

Evaluation of the Efficacy of Four Essential Oils Against *Rhizoctonia Solani*, A Telluric Pathogen of Tomato

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Abstract

In a context of reduction of the abusive use of pesticides in vegetable growing, investigations have shown promising effects of some plant extracts on phytopathogenic microorganisms. The objective of this study was to evaluate the antifungal activity of essential oils extracted from *Cymbopogon citratus*, *Ocimum basilicum*, *Lippia multiflora* and *Eucalyptus camaldulensis*. Tests were carried out, in vitro, with these essential oils and the synthetic fungicide Banko plus on the mycelial growth of *Rhizoctonia solani*. The antifungal activity of the two essential oils, retained after in vitro test, was evaluated, in vivo, on inoculated plants of the tomato variety UC 82 B. These products were used at their respective MIC in preventive and curative treatment. A split plot device with two factors, treatment and application mode, was used with five replicates. Total inhibition (100%) of mycelial growth of *R. solani*, was obtained with *O. basilicum* from 2000 ppm. Banko plus and the essential oils of *C. citratus* and *O. basilicum* were tested in vivo at their respective MIC of 4000 ppm, 4000 ppm and 2000 ppm against *R. solani* as a preventive and curative treatment. The essential oil of *O. basilicum* as a preventative (2000 ppm) and the synthetic fungicide Banko plus as curative (4000 ppm) reduced the mortality rate to 100% and then improved the growth parameters of the plant. The essential oil of *O. basilicum* could be used in biological control of *R. solani*.

Keywords: Antifungal activities, essential oils, *Rhizoctonia solani*, *Lycopersicon esculentum*, Korhogo

INTRODUCTION

Vegetable crops play an important role in human nutrition and contribute significantly to family income in West Africa (Yarou *et al.*, 2017). According to James *et al.* (2010) and Yohou *et al.* (2015), they are considered sovereignty crops, playing a primary role in most nutrition and poverty alleviation programs. In the north of Côte d'Ivoire, specifically in the city of Korhogo, urban market gardening plays an important role. The market garden produce produced there supplies many cities in the country with fresh vegetables (Soro *et al.*, 2018). Among these vegetables, the tomato occupies a place of choice. Indeed, it is the most consumed vegetable and had been industrialized in Côte d'Ivoire through SODEFEL before being held today by the producers themselves and especially women (Doumbouya *et al.*, 2012). National tomato production has increased from 34,734 tons in 2013 to approximately 44,078 tons in 2018. Despite this increase, production remains insufficient and is a concern for the entire country. This insufficiency is related to various reasons including the huge losses caused by pests and especially diseases of fungal order. This insufficiency is linked to various reasons, including the enormous losses caused by bio-aggressors and especially fungal diseases. They not only depreciate the quality but also decrease the quantity of the crops. To boost their production and improve their yield, most market gardeners resort to the systematic use of an

array of phytosanitary products (Kanda *et al.*, 2009; Soro *et al.*, 2018). However, the preservation of biodiversity and the growing demands of consumers, raises the controversy around the use of pesticides because of their harmful effects on human and animal health and on the environment. The need to find other control strategies is necessary (Cissé and Dasylyva, 2017). Investigations on essential oils from aromatic plants have shown their effectiveness in traditional medicine but also in agriculture. In addition, extracts and essential oils of various plant species have shown convincing results on pests and especially on fungal pathogens (Doumbouya *et al.*, 2012; Kassi *et al.*, 2014; Badji, 2015; Faye, 2015; Cissé, 2016). It is in this context that this study was conducted to evaluate, *in vitro* and *in vivo*, the antifungal activity of essential oils extracted from certain plants against *Rhizoctonia solani*.

MATERIALS AND METHODS

Fungal strain and plant material

The fungal material consisted of the pathogen *Rhizoctonia solani* isolated from infected tomato plants from the market garden area of the "Petit Paris" district (Korhogo). The tomato variety used in the *in vivo* evaluation of the antifungal activity of the fungal strains was the cultivar UC 82 B. This is the variety of tomato essentially cultivated by market gardeners in the Korhogo market garden production zones. The seeds of this tomato variety were obtained from the seed company SEMIVOIRE, in Korhogo (Ivory Coast). Its germination rate, tested in the laboratory on blotting paper, was 95%.

