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

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5 Abstract

6 The high cost of chemical pesticides and their negative impact on the environment prompted
7 the search for natural pesticides from plants. The objective of our study was to control rice
8 seed pathogenic fungi and bacteria using aqueous extract and essential oil from *Tithonia*
9 *diversifolia* leaves. We obtained aqueous extract and essential oil, respectively, by maceration
10 and hydrodistillation; the antimicrobial activities were determined *in vitro* on a solid medium
11 by the food poisoning method. The secondary metabolites were determined by qualitative and
12 quantitative assays; the chemical composition of the essential oil obtained from *Tithonia*
13 *diversifolia* was studied using gas chromatography coupled with mass spectrometry. The
14 results showed that phenols, tannins, flavonoids, alkaloids, terpenoids, sugars and saponins
15 were present in the aqueous extract. The essential oil contained mainly hydrocarbonated,
16 oxygenated monoterpenes, terpenoids and sesquiterpenes. α -terpineol (20.3%), eucalyptol
17 (14.6%), camphor (14.3%) and α -pinene (13.5%) as the main compounds. Regarding the
18 antimicrobial activity, all tested bacteria were sensitive to aqueous extract and essential oil.
19 The activity of the aqueous extract on the tested fungi showed an inhibitory concentration 50
20 (IC₅₀) of 50 mg/mL against *Bipolaris oryzae* and *Fusarium moniliforme*. The activity of the
21 essential oil on bacteria and fungi showed MIC of 125 μ g/mL (*Xanthomonas oryzae* pv.
22 *oryzae* and *Pseudomonas fuscovaginae*) and MFC of 5,000 μ g/mL (*Bipolaris oryzae* and
23 *Fusarium moniliforme*). These results allow us to consider *Tithonia diversifolia* as a potential
24 source of natural biopesticides against rice seed-borne pathogens.

25 **Keywords:** *Tithonia diversifolia*, Seed-borne pathogens, Biopesticides, Secondary
26 metabolites.

27 Declarations

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33 Cameroon.

34 **Code availability.** Not applicable.

35 **Authors' contributions.** Laboratory experiments were carried out by Dongmo Nanfack
36 Albert, Emilio Stefani, and Nguefack Julienne. The essential oil was characterized by and
37 Fouelefack Romain François and Nkengfack Augustin Ephrem. The manuscript was written
38 by Emilio Stefani, Azah Udom Rene, and Dongmo Lekagne Blaise Joseph.

39 Introduction

40 The rice demand in Cameroon has more than doubled over the last decade; milled rice
41 imports rose from 469,450 to 728,433 tons, while the paddy yield fell from 2.74 to 1.33
42 tons/ha from 2009 to 2017, respectively. (FAOSTAT 2019). Yield reductions are mainly due
43 to the increasing impact of pests and diseases and their limited control, especially in the case
44 of seed-borne pathogens (Oerke 2006).

45 Disease surveys of rice grown in Cameroon revealed the existence of brown spot (*Bipolaris*
46 *oryzae*) and bakanae disease (*Fusarium moniliforme*), which can respectively lead to a yield
47 reduction of about 67% and 20% (Barnwal et al. 2013; Nguefack et al. 2013). Bacterial leaf
48 blight (*Xanthomonas oryzae* pv. *oryzae*) is present in Cameroon, as in several rice-growing
49 areas worldwide, and can lead to a yield loss of 30-35% (Jones et al. 1993; Sere et al. 2005)
50 or even rise to 50% or more, depending on variety, growth stage, and climatic conditions
51 (Kala et al. 2015). *Pseudomonas fuscovaginae*, the causal agent of sheath brown rot, although
52 not yet officially reported in Cameroon is an emerging threat for rice cultivation (CABI
53 2019). Like *X. oryzae* pv. *oryzae*, *P. fuscovaginae* is a seed-borne and seed-transmitted
54 pathogen of rice, and contribute to the reduction of the photosynthetic capacity of plants
55 (Lamichhane et al. 2015; Słomnicka et al. 2018), thus causing severe yield losses, estimated
56 from 30% to 60%, depending on the species susceptibility (Olczak-Woltman et al. 2008).

57 In Cameroon, as in other developing countries, synthetic pesticides used in plant disease
58 management are frequently unavailable, expensive for poor farmers, and often have negative
59 effects on the ecosystems, including their action on untargeted organisms and the
60 development of pathogen resistance (Wasim 2009). Nowadays, the development and
61 implementation of innovative and sustainable pest management strategies, based on the use
62 of plant extracts as an alternative to synthetic agrochemicals, is becoming more and more
63 explored. Plant extracts are important sources of new agrochemicals with satisfying

64 antimicrobial properties for the control of plant diseases (Fouelefack et al. 2018; Mekam et
65 al. 2019). Plant extracts are usually broad-spectrum antimicrobials, eco-friendly and with
66 minor effects as environmental pollutants; sometimes they are beneficial to soil organisms
67 (Sharma et al. 2015).

