essential oil was evaluated as follows: strong activity, when mycelial growth inhibition was > 50%; weak activity when mycelial growth inhibition was < 50% or not active when no inhibition was observed (Nyegue 2006).

**Determination of the minimum inhibitory concentrations, minimum bactericidal concentration and minimum fungicidal concentration**

The modified microdilution method described by CLSI (2007) was used for the determination of the minimum inhibitory concentrations (MIC). The MIC was defined as the lowest concentration of aqueous extract or essential oil visibly inhibiting bacterial growth after 48 h of incubation at 27 °C. Into each well, 100 μL of broth enriched with 5% red phenol was added. Then, 100 μL of aqueous extract or essential oil were added in every first well of the microplate. Geometric dilutions ranging from 50 to 0.781 mg/mL were carried out, and subsequently, 100 μL of media containing 10° CFU/mL of the target strain was added to all wells to yield 25 to 0.0152 mg/mL of concentration. The plates were then incubated at 27 °C for 48 h. For both extract and essential oil, the experiment was done in triplicate. A colour change from red to yellow indicated a bacterial growth. To obtain the minimum bactericidal concentration (MBC), 20 μL of each well coloured in red was spotted on the agar surface and incubated at 27 °C for 48 h. The MBC was defined as the lowest concentration of aqueous extract and essential oil where less than 10 colonies growing in the plate were counted. The ratio MBC/MIC was calculated.

The MICs of the fungi were determined directly on PDA supplemented with aqueous extract and essential oil. MICs were the lowest concentrations of aqueous extract or essential oil inhibiting visible growth of the target fungi on the agar plate after 7 days of growth. To determine the minimum fungicidal concentration (MFC), the explants present in the plate considered as MIC were subcultured in non-supplement PDA plates. After 4 days of incubation at 27 °C, the absence of mycelial growth indicates the MFC. The ratio MFC/MIC was then calculated: according to Avril et al. (2002), adapted to fungi by Nyegue (2006), for MFC/MIC < 4 the sample is classified as "fungicidal", when the values are in the range 4 < MFC/MIC < 16, the sample is considered "fungistatic" and finally when MFC/MIC > 32, it is called "tolerant".

**Statistical analyses**

Average and standard deviations have been calculated using Excel 2007 software. The graphs were made using SigmaPlot and GraphPad Prism software. Analysis of data variance and comparison of means using the Post Hoc (LSD) test performed at the 5% probability level (\( p < 0.05 \)), using IBM-SPSS16.0 software.

**Results**

**Preparation of aqueous extract from *T. diversifolia* leaves**

The crude aqueous extract of *T. diversifolia* leaves was obtained by maceration of powdered dry leaves. Dried aqueous extract consisted of a dark green powder; its average yield was 29.75% of the dry leaves weight.

**Qualitative phytochemical screening of aqueous extract of *T. diversifolia***

The qualitative phytochemical screening results showed that phenols, flavonoids, terpenoids, tannins, saponins, anthocyanins, glycosides and alkaloids were all present in the aqueous extract of *T. diversifolia* leaves.

**Quantitative phytochemical content of phenols and flavonoids**

The calculated amount of phenols and flavonoids in the aqueous extract varied in a concentration dependant manner. For phenols, it ranged from 19.33 to 274.4 mg of GAE/g of dry powder, and for flavonoids, it ranged from 10.6 to
102.4 mg of CE/g of dry sample (Fig. 1). Therefore, the average content of total phenols was 146.9 mg of GAE/g and the average content of total flavonoids was 56.5 mg of CE/g of dry powder. Total phenolics content was approximately three times higher than the content of flavonoids.

**Extraction and characterization of essential oil**

The mean extraction yield for the essential oil obtained by hydrodistillation was 6% of the initial fresh biomass. The organoleptic and physical characteristics of the essential oil were determined: the oil was liquid and volatile, the colour was pale yellow and the smell was pungent. The density (g/mL) of the essential oil was 0.7.

**Chemical composition of essential oil**

The chromatographic profile of the essential oil is shown in Table 1. The chemical composition of essential oil showed that it was mainly composed of hydrocarbonated, oxygenated monoterpenes and with some sesquiterpenes.