Essential oils and synthetic products

The essential oils were extracted from the plant species *Ocimum gratissimum*, *Cymbopogon citratus*, *Eucalyptus camadulensis* and *Lippia multiflora*. Banko plus fungicide, a combination of chlorothalonil (550 g/L) and Carbendazim (100 g/L), known for its efficacy in treating fungal pathogens in vegetable crops, served as a positive control.

Preparation of the fungal cultures and the different doses for the tests

The culture medium used for the tests was synthetic PDA medium (Potato Dextrose Agar; 39 g : 1000 ml). This medium was homogenized by shaking, boiled to completely dissolve the powders and then sterilized by autoclaving at 121°C for 30 minutes. Once the medium was warmed, an antibiotic, "Moxilfloxacin hydrochloride" (50 mg : 1000 ml), was added to prevent bacterial growth. Amendment of the culture medium with the essential oils or the synthetic fungicide was performed according to the technique described by Soro *et al.* (2011). The choice of concentrations was made based on previous studies (Doumbouya *et al.*, 2012; Diagne, 2015 and Tiendrebeogo *et al.*, 2017). Thus, the concentrations of the products (synthetic fungicide and essential oils) selected to be tested against the pathogens were 250 ppm, 500 ppm, 1000 ppm, 2000 ppm, 4000 ppm and 6000 ppm.

Preparation of the different doses of essential oils and the synthetic product

The preparation of the stock solutions of each essential oil was carried out using DMSO. Thus, 1ml of DMSO was mixed with 5 ml of essential oil and then homogenized by manual shaking for 5 min. This step allows to make the essential oils miscible in the culture medium. Each dose taken from the stock solution was then added to 100ml of PDA just before pouring it into the 90mm diameter Petri dishes. Concerning the preparation of the stock solution of the synthetic fungicide (Banko plus), a dilution is made with sterilized distilled water. Thus 1.4 ml of Banko plus is dissolved in 84.5 ml of sterilized distilled water. From this Banko plus stock solution, each volume taken, in the objective of the retained concentrations, was added to 100 ml of the culture medium (PDA) and mixed just before pouring it into 90 mm diameter Petri dishes.

Mycelial growth inhibition test

A 5 mm diameter mycelial disc was collected from the peripheral growth of the fungus and placed in the center of new Petri dishes containing 15 ml of PDA with added essential oil or Banko plus. For each product, six (6) concentrations were tested, with five (5) replicates per concentration, on the *Rhizoctonia solani* strain. The control petri dishes were made under the same conditions and consisted only of PDA

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medium with DMSO added. The seeded plates were incubated in a photoperiod of 12 h at room temperature. Mycelial growth was measured every 24 hours. For this purpose, two perpendicular diameters passing through the middle of the mycelial disc were drawn on the reverse side of the Petri dish. The daily growth of the mycelium was measured along the axis of the two diameters. These measurements were made over four (4) days. The experiment was repeated two (2) times. The diameter measurements were used to calculate the rate of inhibition of mycelial growth of the pathogen compared to the control using the formula below.

$$TIC = \frac{T - E}{T} \times 100$$

TIC = Rat
 fungal growth (mm) in control medium

T = average
 E = average growth of the
 fungus (mm) in the culture medium at the concentration (C) of the essential oil or synthetic fungicide.

At the end of the mycelial growth inhibition test, all the explants that did not grow on the culture media amended with the different products were transplanted onto the new PDA medium. The aim is to determine if the product is fungicidal in case of no growth of the explant or fungistatic in case of growth of the explant.

Effect of essential oils and synthetic product on the disease caused by *Rhizoctonia solani*

The essential oils *C. citratus* and *O. basilicum* as well as the synthetic fungicide Banko plus, were selected for testing under semi-controlled conditions (greenhouse) on the tomato variety UC 82 B. For each product tested, the lowest concentration that had a total (fungistatic) inhibition rate of mycelial growth observed in the in vitro inhibition test (MIC) was selected.

Preparation of tomato plants and the culture substrat of plants

Seeds of the tomato cultivar tested (UC82B) were superficially disinfected by soaking in 80 % (vol/vol) ethanol for 5 minutes (Benhamou *et al.*, 1997), then rinsed thoroughly with sterile distilled water to remove remnants of pesticides used in seed treatment. Seeds were then sown, in a greenhouse (28±2°C; 12h photoperiod), in honeycombed plates containing sterile commercial potting soil. Watering was done with tap water daily and the plants were transplanted one month after sowing. The transplanting substrate was a mixture of growing soil and sterilized potting soil. This cultivation soil was taken from a plot in the market gardening district "Sinistré" of Korhogo (Côte d'Ivoire). The sampling was done randomly, in different places on the plot. The proportion of the mixture of cultivation soil and potting soil is 2/1. The substrate thus mixed is distributed in the transplanting pots (500 ml) pierced at the base to allow drainage.