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

78 **Materials and methods**

79 **Plant material and media**

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

86 Culture media (Potato dextrose agar, Nutrient sucrose agar, and the reference antibiotic
87 (gentamycin) for biocontrol activities were purchased from Sigma-Aldrich (Milan, Italy).

88 Deionized water was obtained from a Milli-Q System (Bedford, MA, USA). Reference
89 fungicide Banko plus[®] was purchase from ADER, Douala, Cameroon.

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

91 The aqueous plant extract was obtained by maceration in distilled water. One hundred grams
92 (100 g) of powder of *T. diversifolia* leaves were weighed and macerated into 600 mL (1:6,
93 w/v) of distilled water under a magnetic stirrer at 120 rpm for 24 h, at a temperature of 25 °C.
94 After filtration through a Whatman No. 1 paper, the filtrate was centrifuged at 5,000 rpm for
95 5 min and the supernatant was collected and dried in an oven at 48 ± 2 °C overnight. The
96 extraction yield was calculated by weighing the dried extract per total mass of powder used
97 and extracts were stored at 4 °C until use.

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

102 **Phytochemical Screening**

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

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

108 The determination of phenols and flavonoids was chosen since the majority of the biological
109 properties of the plant are attributed to them (Boizot and Charpontier 2006). *T. diversifolia*
110 leaf powder was weighed and dissolved in the corresponding volume of distilled water to
111 obtain different concentrations (1%, 3%, 5%, and 10%). After 24 h, the mixture was decanted

112 and filtered. The filtrate was kept at 4 °C for the determination of the phenol and flavonoid
113 content.

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

123 The total flavonoid content was evaluated using the aluminium chloride protocol as described
124 by Enujiugha (2010). Briefly, 0.25 mL of aqueous extract (as prepared above) was mixed
125 with 1.25 mL distilled of water (1:5, v/v) and 50% NaNO₃ (75 µL) and allowed to stay for 6
126 min at room temperature. Then, 150 µL of a 10% aluminium chloride solution was added in
127 the mixture and incubated for 5 min at room temperature. The reaction solution was brought
128 to 5 mL with distilled water and 0.5 mL of 1M sodium hydroxide was added. After
129 homogenization, the absorbance was measured at $\lambda = 510$ nm using a spectrophotometer (UV
130 160, Shimadzu, Japan). The total flavonoid content of each treatment was expressed as
131 catechin equivalents (mg of CE/g of dry powder) using a calibration curve [OD = f (weight of
132 catechin)].

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

134 The composition of the essential oil from *T. diversifolia* leaves was determined by using an
135 analytical gas-chromatography (GC-FID) and gas-chromatography coupled with mass
136 spectrometry (GC/MS) techniques. The column used and experimental conditions were both

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

149 **Antimicrobial activity *in vitro***

150 **Bacterial and fungal strains**

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

159 **Antibacterial activity**

160 To check the possible antibacterial activity of both aqueous extract and essential oil, assays
161 were done using the modified disk diffusion method (CLSI 2007). This method is based on

162 the diffusion of extracts from filter paper discs in contact with the solid culture medium
163 (NSA) into Petri dishes, previously inoculated with a bacterial inoculum (10^6 CFU/mL).
164 Essential oil was diluted in 5% Tween 20 (v/v) to obtain a concentration of 100 mg/mL; the
165 same concentration was used for the aqueous extract (100 mg/mL). Then, 10 μ L of essential
166 oil and 30 μ L of extract were spotted on different sterilized paper disks, before plating them
167 on the agar surface with the two phytopathogenic bacteria. Gentamicin (1 mg/mL) was used
168 as a control (5 μ L were spotted on sterilized paper disks, before plating them on the agar
169 surface). The inoculated Petri dishes were then incubated at 27 °C and the antibacterial
170 inhibition was assessed after 48 h by measuring the inhibition haloes. The microbial
171 sensitivity was classified according to the diameter of the zones of inhibition as follows: not
172 sensitive for diameters less than 8 mm; sensitive for diameters between 9-14 mm; very
173 sensitive for diameters between 15-19 mm and extremely sensitive for diameters \geq 20 mm
174 (Moreira et al. 2005).