The GC profile of the essential oil showed that terpenes/terpenoids are the main constituents (Table 1). Alpha-terpinene, a monoterpane alcohol, was the component detected in highest amount (20.3%); with terpinen–4-ol (1.8%) its isomeric form, they represent almost one-fourth of the chemical composition of this essential oil. Another major component of the oil was α-pinene (13.5%); considering that another detected molecule was camphor (14.3%), a cyclic ketone derivative from the oxidation of α-pinene, both (α-pinene and camphor) substances may be considered the major component of *T. diversifolia* essential oil. Sesquiterpenes were also found in a not negligible amount: spathulenol, globulol and ledol reach together a percentage of 10.5%. Therefore, monoterpenes and sesquiterpenes represent more than 60% of the essential oil components.

**In vitro antibacterial activity of aqueous extract and essential oil**

The aqueous extract and the essential oil from *T. diversifolia* were tested in vitro for their putative antibacterial activity. Both extracts showed remarkable antibacterial activity against the two bacteria tested: such activity was revealed by the formation of a clear and large inhibition halo around the paper discs soaked with the extracts. The antimicrobial

![Fig. 1](image1.png)

**Fig. 1** Content of phenols and flavonoids in the aqueous extracts of *Tithonia diversifolia* leaves. (Mean ± SD, n = 4)

![Fig. 2](image2.png)

**Fig. 2** In vitro antibacterial activity of the aqueous extract from *Tithonia diversifolia* against *Pseudomonas fuscovaginae* and *Xanthomonas oryzae* pv. *oryzae*. 1 = aqueous extract (100 mg/mL), 2 = essential oil (100 mg/mL), G= Gentamicin (1 mg/mL)

| Formula | Name of chemical compounds | RI   | Percentage |
|---------|----------------------------|------|------------|
| C_{10}H_{16} | α-Pinene                  | 917.7| 13.5       |
| C_{6}H_{6}O | Phenol                     | 968.9| 0.4        |
| C_{10}H_{14} | p-cymene                  | 1022.6| 2.2  |
| C_{10}H_{18}O | Eucalyptol, (cineole)     | 1034.7| 14.6      |
| C_{10}H_{16} | γ-terpinene               | 1072.4| 0.5       |
| C_{10}H_{20}O | Epoxy linalool            | 1093.1| 1.3       |
| C_{10}H_{18}O | Linalool, oxide           | 1111.9| 1.5       |
| C_{10}H_{18}O | Linalool, hydrate         | 1121.8| 0.7       |
| C_{10}H_{16} | α-campholenaldehyde      | 1153.2| 2.6       |
| C_{10}H_{18}O | Pinocarveol               | 1168.4| 5.1       |
| C_{10}H_{16}O | Camphor                   | 1194.7| 14.3      |
| C_{10}H_{18}O | Terpinen–4-ol            | 1203.5| 1.8       |
| C_{10}H_{16}O | α-Terpineol              | 1220.6| 20.3      |
| C_{10}H_{18}O | Myrtenol                  | 1225.6| 0.3       |
| C_{10}H_{16}O | Carvacrol                 | 1324.7| 0.8       |
| C_{10}H_{20}O | Spathulenol              | 1615.4| 3.3       |
| C_{10}H_{20}O | Globulol                  | 1622.7| 1.5       |
| C_{10}H_{20}O | Ledol                    | 1631.7| 5.7       |
| C_{11}H_{20}O | 2-Naphthalenemethanol    | 1689.8| 1.2       |
activity of both extracts against both phytopathogenic bacteria was comparable, since the inhibitory haloes showed a similar area in any replicate plates. Interestingly, the tested extracts proved a superior antibacterial activity than the antibiotic gentamicin, at the given concentrations (Fig. 2).

The results obtained from the activity of the aqueous extract and the essential oil on targeted bacteria are qualitatively illustrated in Fig. 3. Histograms show that the two bacterial strains were strongly inhibited by both the aqueous extract and the essential oil of T. diversifolia. The inhibition diameters of the aqueous extract and the essential oil ranged from 21 to 24 mm. Interestingly, bacterial sensitivity to both extracts was higher than that of gentamicin, for which the inhibition diameters vary from 12 to 15 mm for both bacteria. Such inhibition was reached using the concentration of 100 mg/mL for both extracts, compared to a concentration of gentamicin of 1 mg/mL.

Determinations of minimum inhibitory concentration and minimum bactericidal concentration

The inhibition parameters of aqueous extract, MIC and MBC, were not assessed, but those of essential oil were determined. The latter data made it possible to calculate the ratio MBC/MIC. This relationship made it possible to characterize a bactericidal, bacteriostatic action or to determine the “tolerance” of a strain (Table 2).