Preparation of the inoculation solution fungal for the plants and treatment products

Rhizoctonia solani inoculum was produced using its mycelium, harvested from four (4) days culture in petri dish, by adding sterile distilled water. The technique consists in putting 5 ml of sterile distilled water in the petri dish containing a 4 days old strain and scraping the surface of the mycelium with a sterile scraper. The solution (mycelium + sterile distilled water) is collected in a tube and vortexed to obtain a mycelial suspension. Two (2) ml of this suspension is collected and then diluted three (3) times. The inoculum concentration is calibrated to 4.10⁵ CFU/ml (Colony Forming Unit per milliliter) using a Malassez type hematimeter (Soro, 2014). Against the *Rhizoctonia solani* agent, the products (*O. basilicum*, *C. citratus* and Banko plus) were used at their respective MIC (2000 ppm, 4000 ppm and 4000 ppm). Thus, for a final solution of 125 ml, a volume of 0.25 ml of essential oil of *O. basilicum* and 0.5 ml of *C. citratus* was used. Each volume of essential oil is transited in tween 20 (for miscibility) to obtain a volume of 5 ml to be added in 120 ml of sterile distilled water. For the Banko plus treatment, 0.5 ml of product was diluted in 124.5 ml of sterile distilled water.

Inoculation of the substrate and antifungal treatments of the plants

The inoculation technique consisted in spraying 1ml of fungal solution in each transplanting hole made in the pots which will be closed by a thin layer of substrate. A light watering was carried out, in order to

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support the development of the fungus. After three days of incubation, the 21-day-old nursery plants were transplanted into the pots of inoculated or uninoculated substrate (control). The effect of essential oils was compared to that of the synthetic fungicide (Banko plus) through two control methods (preventive and curative). In preventive treatment, the products were applied before transplanting. It consisted in spraying, before transplanting, two (2) ml of the considered product in the transplanting hole infected by the *Rhizoctonia solani* strain. Two (2) types of control plants were made. The transplant hole of the first type (infected) was sprayed with 2 ml of an aqueous solution of tween 20 (0.5 ml per 12 ml of sterile distilled water). The second type (uninfected) was sprayed with 2 ml of sterile distilled water. The procedure, in curative treatment, consists in spraying from the stem to the leaves, 2 ml of the product used, at its minimal inhibitory concentration, three (3) days after transplanting of the plants. Two types of control plants were also made. Plants of the first type (infected) were sprayed with 2 ml of an aqueous solution of tween 20 (0.5 ml per 12 ml of sterile distilled water). The second type (uninfected) was sprayed with 2 ml of sterile distilled water.

Experimental device

The transplanted plants were watered twice (2) a day during the whole experiment with tap water. The pots were placed under the greenhouse according to a split-plot design that takes into account 2 factors: treatments (products-dose), mode of application (preventive-curative). Ten (10) plants per treatment according to the strains were used in this trial. Two types of controls were made: an inoculated and untreated control and an uninoculated and untreated control. The experiment was repeated two (2) times.

Monitoring the development of the disease

Monitoring of growth parameters and pathogen symptoms was done every other day starting from the first week after transplanting. To evaluate the effect of the products against the development of the disease, the growth indicators were: height, stem neck diameter and number of leaves of the plants. The indicators of the expression of the symptoms considered were: incidence and severity of the disease.

Effects of treatments on the agronomic parameters of the plants

This assessment was done, by measuring two days after transplanting the growth indicators until flowering (Aka, 2018). Plant height was measured with a tape measure from the cotyledonary leaves to the V formed between the last unbloomed leaf and the second to last fully bloomed leaf. The average height (Hm) was calculated per treatment (Aka, 2018).

$$Hm = \frac{\sum Hi}{Nt}$$

Hi is the height of each plant of the considered treatment and Nt the total number of observed plants of the treatment.