175 **Antifungal activity**

176 The antifungal activity of the aqueous extract and the essential oil from *T. diversifolia* were
177 checked *in vitro* by measuring the inhibition of the mycelial growth on PDA, supplemented
178 with increasing concentrations of the extract and essential oil. Three increasing
179 concentrations of the aqueous extract were used: 10, 50, and 100 mg/mL; five increasing
180 concentrations were considered for the essential oil: 625; 1,250; 2,500; 5,000; and 10,000
181 μ g/mL. The positive control used was Banko plus[®], the most common fungicide indicated for
182 rice (Chlorothalonil 550 g/L - Carbendazm 100 g/L as active substances) at following
183 concentrations: 62.5; 125; 250; 500; and 1,000 μ g/mL; the negative control was represented
184 by PDA plates supplemented with sterile distilled water. Each agar plate was then inoculated
185 with a 5 mm mycelium plug taken from the margin of a 6-days old culture of each fungus and
186 kept in an incubator at 27 °C. Growth inhibition was assessed after seven days: this was done

187 by measuring the two perpendicular diameters of the fungal colony (Nyegue et al. 2014). The
188 mycelium growth inhibition relative to the controls was then calculated according to the
189 following equation:

$$190 \text{ Mycelium growth inhibition (\%)} = (D-d)/D \times 100$$

191 where, D = mycelium diameter in the control PDA plate, d = mycelium diameter in the
192 amended PDA plate. The tests were carried out in triplicates and the experiments were
193 independently repeated 3 times.

194 The concentration of plant extracts required to inhibit by 50% the fungal growth (IC₅₀) was
195 determined by plotting the growth inhibition percentage as a function of final plant extract
196 concentration (base-10 logarithm). IC₅₀ values were expressed as mg of extract/mL. The
197 antifungal activity of the aqueous extract and essential oil was evaluated as follow: strong
198 activity, when mycelial growth inhibition was > 50%; weak activity when mycelial growth
199 inhibition was <50% or not active when no inhibition was observed (Nyegue 2006).

200 **Determination of the Minimum Inhibitory Concentrations, Minimum Bactericidal** 201 **Concentration and Minimum Fungicidal Concentration**

202 The modified microdilution method described by CLSI (2007) was used for the determination
203 of the minimum inhibitory concentrations (MIC). The MIC was defined as the lowest
204 concentration of aqueous extract or essential oil visibly inhibiting bacterial growth after 48 h
205 of incubation at 27 °C. Into each well, 100 µL of broth enriched with 5% red phenol was
206 added. Then, 100 µL of aqueous extract or essential oil were added in every first well of the
207 microplate. Geometric dilutions ranging from 50 to 0.781 mg/mL were carried out and
208 subsequently, 100 µL of media containing 10⁶ CFU/mL of the target strain was added to all
209 wells to yield 25 to 0.0152 mg/mL of concentration. The plates were then incubated at 27 °C
210 for 48 h. For both extract and essential oil, the experiment was done in triplicate. A colour
211 change from red to yellow indicated a bacterial growth. To obtain the minimum bactericidal

212 concentration (MBC), 20 μ L of each well coloured in red was spotted on the agar surface and
213 incubated at 27 °C for 48 h. The MBC was defined as the lowest concentration of aqueous
214 extract and essential oil where less than 10 colonies growing in the plate were counted. The
215 ratio MBC/MIC was calculated.

216 The MICs of the fungi were determined directly on PDA supplemented with aqueous extract
217 and essential oil. MICs were the lowest concentrations of aqueous extract or essential oil
218 inhibiting visible growth of the target fungi on the agar plate after 7 days of growth. To
219 determine the minimum fungicidal concentration (MFC), the explants present in the plate
220 considered as MIC were subcultured in non-supplement PDA plates. After 4 days of
221 incubation at 27 °C, the absence of mycelial growth indicates the MFC. The ratio MFC/MIC
222 was then calculated: according to Avril and Fauchere (2002), adapted to fungi by Nyegue
223 (2006), for $MFC/MIC < 4$ the sample is classified as "fungicidal", when the values are in the
224 range $4 < MFC/MIC < 16$, the sample is considered "fungistatic", and finally when MFC/MIC
225 > 32 , it is called "tolerant".

226 **Statistical analyses**

227 Average and standard deviations have been calculated using Excel 2007 software. The graphs
228 were made using SigmaPlot and GraphPad Prism software. Analysis of data variance and
229 comparison of means using the Post Hoc (LSD) test performed at the 5% probability level (p
230 < 0.05), using IBM-SPSS16.0 software.

231 **Results**

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

233 The crude aqueous extract of *T. diversifolia* leaves was obtained by maceration of powdered
234 dry leaves. Dried aqueous extract consisted of a dark green powder; its average yield was
235 29.75% of the dry leaves weight.