Table 2 shows that the MIC for both phytopathogenic bacteria was 125 µg/mL for the essential oil; the reference antibiotic (gentamicin) gave a MIC of 31.25 µg/mL, again for both bacteria. The measurement of the MBC/MIC ratio, with values of 2 (X. oryzae pv. oryzae) and 1 (P. fuscovaginae), showed that the essential oil of T. diversifolia can be considered as bactericidal, according to the scale of Avril et al. (2002). As expected, gentamicin proved its antibiotic effect against both bacteria, with a MBC/MIC ratio of 1.

In vitro antifungal activity of aqueous extract and essential oil

In general, aqueous extract and essential oil of T. diversifolia showed good inhibitory activity against both B. oryzae and F. moniliforme. Such inhibitory activity increased in a dose-dependent manner (Fig. 4). A concentration of 100 mg/mL of the plant extract inhibited the mycelial growth of B. oryzae and reduced its growth rate by approximately 68.44% (Fig. 4b). Similar results were observed against F. moniliforme: a concentration of 100 mg/mL reduced the mycelial growth by approximately 70.69%. The essential oil demonstrated a more evident antifungal activity against both pathogens. It was active at a concentration of 625 µg/mL and produced a total fungal inhibition at a concentration of 5000 µg/mL and above (Fig. 4a).

Figure 5 shows the quantitative mycelium growth inhibition (%) stimulated by the aqueous extract from T. diversifolia leaves against B. oryzae and F. moniliforme. The mycelium growth inhibition ranged from 25% at 10 mg/mL to 68.44% at 100 mg/mL of aqueous extract on B. oryzae and from 20% at 10 mg/mL to 70.69% at 100 mg/mL of aqueous extract on F. moniliforme. Inhibitory concentration 50 (IC50) was determined, and it was calculated at 50 mg/mL on B. oryzae and F. moniliforme. According to the scale of Nyegue (2006), the aqueous extract exhibited a strong antimicrobial activity with IC50 > 50%. A total growth inhibition of B. oryzae and F. moniliforme was not reached using aqueous extracts at the dilutions tested (Fig. 5).

Figure 6 shows the mycelium growth inhibition (%) obtained by using the essential oil of T. diversifolia leaves against B. oryzae and F. moniliforme. The mycelium growth inhibition ranged from 58.28% at 625 µg/mL to 100% at
5000 μg/mL of essential oil concentrations on *B. oryzae* and from 56.87% at 625 μg/mL to 100% at 5000 μg/mL of essential oil concentrations on *F. moniliforme*. Therefore, the sensitivity of both fungi to the essential oil was quite similar. Inhibitory concentration 50 (IC50) was then determined and resulted to be 625 μg/mL for both *B. oryzae* and *F. moniliforme*, with a percentage inhibition of 58.29% and 56.87%, respectively. According to the scale of Nyegue et al. (2006), the essential oil showed a very strong antimicrobial activity, with an IC50 > 50%. The minimal inhibition concentration was 5000 μg/mL. Also, we obtained complete fungal inhibition at 5000 μg/mL, which corresponds to the MFC (Fig. 6).
Some authors have demonstrated their biological activity, T. diversifolia and its leaves have a fungicidal activity. Its leaves are very rich in phenols flavonoids, terpenoids, alkaloids, glycosides, saponins and tannins; these results are in agreement with the findings of Olutobi et al. (2012), who reported the presence of similar phytochemical compounds in the methanolic extract of T. diversifolia leaves. Some authors have demonstrated their biological activity, among which antibacterial (Desi et al. 2017) and antifungal (Mekam et al. 2019).

In this study, the quantitative analysis of phenols and flavonoids yielded 274.47 mg of GAE/g and 102.4 mg of CE/g of dry powder, respectively. While using T. diversifolia water extract in their study, Olayinka et al. (2015) obtained a phenols level (64.58 mg of GAE/g of dry powder) and flavonoids (851.67 mg of CE/g of dry powder). This could be explained by the fact that plants under conditions of stress induced by biotic and abiotic factors may show changes in the production of different classes of metabolite or sometime due to the technology used to assay the secondary metabolites (Lapornik et al. 2005; Arbona et al. 2013; Osama 2018; Mekam et al. 2019).