Stem diameter of the plants was measured with a caliper. These measurements were made above the cotyledonary leaves (at the collar). The average diameter (Dm) was calculated per treatment :

$$Dm = \frac{\sum Di}{Nt}$$

Di is the diameter of each pl treatment.

nd Nt is the total number of plants observed in the

Live leaves were counted on each plant per treatment (Aka, 2018). The average number of live leaves (Nm) on the plants was calculated using the following formula:

$$Nm = \frac{\sum Ni}{Nt}$$

Ni corresponds to the number of living leaves of each plant of the treatment considered and Nt is the total number of plants observed in the treatment.

The evaluation of the treatments on the flowering time was carried out over two weeks. It allowed to determine the earliness of flowering according to the products. The flowering time variable was evaluated in number of days after sowing (Djidji *et al.*, 2010). The average day of flowering (Fm) was calculated per treatment :

$$Fm$$

$$\frac{\sum Ni}{Nt}$$

Fi is the flowering date of each plant in the treatment considered; Nt is the total number of plants observed in the treatment.

Evaluation of the severity and incidence of the disease according to the treatments

The severity index was evaluated according to the symptomatic status of the tomato plants. Disease development was assessed using a symptom rating scale based on that proposed by Vakalounakis and Fragakiadakis (1999).

0: healthy plant ;	1: slight
yellowing, slight rot of the pivot and secondary roots and rot of the crown ;	2: yellowing of
leaves and stem with or without wilting or stunting of plants ;	3: death of the plant.

The disease severity index (SI) is obtained using the following formula (Song *et al.*, 2004):

$$IS(\%) = \frac{\sum (Ni \cdot Zi)}{(Nt \cdot Z)}$$

Ni is the number of plants with the same score; Zi: score (0; 1; 2; 3); Nt: total number of plants considered for each treatment; Z: highest score (3).

The incidence of disease represents the proportion of the number of diseased plants to the total number of plants considered in the experiment (Aka, 2018). It was evaluated using the following formula:

$$IM(\%) = \frac{Npm}{Ntp}$$

With MI: average incidence, Npm: number of diseased plants and Ntp: total number of plants considered

Statistical analysis

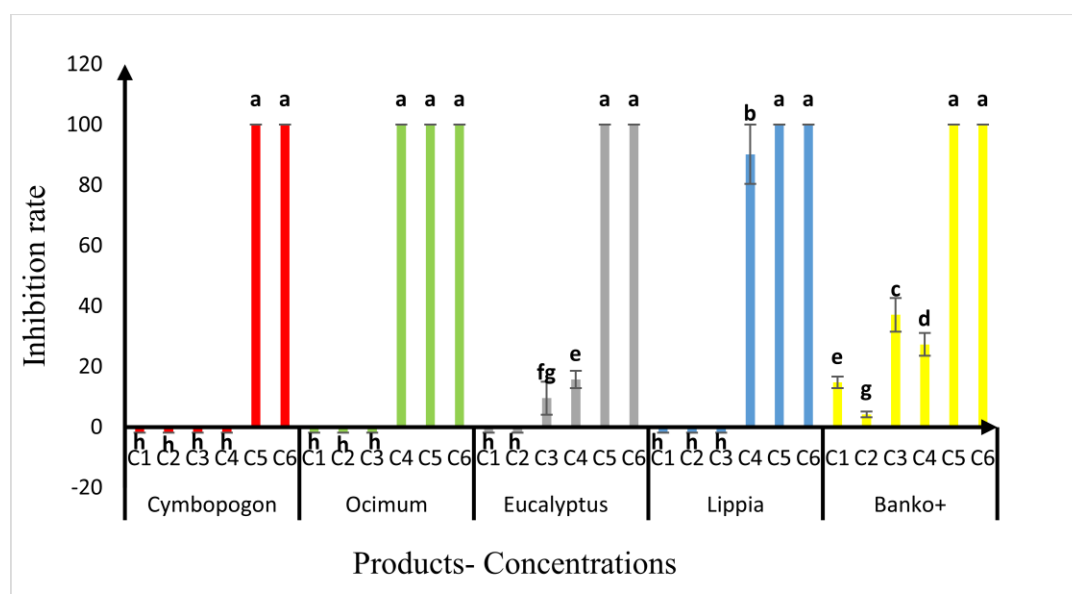
Data from all observations were entered and organized using Excel 2013 spreadsheet software. Analysis of variance was performed using Statistica version 7.1 software. The Newman Keuls test of comparison of means at the 5% threshold was performed.