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

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

240 **Quantitative phytochemical content of phenols and flavonoids**

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

247 **Extraction and characterization of essential oil**

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

252 **Chemical composition of essential oil**

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

256 The GC profile of the essential oil showed that terpenes/terpenoids are the main constituents
257 (Table 1). Alpha-terpineol, a monoterpene alcohol, was the component detected in highest
258 amount (20.3%); with terpinen-4-ol (1.8%) its isomeric form, they represent almost one-
259 fourth of the chemical composition of this essential oil. Another major component of the oil
260 was α -pinene (13.5%); considering that another detected molecule was camphor (14.3%), a
261 cyclic ketone derivative from the oxidation of α -pinene, both (α -pinene and camphor)

262 substances may be considered the major component of *T. diversifolia* essential oil.
263 Sesquiterpenes were also found in a not negligible amount: spathulenol, globulol and ledol
264 reach together a percentage of 10.5%. Therefore, monoterpenes and sesquiterpenes represent
265 more than 60% of the essential oil components.

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

267 The aqueous extract and the essential oil from *T. diversifolia* were tested *in vitro* for their
268 putative antibacterial activity. Both extracts showed remarkable antibacterial activity against
269 the two bacteria tested: such activity was revealed by the formation of a clear and large
270 inhibition halo around the paper discs soaked with the extracts. The antimicrobial activity of
271 both extracts against both phytopathogenic bacteria was comparable, since the inhibitory
272 haloes showed a similar area in any replicate plates. Interestingly, the tested tracts proved a
273 superior antibacterial activity than the antibiotic gentamicin, at the given concentrations (Fig.
274 2).

275 The results obtained from the activity of the aqueous extract and the essential oil on targeted
276 bacteria are quantitatively illustrated in figure 3. Histograms show that the two bacterial
277 strains were strongly inhibited by both the aqueous extract and the essential oil of *T.*
278 *diversifolia*. The inhibition diameters of the aqueous extract and the essential oil ranged from
279 21 to 24 mm. Interestingly, bacterial sensitivity to both extracts was higher than that of
280 gentamicin, for which the inhibition diameters vary from 12 to 15 mm for both bacteria. Such
281 inhibition was reached using the concentration of 100 mg/mL for both extracts, compared to
282 a concentration of gentamicin of 1 mg/mL.

283 **Determination of Minimum Inhibitory Concentration and Minimum Bactericidal** 284 **Concentration**

285 The inhibition parameters of aqueous extract, MIC and MBC, were not assessed, but those of
286 essential oil were determined. The latter data made it possible to calculate the ratio

287 MBC/MIC. This relationship made it possible to characterize a bactericidal, bacteriostatic
288 action or to determine the “tolerance” of a strain (Table 2).

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

295 ***In vitro* antifungal activity of aqueous extract and essential oil**

296 In general, aqueous extract and essential oil of *T. diversifolia* showed good inhibitory activity
297 against both *B. oryzae* and *F. moniliforme*. Such inhibitory activity increased in a dose-
298 dependent manner (Fig. 4). A concentration of 100 mg/mL of the plant extract inhibited the
299 mycelial growth of *B. oryzae* and reduced its growth rate by approximately 68.44%. Similar
300 results were observed against *F. moniliforme*: a concentration of 100 mg/mL reduced the
301 mycelial growth by approximately 70.69%. The essential oil demonstrated a more evident
302 antifungal activity against both pathogens. It was active at a concentration of 625 µg/mL, and
303 produced a total fungal inhibition at a concentration of 5,000 µg/mL and above (Fig. 4).

304 Figure 5 shows the quantitative mycelium growth inhibition (%) stimulated by the aqueous
305 extract from *T. diversifolia* leaves against *B. oryzae* and *F. moniliforme*. The mycelium
306 growth inhibition ranged from 25% at 10 mg/mL to 68.44% at 100 mg/mL of aqueous extract
307 on *B. oryzae* and from 20% at 10 mg/mL to 70.69% at 100 mg/mL of aqueous extract on *F.*
308 *moniliforme*. Inhibitory concentration 50 (IC₅₀) was determined and it was calculated at 50
309 mg/mL on *B. oryzae* and *F. moniliforme*. According to the scale of Nyegue (2006), the
310 aqueous extract exhibited a strong antimicrobial activity with IC₅₀ > 50%. A total growth

311 inhibition of *B. oryzae* and *F. moniliforme* was not reached using aqueous extracts at the
312 dilutions tested (Fig. 5).