The gas chromatographic profile of T. diversifolia essential oil showed a total of 19 compounds; terpenes and terpenoids were the main constituents, accounting for 95% of the composition. In the present study, the main constituents were α-pinene (13.5%), camphor (14.3%), eucalyptol (14.6%) and α-terpineol (20.3%); these results are different from those obtained by Wanzala et al. (2016) in Kenya, Adebayo et al. (2008) in Nigeria and Ingrid et al. (2018) in Brazil, who showed that the essential oil of T. diversifolia is mainly rich in α-pinene in the proportion 63.64%, 4.4% and 45%, respectively. These differences could be due to the difference among geographical areas where the plants grew and were harvested (Arbona et al. 2013). These authors gave no clear details on the handling of T. diversifolia; thus, other intrinsic factors, such as storage condition and age of plants, could considerably influence the composition (Lapornik et al. 2005).

The activity of essential oil is often reduced to the activity of its major compounds, or those likely to be active; however, some minor compounds may act in synergy with the major or other compounds (Sonboli et al. 2006; Lahlou 2004). The antibacterial and antifungal activity observed in this study could be attributed to the presence of the identified major compounds. In fact, α-pinenes destroy the cellular integrity of pathogens, inhibiting both their respiration and the ion transport process, while modifying cell permeability (Andrews et al. 1980); eucalyptol and camphor display antimicrobial effect against phytopathogenic fungi and are widely exploited to control post-harvest diseases and the growth of mycotoxigenic fungi (Rahmouni et al. 2019); α-terpineol was recently shown to possess antimicrobial activity against important phytopathogenic fungi (Song et al. 2019).

The essential oil from T. diversifolia proved to be active against rice pathogenic bacteria, with MICs of 125 µg/mL; according to Tegos et al. (2002), phytochemicals or extracts with MIC values between 100 µg/mL and 1000 µg/mL are considered as antimicrobials of interest. The differences in sensitivity between the fungal and bacterial species concerning the aqueous extract and the essential oil of T. diversifolia leaves observed during our study may be due to intrinsic factors specific to each microorganism (Takeo et al. 2004) or due to the phytochemical profile of the aqueous extract and essential oil; oxygenated molecules like phenols, alkaloids, flavonoids and oxygenated terpenoids are generally more active than lipophilic hydrocarbons (Silva et al. 2012), but

### Table 3 Minimum fungicidal concentration (µg/mL) of the essential oil from *Tithonia diversifolia* and the commercial fungicide Banko Plus®

| Tested product | Inhibition parameters | Pathogens | B. oryzae | F. moniliforme |
|---------------|-----------------------|-----------|-----------|---------------|
| Essential oil (µg/mL) | MIC | 5000 | 5000 |
| | MFC | 5000 | 5000 |
| | MFC/MIC | 1 | 1 |
| Banko Plus® (µg/mL) | MIC | 1000 | 500 |
| | MFC | 1000 | 500 |
| | MFC/MIC | 1 | 1 |

### Determination of minimum inhibition concentration and minimum fungicidal concentration

As shown in Table 3, the MFC obtained in our experiments using the essential oil was 5000 µg/mL; this value was obtained for both fungi tested. The calculated ratio MFC/MIC was 1; according to the scale of Avril et al. (2002), the essential oil of T. diversifolia leaves has a fungicidal activity. Therefore, in our experiments, the activity of the essential oil was comparable to the antifungal action of the reference fungicide (Banko Plus®), since both their calculated MFC/MIC were less than 4 (Table 3).

### Discussion

Plant-derived biomolecules have drawn great attention during the last 15 years, due to their general antimicrobial properties; indeed, they have been suggested as prospective compounds to be used during the development of innovative biopesticides and in the implementation of sustainable strategies to control phytopathogenic fungi and bacteria (Reignault et al. 2007; Martinez 2012). The present study showed that the aqueous extract of T. diversifolia and its essential oil possess a pronounced antimicrobial activity and may be considered sources of bioactive phytochemicals. Its leaves are very rich in phenols, flavonoids, terpenoids, alkaloids, glycosides, saponins and tannins; these results are in agreement with the findings of Olutobi et al. (2012), who reported the presence of similar phytochemical compounds in the methanolic extract of T. diversifolia leaves. Some authors have demonstrated their biological activity, among which antibacterial (Desi et al. 2017) and antifungal (Mekam et al. 2019).

In this study, the quantitative analysis of phenols and flavonoids yielded 274.47 mg of GAE/g and 102.4 mg of CE/g of dry powder, respectively. While using T. diversifolia water extract in their study, Olayinka et al. (2015) obtained a phenols level (64.58 mg of GAE/g of dry powder) and flavonoids (851.67 mg of CE/g of dry powder). This could be explained by the fact that plants under conditions of stress induced by biotic and abiotic factors may show changes in the production of different classes of metabolite or sometime due to the technology used to assay the secondary metabolites (Lapornik et al. 2005; Arbona et al. 2013; Osama 2018; Mekam et al. 2019).