RESULTS AND DISCUSSION

Results

Inhibition of mycelial growth of *Rhizoctonia solani*

The analysis of figure 1 highlights the total inhibition of the products (Banko plus, *Cymbopogon citratus*, *Eucalyptus camaldulensis* and *Lippia multiflora*) at concentrations of 4000 ppm and 6000 ppm on the mycelial growth of *Rhizoctonia solani*. Total inhibition was, however, obtained at the concentration of 2000 ppm with the essential oil *O. basilicum*. Negative inhibition rates were obtained at 250 ppm, 500 ppm, 1000 ppm and 2000 ppm for *C. citratus*; 250 ppm, 500 ppm and 1000 ppm for *O. basilicum* and *L. multiflora*; 250 ppm and 500 ppm for *E. camaldulensis*. With an MIC of 2000 ppm (Table 1), *O. basilicum* essential oil showed the highest inhibition activity. Differences in inhibition rates of *R. solani* were highly significant at a probability ($P < 0.001$) at the 5% threshold (Newman Keuls test).



C1 = 250 ppm C2 = 500 ppm C3 = 1000 ppm C4 = 2000 ppm C5 = 4000 ppm C6 = 6000 ppm

Figure 1: Rate of inhibition of mycelial growth of *R. solani* as a function of products and concentrations

Table 1: Minimum inhibitory concentrations for mycelial growth of *R. solani* by

Produits	MIC (ppm)
<i>Ocimum basilicum</i>	2000
<i>Cymbopogon citratus</i>	4000
<i>Lippia multiflora</i>	4000
<i>Eucalyptus camaldulensis</i>	4000
Banko plus	4000

Effect of treatments on the development, severity index and incidence of *Rhizoctonia solani* disease

Five (3) days after transplantation, the first symptoms of the disease were observed on inoculated control plants (Te + Rhiz). These symptoms were observed, ten (10) days later, on plants treated as a preventive measure with the essential oil *C. citratus*. They are characterized by the presence of a yellowing at the level of the collar of the plants followed by a browning and the rotting of the affected zone. The plants thus contaminated by the fungus end up by wilting and then totally dried out in a few days. The severity index values (Figure 2) were higher with the untreated inoculated plants (92%). On the other hand, plants treated

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as a preventive with *C. citratus* essential oil showed the highest severity index (83.33%) compared to the other treatments. For all treated plants, the severity index varied according to the treatment and the application method. The low severity indices were obtained both in curative and preventive treatments. However, the essential oil of *O. basilicum*, in preventive treatment, induced a lower severity index than in curative treatment.

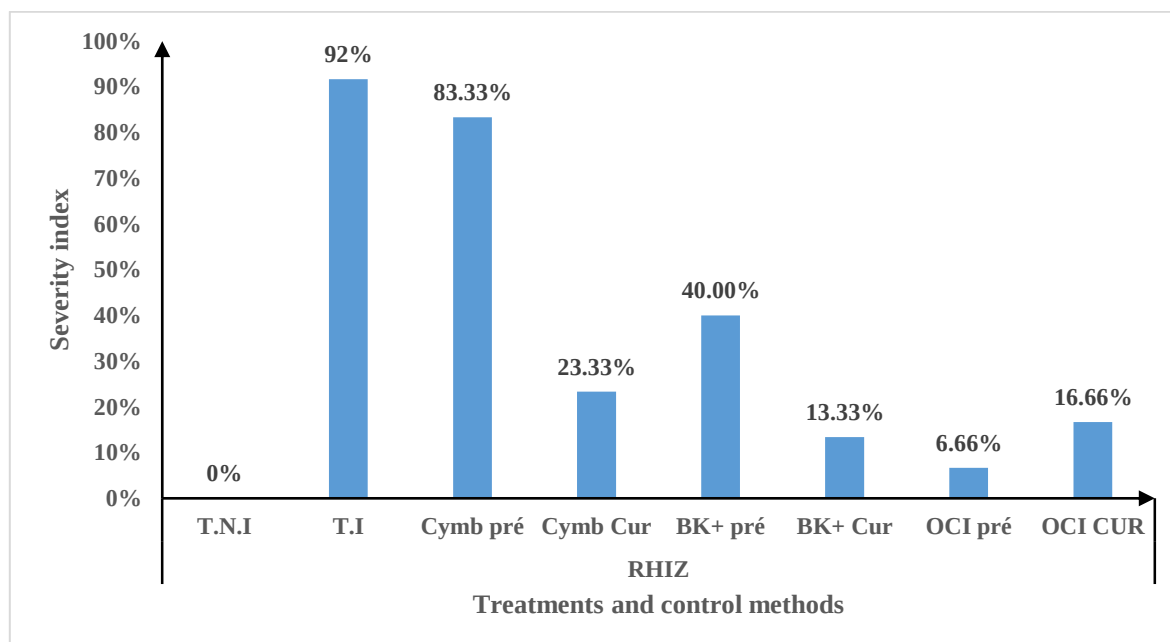


Figure 2: Rhizoctonia disease severity index according to product and application method