313 Figure 6 shows the mycelium growth inhibition (%) obtained by using the essential oil of *T.*
314 *diversifolia* leaves against *B. oryzae* and *F. moniliforme*. The mycelium growth inhibition
315 ranged from 58.28% at 625 µg/mL to 100% at 5,000 µg/mL of essential oil concentrations on
316 *B. oryzae* and from 56.87% at 625 µg/mL to 100% at 5,000 µg/mL of essential oil
317 concentrations on *F. moniliforme*. Therefore, the sensitivity of both fungi to the essential oil
318 was quite similar. Inhibitory concentration 50 (IC₅₀) was then determined and resulted to be
319 625 µg/mL for both *B. oryzae* and *F. moniliforme*, with a percentage inhibition of 58.29%
320 and 56.87%, respectively. According to the scale of Nyegue et al. (2006), the essential oil
321 showed a very strong antimicrobial activity, with an IC₅₀ > 50%. The minimal inhibition
322 concentration was 5,000 µg/mL. Also, we obtained complete fungal inhibition at 5,000
323 µg/mL, which corresponds to the MFC (Fig. 6).

324 **Determination of Minimum Inhibition Concentration and Minimum Fungicidal** 325 **Concentration**

326 As shown in table 3 the MFC obtained in our experiments using the essential oil was 5,000
327 µg/mL; this value was obtained for both fungi tested. The calculated ratio MFC/MIC was 1;
328 according to the scale of Avril and Fauchere (2002), the essential oil of *T. diversifolia* leaves
329 has a fungicidal activity. Therefore, in our experiments, the activity of the essential oil was
330 comparable to the antifungal action of the reference fungicide (Banko plus[®]), since both their
331 calculated MFC/MIC were less than 4 (Table 3).

332 **Discussion**

333 Plant-derived biomolecules have drawn great attention during the last 15 years, due to their
334 general antimicrobial properties; indeed, they have been suggested as prospective compounds
335 to be used during the development of innovative biopesticides and in the implementation of

336 sustainable strategies to control phytopathogenic fungi and bacteria (Reignault and Walters
337 2007; Martinez 2012). The present study showed that the aqueous extract of *T. diversifolia*
338 and its essential oil possess a pronounced antimicrobial activity and may be considered
339 sources of bioactive phytochemicals. Its leaves are very rich in phenols, flavonoids,
340 terpenoids, alkaloids, glycosides, saponins, and tannins; these results are in agreement with
341 the findings of Olutobi and Olasupo (2012), who reported the presence of similar
342 phytochemical compounds in the methanolic extract of *T. diversifolia* leaves. Some authors
343 have demonstrated their biological activity, among which antibacterial (Desi et al. 2017) and
344 antifungal (Saini et al. 2009; Mekam et al. 2019).

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

353 The gas chromatographic profile of *T. diversifolia* essential oil showed a total of 19
354 compounds; terpenes and terpenoids were the main constituents, accounting for 95% of the
355 composition. In the present study, the main constituents were α -pinene (13.5%), camphor
356 (14.3%), eucalyptol (14.6%) and α -terpineol (20.3%): these results are different from those
357 obtained by Wanzala et al. (2016) in Kenya, Adebayo et al. (2008) in Nigeria and Ingrid et al.
358 (2018) in Brazil, who showed that the essential oil of *T. diversifolia* is mainly rich in α -
359 pinene in the proportion 63.64%, 4.4% and 45%, respectively. These differences could be due
360 to the difference among geographical areas where the plants grew and were harvested

361 (Arbona et al. 2013). These authors gave no clear details on the handling of *T. diversifolia*;
362 thus, other intrinsic factors, such as storage condition and age of plants, could considerably
363 influence the composition (Lapornik et al. 2005).

364 The activity of essential oil is often reduced to the activity of its major compounds, or those
365 likely to be active; however, some minor compounds may act in synergy with the major or
366 other compounds (Sonboli et al. 2006; Lahlou 2004). The antibacterial and antifungal activity
367 observed in this study could be attributed to the presence of the identified major compounds.

368 In fact, α -pinenes destroy the cellular integrity of pathogens, inhibiting both their respiration
369 and the ion transport process, while modifying cell permeability (Andrews et al. 1980);
370 eucalyptol and camphor display antimicrobial effect against phytopathogenic fungi and are
371 widely exploited to control post-harvest diseases and the growth of mycotoxigenic fungi
372 (Rahmouni et al. 2019); α -terpineol was recently shown to possess antimicrobial activity
373 against important phytopathogenic fungi (Song et al. 2019).

374 The essential oil from *T. diversifolia* proved to be active against rice pathogenic bacteria, with
375 MICs of 125 $\mu\text{g/mL}$; according to Tegos et al. (2002), phytochemicals or extracts with MIC
376 values between 100 $\mu\text{g/mL}$ and 1,000 $\mu\text{g/mL}$ are considered as antimicrobials of interest. The
377 differences in sensitivity between the fungal and bacterial species concerning the aqueous
378 extract and the essential oil of *T. diversifolia* leaves observed during our study may be due to
379 intrinsic factors specific to each microorganism (Takeo et al. 2004) or due to the
380 phytochemical profile of the aqueous extract and essential oil; oxygenated molecules like
381 phenols, alkaloids, flavonoids, oxygenated terpenoids are generally more active than lipophilic
382 hydrocarbons (Silva et al. 2012), but the high concentration of the latter makes the essential
383 oil more active.