The gas chromatographic profile of T. diversifolia essential oil showed a total of 19 compounds; terpenes and terpenoids were the main constituents, accounting for 95% of the composition. In the present study, the main constituents were α-pinene (13.5%), camphor (14.3%), eucalyptol (14.6%) and α-terpineol (20.3%); these results are different from those obtained by Wanzala et al. (2016) in Kenya, Adebayo et al. (2008) in Nigeria and Ingrid et al. (2018) in Brazil, who showed that the essential oil of T. diversifolia is mainly rich in α-pinene in the proportion 63.64%, 4.4% and 45%, respectively. These differences could be due to the difference among geographical areas where the plants grew and were harvested (Arbona et al. 2013). These authors gave no clear details on the handling of T. diversifolia; thus, other intrinsic factors, such as storage condition and age of plants, could considerably influence the composition (Lapornik et al. 2005).

The activity of essential oil is often reduced to the activity of its major compounds, or those likely to be active; however, some minor compounds may act in synergy with the major or other compounds (Sonboli et al. 2006; Lahlou 2004). The antibacterial and antifungal activity observed in this study could be attributed to the presence of the identified major compounds. In fact, α-pinenes destroy the cellular integrity of pathogens, inhibiting both their respiration and the ion transport process, while modifying cell permeability (Andrews et al. 1980); eucalyptol and camphor display antimicrobial effect against phytopathogenic fungi and are widely exploited to control post-harvest diseases and the growth of mycotoxigenic fungi (Rahmouni et al. 2019); α-terpineol was recently shown to possess antimicrobial activity against important phytopathogenic fungi (Song et al. 2019).

The essential oil from T. diversifolia proved to be active against rice pathogenic bacteria, with MICs of 125 µg/mL; according to Tegos et al. (2002), phytochemicals or extracts with MIC values between 100 µg/mL and 1000 µg/mL are considered as antimicrobials of interest. The differences in sensitivity between the fungal and bacterial species concerning the aqueous extract and the essential oil of T. diversifolia leaves observed during our study may be due to intrinsic factors specific to each microorganism (Takeo et al. 2004) or due to the phytochemical profile of the aqueous extract and essential oil; oxygenated molecules like phenols, alkaloids, flavonoids and oxygenated terpenoids are generally more active than lipophilic hydrocarbons (Silva et al. 2012), but

---

© Springer
the high concentration of the latter makes the essential oil more active.

The essential oil was more active as antimicrobial compound than the aqueous extract against the two target bacterial strains (\textit{X. oryzae pv. oryzae} and \textit{P. fucovaginae}) and the two rice pathogenic fungi (\textit{B. oryzae} and \textit{F. moniliforme}); the MICs of our essential oil were 125 \( \mu \text{g/mL} \) and 5000 \( \mu \text{g/mL} \) against bacteria and fungi strains, respectively. These MICs were higher compared to those reported by Ingrid et al. (2018) and Oludare et al. (2016), who also worked with \textit{T. diversifolia} essential oil; they found MICs of 1000 \( \mu \text{g/mL} \) against \textit{Streptococcus mitis} and 72,000 \( \mu \text{g/mL} \) against \textit{Fusarium solani}. Thus, the activity of essential oil of \textit{T. diversifolia} is microorganism-dependent (Miranda et al. 2016).

In the present study, a significant mean inhibition halo of 22 mm for \textit{X. oryzae pv. oryzae} was observed with 3 mg/mL of the aqueous extract \textit{T. diversifolia} leaves. This result differed from that reported by Desi et al. (2017) who showed that, up to 10 mg/mL, the aqueous and methanol extracts of leaves of \textit{T. diversifolia} harvested in Nigeria had no effect on \textit{X. oryzae pv. oryzae}. The noticed difference in the activity may also be due to the genetic diversity within \textit{X. oryzae pv. oryzae} populations, the population structure and the biology of the phytopathogenic bacteria (Lapornik et al. 2005).