T.N.I: Non-inoculated control; T.I: Inoculated control; Cymb pré: Cymbopogon citratus in preventive treatment; Cymb Cur: Cymbopogon citratus in curative treatment; Bank+ pré: Banko plus in preventive treatment; Bank+ Cur: Banko plus in curative treatment

Figure 3 illustrates the effect of different products and application methods on Rhizoctonia incidence in tomato. Sixty (60) days after transplanting, plants transplanted to the substrate inoculated with *R. solani* and untreated (T+ Rhiz) showed high mortality rates (40%). However, the highest mortality rate was observed with the essential oil of *C. citratus* as a preventive treatment (83%). The synthetic fungicide Banko plus in both preventive and curative treatments and *O. basilicum* oil in preventive treatment showed no mortality.

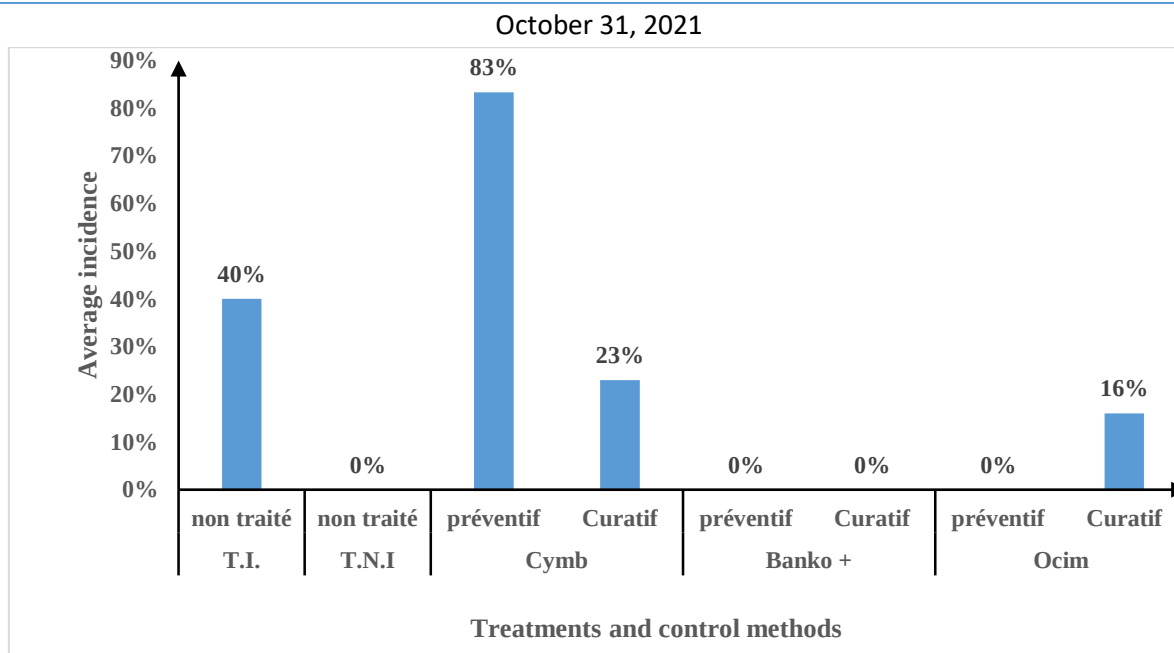


Figure 3: Incidence of Rhizoctonia disease on tomato plants according to product and application method

T.N.I: Non-inoculated control; T.I: Inoculated control; Cymb préventif: *Cymbopogon citratus* in preventive treatment; Cymb Curatif: *Cymbopogon citratus* in curative treatment; Bank+ préventif: Banko plus in preventive treatment; Bank+ Curatif: Banko plus in curative treatment ; Ocim préventif : *Ocimum basilicum* in preventive treatment ; Ocim curatif : *Ocimum basilicum* in curative treatment