384 The essential oil was more active as antimicrobial compound than the aqueous extract against
385 the two target bacterial strains (*X. oryzae* pv. *oryzae* and *P. fucovaginae*) and the two rice

386 pathogenic fungi (*B. oryzae* and *F. moniliforme*); the MICs of our essential oil were 125
387 µg/mL and 5,000 µg/mL against bacteria and fungi strains, respectively. These MICs were
388 higher compared to those reported by Ingrid et al. (2018) and Oludare et al. (2016), who also
389 worked with *T. diversifolia* essential oil; they found MICs of 1,000 µg/mL against
390 *Streptococcus mitis* and 72,000 µg/mL against *Fusarium solani*. Thus, the activity of
391 essential oil of *T. diversifolia* is microorganisms dependent (Miranda et al. 2016).

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

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

409 The powerful antibacterial and antifungal activities of *T. diversifolia* extracts opens new
410 chances for African farmers to manage the most destructive rice pathogens and, additionally,

411 may stimulate new opportunities in the development of locally based small/medium sized
412 industries devoted to make use of a common local botanic resource. In order to reinforce this
413 approach towards the production of Traditional Improved Pesticides (TIP) and the discovery
414 of new growth potentiating substances, these results call us to complete this work and consider
415 the evaluation of active fractions on pathogen reductions for future studies. In particular, such
416 plant bioactive extracts might be taken into consideration when developing seed treatments, in
417 order to effectively decrease the primary inoculum of these seed-transmitted pathogens;
418 therefore, the next research step would be to observe their phytotoxicity on the germination
419 and physiology of the rice seedlings.

420 **References**

- 421 Adebayo AG, Tira-Picosb V, Nogueira JMF (2008) Analysis of chemical constituents of
422 *Tithonia rotundifolia* leaf essential oil found in Nigeria. Nat Product Comm 3(9): 1537-
423 1538
- 424 Andrews RE, Parks LW, Spence KD (1980) Some effects of Douglas fir terpenes on certain
425 microorganisms. Appl Environ Microbiol 40(2): 301-304
- 426 Arbona V, Manzi M, Ollas CD, Gómez-Cadenas A (2013) Metabolomics as a tool to
427 investigate abiotic stress tolerance in plants. Inter J Molecul Sci 14: 4885-4911
- 428 Avril JL, Fauchere JL (2002) General and medical bacteriology. Ellipses, Paris, France.
- 429 Barnwal MK, Kotasthane A, Magculia N, Mukherjee PK, Savary S, Sharma AK, Singh HB,
430 Singh US, Sparks AH, Variar M, Zaidi N (2013) A review on crop losses, epidemiology
431 and disease management of rice brown spot to identify research priorities and knowledge
432 gaps. Eur J Plant Patholog 136: 443-457
- 433 Boizot N, Charpontier JP (2006) Rapid method for assessing the phenolic compound content
434 of organs in a forest tree. The INRA Tech. Notebook, pp 79-82

435 CABI (2019) Invasive Species Compendium. Wallingford, UK: CAB International.
436 www.cabi.org/isc. Accessed 10 November 2019

437 CLSI (Clinical and Laboratory Standards Institute) (2007) Performance standards for
438 antimicrobial disk and dilution susceptibility test methods for antimicrobial susceptibility
439 testing for bacterial isolation from animal-Approved standard, 3thedn. CLSI document
440 M11-A7-Clinical and Laboratory Standards Institute, Wayne PA (USA), pp 50-71

441 Desi R, Suharto, Hardian SA (2017) Antimicrobial activity of *Tithonia diversifolia*,
442 *Elephantopus scaber*, and *Kigelia africana* against plant pathogens. Front Environ
443 Microbiol 3(4): 56-61

444 Edeoga H, Okwo D, Mbaebie B (2005) Phytochemical constituents of Nigerian medicinal
445 plants. Afri J Biotech 4: 685-688

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

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

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

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

456 Ingrid PS, Chagas-Paula DA, Renata FJT, Eliane de OS, Mariza AM, Barbosa de Oliveira R,
457 Augusto CCS, Jairo KB, Niede AJCF, Da Costa FB (2018) Essential oils from *Tithonia*
458 *diversifolia* display potent anti-oedematogenic effects and inhibit acid production by
459 cariogenic bacteria. J Essential Oil Res. [https://doi.org/ 10.1080/10412905.2018.1500315](https://doi.org/10.1080/10412905.2018.1500315)