In this study, no MIC was obtained against the two target bacterial strains with the aqueous extract of \textit{T. diversifolia}. The findings reported by Obafemi et al. (2006) in Nigeria showed that the methanol and ethanol extracts from the leaves of \textit{T. diversifolia} had a significant inhibitory activity against clinical Gram positive bacteria (\textit{Clostridium sporogenes} with MIC of 15.6 \( \mu \text{g/mL} \) and \textit{Streptococcus faecalis}, with MIC of 72.5 \( \mu \text{g/mL} \)) and Gram negative bacteria (\textit{Pseudomonas aeruginosa}, with MIC of 15.6 \( \mu \text{g/mL} \)). Thus, the human pathogenic bacteria seem to be more sensitive to \textit{T. diversifolia} extracts when compared to plant pathogenic bacteria; this may be due to the high concentration in bioactive constituents (\textit{e.g.} feruloyl, coniferin) with antimicrobial activity generally present in ethanol and methanol extracts, as compared to aqueous extracts (Mekam et al. 2019).

The powerful antibacterial and antifungal activity of \textit{T. diversifolia} extracts opens new choices for African farmers to manage the most destructive rice pathogens and, additionally, may stimulate new opportunities in the development of locally based small/medium-sized industries devoted to make use of a common local botanic resource. In order to reinforce this approach towards the production of traditional improved pesticides and the discovery of new growth potentiating substances, these results call us to complete this work and consider the evaluation of active fractions on pathogen reductions for future studies. In particular, such plant bioactive extracts might be taken into consideration when developing seed treatments, in order to effectively decrease the primary inoculum of these seed-transmitted pathogens; therefore, the next research step would be to observe their phytotoxicity on the germination and physiology of the rice seedlings.

\textbf{Supplementary Information} The online version contains supplementary material available at https://doi.org/10.1007/s41348-021-00439-w.

\textbf{Acknowledgment} The European Commission is kindly acknowledged for financially supporting early career scientists’ mobility between the University of Modena and Reggio Emilia (Italy) and the University of Yaoundé I (Cameroon).

\textbf{Author contributions} Laboratory experiments were carried out by Dongmo Nanfack Albert, Emilio Stefani and Nguefack Julienne. The essential oil was characterized by Foulefack Romain François and Nkengfack Augustin Ephrem. The manuscript was written by Emilio Stefani, Azah Udom Rene and Dongmo LeKagne Blaise Joseph.

\textbf{Funding} European Commission, in the framework of the Erasmus + ICM KA107 project, Grant Agreement no. 2017–1-IT02-KA107-036227.

\section*{Compliance with ethical standards}

\textbf{Conflict of interest} The authors declare no conflict of interest.

\textbf{Availability of data and material} Department of Biochemistry, University of Yaoundé I, Cameroon.

\section*{References}

Adebayo AG, Tira-Picosb V, Nogueira JMF (2008) Analysis of chemical constituents of \textit{Tithonia rotundifolia} leaf essential oil found in Nigeria. Nat Product Comm 3(9):1537–1538

Andrews RE, Parks LW, Spence KD (1980) Some effects of Douglas fir terpenes on certain microorganisms. Appl Environ Microbiol 40(2):301–304

Arbona V, Manzi M, Ollas CD, Gómez-Cadenas A (2013) Metabolo-mics as a tool to investigate abiotic stress tolerance in plants. Int J Mol Sci 14:4885–4911

Avril JL, Fauchere JL (2002) General and medical bacteriology. Ellipses, Paris, France

Barnwal MK, Kotasthane A, Magculia N, Mukherjee PK, Savary S, Sharma AK, Singh HB, Singh US, Sparks AH, Variar M, Zaidi N (2013) A review on crop losses, epidemiology and disease management of rice brown spot to identify research priorities and knowledge gaps. Eur J Plant Pathol 136:443–457

Boizot N, Charpontier JP (2006) Rapid method for assessing the phe-nolic compound content of organs in a forest tree. The INRA Tech. Notebook. 79–82

CABI (2019) Invasive Species Compendium. Wallingford, UK: CABI International. \url{www.cabi.org/isc}. Accessed 10 November 2019

CLSI (Clinical and Laboratory Standards Institute) (2007) Performance standards for antimicrobial disk and dilution susceptibility test methods for antimicrobial susceptibility testing for bacterial isolation from animal-Approved standard, 3\textsuperscript{rd}edn. CLSI document M11-A7. Clinical and Laboratory Standards Institute, Wayne PA (USA). 50–71
Desi R, Suharto HSA (2017) Antimicrobial activity of *Tithonia diversifolia*, *Elephantopus scaber* and *Kigelia africana* against plant pathogens. Front Environ Microbiol 3(4):56–61

Edoega H, Okwo D, Mbaebie B (2005) Phytochemical constituents of Nigerian medicinal plants. Afri J Biotech 4:685–688