Effects of treatments on agronomic parameters of tomato plants

Effects of products on growth parameters of tomato plants

The effects of the different treatments on the growth parameters of the plants, sixty (60) days after transplanting, are presented in Table 2. The Infected plants and subjected to the different modes of application (preventive and curative) of Banko plus (4000 ppm), *O. basilicum* (2000 ppm) and *C. citratus* (4000 ppm), recorded high average heights. However, the highest values were obtained in curative treatment with Banko plus (21.17 cm) and preventive treatment with *O. basilicum* essential oil (22.16 cm). The average height growth of uninoculated and untreated control plants and those inoculated and treated curatively with *C. citratus* and *O. basilicum* oils ranged from 16 to 19 cm. As for the inoculated and untreated control plants and the inoculated plants that underwent a preventive treatment with Banko plus and *C. citratus*, the growth in height was low with average values of 10.60 cm, 14.97 cm and 12.88 cm respectively.

Table 2: Effects of products and application methods on growth parameters of tomato plants inoculated with *R. solani*, 60 days after transplanting

Treatments	Average height	Average diameter	Average number of leaves
BK+ Cur	21,17 a	4,45a	10,33ab
Ocim-Pré	22,16 a	3,96a	11,76a
Cymb-Pré	12,88 d	4,32a	11a
Témoin-nonIn	18,60b	3,02b	11,50a
Ocim-Cur	17,02bc	4,05a	9,30b
Cymb-Cur	16,74bc	3,99a	9,20b
BK+ Pré	14,97cd	3,89a	10,26ab
Témoin-In	10,60e	2,86b	7c

The values affected by the same letter are not significantly different at the 5% threshold using the Newman and Keuls test for the same parameter and for the same product associated with the application mode

Bank+ Cur: Banko plus in curative treatment ; Bank+ Pré: Banko plus in preventive treatment; Ocim-Pré : *Ocimum basilicum* in preventive treatment ; Ocim-Cur : *Ocimum basilicum* in curative treatment Témoin-nonIn: Non-inoculated control; Témoin-In: Inoculated control; Cymb-Pré: *Cymbopogon citratus* in preventive treatment; Cymb-Cur: *Cymbopogon citratus* in curative treatment;

Statistically identical, the highest average values of plants collar diameters were obtained, respectively, with Banko plus in curative treatment (4.45 mm), *C. citratus* in preventive treatment (4.32 mm), *O. basilicum* in curative treatment (4.05 mm), *C. citratus* in curative treatment (3.99 mm), *O. basilicum* in preventive treatment (3.96 mm) and Banko plus in preventive treatment (3.89 mm). However, the two types of control showed the lowest values, with the lowest value shown by the inoculated control plants (2.86 mm).

Concerning the average number of living leaves, significant differences at the 5% threshold were also observed between the different treatments associated with the application modes (Table 2). The highest mean value was obtained with *O. basilicum* in preventive treatment (11.76). The mean number of leaves was statistically identical between the uninoculated and untreated control plants (11.5), the plants treated with *C. citratus* as a preventive treatment (11) and the plants treated with Banko plus as curative and preventive treatment (10.33; 10.26). The lowest value was obtained by inoculated and untreated control plants (7).

Effects of treatments on the average flowering time of tomato plants

Table 3 shows the effect of the different treatments on the average flowering time of tomato plants inoculated with *Rhizoctonia solani*. The analysis of variance showed that there was a significant difference between the flowering time of the different plants treated with preventive and curative Banko plus (68th and 60.8th day). No differences were observed between the uninoculated control and the Banko plus preventive treatments. No significant difference was also observed between the other treatments and the non-inoculated control. In effect, Banko plus as curative treatment induced the shortest flowering time (60th days) compared to the non-inoculated control (69th days). This delay is statistically identical to those obtained in curative treatment with essential oils of *O. basilicum* (63th day), *C. citratus* (63.93th day), but also to those obtained in preventive treatment with *C. citratus* (64th day) and *O. basilicum* (66.66th day). The longest time to flowering (69th day) was obtained with the non-inoculated control plants. Among the products and modes of application, Banko plus and *O. basilicum* essential oil in curative treatment induced more effect on flower appearance (Table 3).