460 Jones MP, Jeutong F, Tchatchoua J (1993) A survey of rice diseases in Cameroon. *Plant Dis*
461 77: 133-136

462 Kaho F, Nyambi NG, Yemefack M, Yongue-Fouateu R, Amang- Abang J, Bilong P, Tonyé J
463 (2009) Screening of seven plant species for short term improved fallow in the humid forest
464 zone of Cameroon. *Commun Soil Sci Plant Anal* 40: 1-10

465 Kala A, Soosairaj S, Mathiyazhagan S, Raja P (2015) Isolation and identification of
466 *Xanthomonas oryzae* pv. *oryzae* the causal agent of rice bacterial leaf blight and its
467 activities against of six medicinal plants. *Asi J Plant Sci Res* 5(6): 80-83

468 Kovàts E (1958) Characterization of organic compounds by gas chromatography. Part 1.
469 Retention indices of aliphatic halides, alcohols, aldehydes, and ketones. *Helveti Chimi Act*
470 41: 1915-1932

471 Lahlou M (2004) Methods to study the photochemistry and bioactivity of the essential oils.
472 *Phytother Res* 18: 435-448

473 Lamichhane JR, Messéan A, Morris CE (2015) Insights into epidemiology and control of
474 diseases of annual plants caused by the *Pseudomonas syringae* species complex. *J Genet*
475 *Plant Pathol* 81: 331-350

476 Lapornik B, Prošek M, Wondra AG (2005) Comparison of extracts prepared from plant by
477 products using different solvents and extraction time. *J Food Engin* 71: 214-222

478 Linthoingambi W, Muthum SS (2013) Antimicrobial activities of different solvent extracts of
479 *Tithonia diversifolia* (Hemsely) A. Gray. *Asi J Plant Sci Res* 3(5): 50-54

480 Martinez JA (2012) Natural Fungicides Obtained from Plants. In: *Fungicides for Plant and*
481 *Animal Diseases* (Dhanasekeran D, Thajuddin N, Panneerselvam A, ed.). Available at:
482 [https://www.intechopen.com/books/fungicides-for-plant-and-animal-diseases/natural-](https://www.intechopen.com/books/fungicides-for-plant-and-animal-diseases/natural-fungicides-obtained-from-plants)
483 [fungicides-obtained-from-plants](https://www.intechopen.com/books/fungicides-for-plant-and-animal-diseases/natural-fungicides-obtained-from-plants). Accessed 10 November 2020

484 Mekam PN, Martini S, Nguéfack J, Tagliazucchi D, Mangoumou GN, Stefani E (2019) The
485 activity of extracts from three tropical plants towards fungi pathogenic to tomato (*Solanum*
486 *lycopersicum*). *Phytopath Medit* 58(3): 573-586

487 Miranda CASF, Cardoso MG, Batista LR, Rodrigues LMA, Figueiredo ACS (2016) Essential
488 oils from leaves of various species: antioxidant and antibacterial properties on growth in
489 pathogenic species. *Reviews Ciênc Agronomic. J Essential Oil Res* 47: 213-220

490 Moreira MR, Ponce AG, Del Valle CE, Roura SI (2005) Inhibitory parameters of essential
491 oils to reduce a foodborne pathogen. *Food Sci Technol J* 38: 565-570

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

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

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

503 Nyegue MA, Ndoyé FFMC, Riwom ES, Hockmeni TC, Etoa FX, Menut C (2014) Chemical
504 composition of essential oils of *Eugenia caryophylla* and *mentha sp cf Piperita* and their
505 *in vitro* antifungal activities on six human pathogenic fungi. *Afri J of Trad Complement*
506 *Alter Med* 11(6): 40-46

507 Oerke EC (2006) Crop losses to pests. *J Agri Sci* 144: 31-43

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

512 Oludare OA, Stephen O, Joshua OO, Abdulwakeel A, Oladipo A (2016) Chemical
513 composition and antimicrobial activities of essential oil extracted from *Tithonia*
514 *diversifolia* (*Asteraceae*) flower. J Biores Bioproduct 1(4): 169-176

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

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

520 Rahmouni A, Saidi R, Khaddor M (2019) Chemical composition and antifungal activity of
521 five essential oils and their major components against *Fusarium oxysporum* f. sp. *albedinis*
522 of Moroccan palm tree. Euro-Medi J Environ Integr, [https://doi.org/ 10.1007/s41207-019-](https://doi.org/10.1007/s41207-019-0117-x)
523 0117-x

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

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

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

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

534 Silva ACR, Lopes PM, Azevedo MMB, Costa DCM, Alviano CS, Alviano DS (2012)
535 Biological activities of α -pinene and β -pinene enantiomers. Molecules 17(6): 6305-6316.