Enjuigah VN (2010) The antioxidant and free radical scavenging capacity of phenolics from African locust bean seeds (*Parkia biglobosa*). Adv Food Sci Food 32(2):7

FAOSTAT (2019) Food and Agriculture Organisation of the United Nations Database. Main series of world statistics. Available online at http://apps.fao.org/. Accessed 20 June 2019

Fouelefack FR, Nguefack J, Dongmo LJB, Dongmo NA, Azah UR, Nkengfack AE (2018) Effects of extracts of *Oxalis barrelieri* L and *Cymbopogon citratus* Stapf, coupled with NaCl sorting on seed health, germination and seedlings vigor of rice (*Oryza sativa* L.). Afri J Agri Res. 13(3):104–114

Harborne JB (1998) Phytochemical methods. A guide of modern techniques of plant analysis, Chapman and Hall, London

Ingrid PS, Chagas-Paula DA, Renata FJT, de Eliane OS, Mariza AM, Barbosa de Oliveira R, Augusto CCS, Jairo KB, Niege AJCF, Da Costa FB (2018) Essential oils from *Tithonia diversifolia* display potent anti-oedematogenic effects and inhibit ac acid production by cariogenic bacteria. J Essential Oil Res. https://doi.org/10.1080/10412905.2018.1503015

Jones MP, Jeutong F, Tchatchoua J (1993) A survey of rice diseases in Cameroon. Plant Dis 77:133–136

Kaho F, Nyambi NG, Yemefack M, Yongue-Fouateu R, Amang-Abang J, Bilong P, Tonyé J (2009) Screening of seven plant species for their antifungal activities on six human pathogenic fungi. Afri J of Trad Complement Alter Med 11(6):40–46

Kerke EC (2006) Crop losses to pests. J Agri Sci 144:31–43

Olayinka BU, Ruijemo DA, Etejere EO (2015) Phytochemical and proximate composition of *Tithonia diversifolia* (Hemsl.). A. Gray. Ann Food Sci Technol 16(1):195–200

Olczak-Woltman H, Schollenberger M, Madry W, Niemirowicz-Szczytt K (2008) Evaluation of cucumber (*Cucumis sativus*) cultivars grown in Eastern Europe and progress in breeding for resistance to angular leaf spot (*Pseudomonas syringae* pv. lachrymans). Eur J Plant Pathol 122:385–393

Oludare OA, Stephen O, Joshua OO, Abdulwaleek A, Oladipo A (2016) Chemical composition and antimicrobial activities of essential oil extracted from *Tithonia diversifolia* (*Asteraceae*) flower. J Biorec Bioprod 1(4):169–176

Olutobi O, Olasupo I (2012) Phytochemical screening and the phytotoxic effect of aqueous extracts of *Tithonia diversifolia* (*Hemsl.*). A. Gray. Int J Biol 4(3):97

Osama AN (2018) Effet des conditions environnementales sur les caractéristiques morpho-physiologiques et la teneur en métabolites secondaires chez Inula monatana : une plante de la médecine traditionnelle Provençale. Dissertation, Université d’Avignon

Rahmooni A, Saidi R, Kheddar M (2019) Chemical composition and antifungal activity of five essential oils and their major components against *Fusarium oxysporum* f. sp. *albedinis* and *Fusarium oxysporum* f. sp *anguli*. Molecules 17(6):6305–6316

Reignault P, Walters D (2007) Topical induction of inducers for disease control. Induced resistance for plant disease control: A sustainable approach to crop protection. Blackwell Publishing, London, pp 179–200

Sere Y, Onasanya A, Verdier V, Akator K, Ouedrago LS (2005) Rice bacterial leaf blight in West Africa: Preliminary studies in farmer fields and screening released varieties for resistance to the bacteria. Asia J Plant Sci 4:577–579

Sharma KK, Singh US, Pankaj S, Ashish K, Lalan S (2015) Seed treatments for sustainable agriculture-A review. J Appl Nat Sci 7(1):521–539

Sidhuhraru P, Becker K (2007) The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* L) wall seed extracts. Food Chem 101:10–19

Silva ACR, Lopes PM, Azevedo MM, Costa DCM, Alviano CS, Alviano DS (2012) Biological activities of a-pine and β-pine enantiomers. Molecules 17(6):6305–6316

Slomnicka R, Olczak-Woltman H, Oskiera M, Schollenberger M, Niemirowicz-Szczytt K, Bartoszewski G (2018) Genome analysis of *Pseudomonas syringae* pv. *lachrymans* strain S15/98 indicates diversity within the pathovar. Eur J Plant Pathol 151:663–676