536 Słomnicka R, Olczak-Woltman H, Oskiera M, Schollenberger M, Niemirowicz-Szczytt K,
537 Bartoszewski G (2018) Genome analysis of *Pseudomonas syringae* pv. *lachrymans* strain
538 814/98 indicates diversity within the pathovar. Eur J Plant Pathol 151: 663-676

539 Sonboli A, Babakhani B, Mehrabian AR (2006) Antimicrobial activity of six constituents of
540 essential oil from *Salvia*. Z Naturforsch [C] 61(3-4): 160-4

541 Song XY, Wang H, Ren F, Wang K, Dou GLX, Yan DH, Strobel G (2019) An endophytic
542 *Diaporthe apiculatum* produces monoterpenes with inhibitory activity against
543 phytopathogenic fungi. Antibiotics, 8(4), <https://doi.org/10.3390/antibiotics8040231>

544 Takeo O, Masato K, Keiko S, Rika O, Junko M, Hiroshi I, Hiroyuki K, Toshi A, Tosshifumi
545 A, Shigeo M (2004) *In vitro* and *in vivo* antimicrobial activities of tricyclic ketolide Te-
546 802 and its analogues. J Antibiotics 57: 518-527

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

550 Wanzala W, Osundwa EM, Alwala OJ, Gakuubi MM (2016) Chemical composition of
551 essential oil of *Tithonia diversifolia* (Hemsl.) A. Gray from the Southern slopes of Mount
552 Elgon in Western Kenya. Indi J Ethno Phytopharm 2 (2): 72-83

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

555 **List of tables**

556 **Table 1** Formula, name of the compounds, retention index (RI) and percentage of the
 557 compound in the essential oil extracted from *Tithonia diversifolia* leaves

	Formula	Name of chemical compounds	RI	Percentage
1	C ₁₀ H ₁₆	α-Pinene	917.7	13.5
2	C ₆ H ₆ O	phenol	968.9	0.4
3	C ₁₀ H ₁₄	p-cymene	1022.6	2.2
4	C ₁₀ H ₁₈ O	eucalyptol, (cineole)	1034.7	14.6
5	C ₁₀ H ₁₆	γ-terpinene	1072.4	0.5
6	C ₁₀ H ₁₈ O ₂	Epoxy linalool	1093.1	1.3
7	C ₁₀ H ₁₈ O ₂	Linalool, oxide	1111.9	1.5
8	C ₁₀ H ₁₈ O	Linalool, hydrate	1121.8	0.7
9	C ₁₀ H ₁₆ O	α-campholena ldehyde	1153.2	2.6
10	C ₁₀ H ₁₆ O	Pinocarveol	1168.4	5.1
11	C ₁₀ H ₁₈ O	Camphor	1194.7	14.3
12	C ₁₀ H ₁₈ O	Terpinen-4-ol	1203.5	1.8
13	C ₁₀ H ₁₈ O	α-Terpineol	1220.6	20.3
14	C ₁₀ H ₁₆ O	Myrtenol	1225.6	0.3
15	C ₁₀ H ₁₄ O	Carvacrol	1324.7	0.8
16	C ₁₅ H ₂₄ O	Spathulenol	1615.4	3.3
17	C ₁₅ H ₂₆ O	Globulol	1622.7	1.5

18	$C_{15}H_{26}O$	Ledol	1631.7	5.7
19	$C_{15}H_{26}O$	2-Naphthalenemethanol	1689.8	1.2

558

559

560 **Table 2** Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration
 561 (MBC) of essential oil from *Tithonia diversifolia* and gentamicin

Tested product	Inhibition parameters	Pathogens	
		<i>X. oryzae</i> pv. <i>oryzae</i>	<i>P. fuscovaginae</i>
Essential oil (µg/mL)	MIC	125	125
	MBC	250	125
	MBC/MIC	2	1
Gentamicin (µg/mL)	MIC	31.25	31.25
	MBC	31.25	31.25
	MBC/MIC	1	1

562

563

564 **Table 3** Minimum fungicidal concentration of essential oil and Banko Plus[®]

Tested product	Inhibition parameters	Pathogens	
		<i>B. oryzae</i>	<i>F. moniliforme</i>
Essential oil (µg/mL)	MIC	5,000	5,000
	MFC	5,000	5,000
	MFC/MIC	1	1
Banko Plus [®] (µg/mL)	MIC	1,000	500
	MFC	1,000	500
	MFC/MIC	1	1

565