Nguefack J, Mfopou MYC, Dongmo LJB, Djoufack MM, Fotio D, Daboy CD, Fouelefack FR (2020) Nitrogen Use Efficiency (NUE) in tomato (*Solanum lycopersicum*) seedlings in response to treatment with extract of *Cymbopogon citratus* and mineralization of *Tithonia diversifolia* leaves and cow dung. Inter J Environ Agri Biotechnol 5(4):2456–1878

Nguefack J, Wulf GE, Dongmo LJB, Fouelefack FR, Fotio D, Mbo J, Torp J (2013) Effect of plant extracts and essential oil on the control of brown spot disease, tillering, number of panicles and yield increase in rice. Eur J Plant Pathol 137:871–882

Nygue MA (2006) Propriétés chimiques et biologiques des huiles essentielles de quelques plantes aromatiques et/ou médicinales du Cameroun: Evaluation de leurs activités antiradicuaires, anti-inflammatoire et antimicrobienne. Dissertation, Université de Montpellier.

Nygue MA, Ndonge FFMC, Riwom ES, Hockmeni TC, Etoa FX, Menut C (2014) Chemical composition of essential oils of *Eugenia caryophylla* and *Mentha sp* cf *piperita* and their in vitro antifungal activities on six human pathogenic fungi. Afri J of Trad Complement Alter Med 11(6):40–46

Okeke EC (2006) Crop losses to pests. J Agri Sci 144:31–43

Selim MA, Sberna N, Olcza-Woltman H, Oskiera M, Schollenberger M, Stapf, coupled with NaCl sorting on seed health, germination and seedlings vigor of rice (*Oryza sativa* L.). Afri J Agri Res. 13(3):104–114

Lahhou M (2004) Methods to study the photochemistry and bioactivity of the essential oils. Phytother Res 18:435–448

Lamichhane JR, Prošek M, Wondra AG (2005) Comparison of extracts prepared from *Tithonia diversifolia* flower. J Biores 7(1):521–539

Menut C (2014) Chemical composition of essential oils of *Parkia biglobosa* (Hemsl.). A. Gray. Inter J Biol 4(3):97

Martinez JA Inter J Biol 4(3):97

Słomnicka R, Olczak-Woltman H, Siemiarczuk M, Schollenberger M, Stasiak J, Bilong P, Tonye J (2009) Screening of seven plant species for their antifungal activities on six human pathogenic fungi. Afri J of Trad Complement Alter Med 11(6):40–46

Mekam PN, Martini S, Nguefack J, Tagliazucchi D, Mangoumou GN, Mfopou MYC, Dongmo LJB, Dongmo LJB, Djoufack MM, Fotio D, Daboy CD, Fouelefack FR (2020) Nitrogen Use Efficiency (NUE) in tomato (*Solanum lycopersicum*) seedlings in response to treatment with extract of *Cymbopogon citratus* and mineralization of *Tithonia diversifolia* leaves and cow dung. Inter J Environ Agri Biotechnol 5(4):2456–1878
Sonbolì A, Babakhani B, Mehrabian AR (2006) Antimicrobial activity of six constituents of essential oil from Salvia. Z Naturforsch [C] 61(3–4):160–164

Song XY, Wang H, Ren F, Wang K, Dou GLX, Yan DH, Strobel G (2019) An endophytic Diaporthe apiculatum produces monoterpens with inhibitory activity against phytopathogenic fungi. Antibiotics. https://doi.org/10.3390/antibiotics8040231

Takeo O, Masato K, Keiko S, Rika O, Junko M, Hiroshi I, Hiroyuki K, Toshi A, Toshifumi A, Shigeo M (2004) In vitro and in vivo antimicrobial activities of tricyclic ketolide Te-802 and its analogues. J Antibiotics 57:518–527

Tegos G, Stermitz FR, Lomovskaya O, Lewis K (2002) Antimicrobial Agents and Chemotherapy (AAC) features interdisciplinary studies that build our understanding of the underlying mechanisms and therapeutic. Antimicrob Agents Chemother 46:3133–3141

Wanzala W, Osundwa EM, Alwala OJ, Gakuubi MM (2016) Chemical composition of essential oil of Tithonia diversifolia (Hems) A. Gray from the Southern slopes of Mount Elgon in Western Kenya. Ind J Ethno Phytopharm 2(2):72–83

Wasim AMD, Dwaiyahan S, Ashim C (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdiscipl Toxicol 2(1):1–12

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.