Table 3: Effect of essential oils and the synthetic product Banko plus on the average flowering time of tomato plants inoculated with *Rhizoctonia solani*

Treatments	Average flowering	
	time	Groups
Témoin-nonIn	69,0000	a
BK+ Pré	68,0000	a
Ocim-Pré	66,6667	a b
Cymb-Pré	64,0000	a b
Cymb-Cur	63,9333	a b
Ocim-Cur	63,0000	a b
BK+ Cur	60,8000	b

The values affected by the same letter are not significantly different at the 5% threshold using the Newman and Keuls test for the same parameter and for the same product associated with the application mode

Bank+ Cur: Banko plus in curative treatment ; Bank+ Pré: Banko plus in preventive treatment; Ocim-Pré : *Ocimum basilicum* in preventive treatment ; Ocim-Cur : *Ocimum basilicum* in curative treatment Témoin-nonIn: Non-inoculated control; Témoin-In: Inoculated control; Cymb-Pré: *Cymbopogon citratus* in preventive treatment; Cymb-Cur: *Cymbopogon citratus* in curative treatment;

Discussion

From the results, it appears that all treatments applied at different concentrations induce some efficacy on the reduction of mycelial growth of *Rhizoctonia solani*. Their effects differ significantly according to the essential oils and the concentration. The inhibition of the growth of the pathogen by the extracts would be due to the fact that these extracts would possess natural organic compounds with antimicrobial activities recognized as reported in several aromatic plants. These results are in agreement with the work of Oyediji *et al.* (1999), Cimanga *et al.* (2002), Ling *et al.* (2003), and Tiendrebeogo *et al.* (2017) who showed that essential oils have antimicrobial, insecticidal, fungicidal and bactericidal activities. The antifungal power of the tested essential oils could thus be attributed to the presence of antifungal components classified in the list of constituents with antifungal activity. But also, by the quality, quantity, and chemical structure of the antifungal molecules contained in each essential oil (Zarith *et al.*, 2018). The essential oil of *O. basilicum* is otherwise predominantly composed of oxygenated monoterpenes represented mainly by estragol, linalool, and methyl eugenol, followed by a hydrocarbon sesquiterpene, bergamotene (Politeo *et al.*, 2007; Ngom *et al.*, 2014). As for the essential oil of *L. multiflora*, its main compounds are generally p-cymene, β -caryophyllene, thymol, γ -terpinene and thymyl acetate (Pelissier *et al.*, 1994; Bayala, 2014). The major constituents of *E. camaldulensis* essential oil, according to Farah *et al.*, 2001 and Hmiri *et al.*, 2011, are 1,8-cineole, α -pinene, followed by γ -terpinene and p-cymene. Degnon *et al.* (2016) report that the essential oil of *Cymbopogon citratus* has geranial, neral, and myrcene as majority compounds. These essential oils are thus characterized by the majority presence of oxygenated monoterpenes which are generally antimicrobial compounds with a broad spectrum of action (Alitonou, 2006). Majority compounds are often responsible for the antifungal activity of the essential oil (Giordani *et al.*, 2008). However, these major or minor compounds can increase the antifungal activity of an essential oil through their synergistic effects.

Our results also revealed negative values for mycelial growth inhibition rates of the agent *Rhizoctonia solani* at certain concentrations of *Cymbopogon citratus* (250 ppm, 500 ppm, 1000 ppm, 2000 ppm), *Ocimum gratissimum* (250 ppm, 500 ppm, 1000 ppm), *Eucalyptus camaldulensis* (250 ppm, 500 ppm), and *Lippia multiflora* (250 ppm, 500 ppm, 1000 ppm). In other words, these oils at these different doses, relatively low,

tend to stimulate the growth of the strains. This would mean that these bio-pesticides instead of being toxic at these specific concentrations become nutrients for the pathogen *Rhizoctonia solani*. Our results are in agreement with Tiendrebeogo *et al.* (2017) who showed that some bio-pesticides such as aqueous extract of *C. citratus* stimulate the growth of *Fusarium moniliforme* and *Biolaris Oryzae*. These bio-pesticides would thus act as sources of nutrients when the active compounds are at too low doses. Such a stimulatory activity has also been reported by Doumbouya *et al.* (2012), on the sporulation of *Fusarium oxysporum* f. sp. *lycopersici*.

The treatment of the disease caused by *Rhizoctonia solani* in greenhouse tomato crops was carried out with the synthetic fungicide Banko plus and the essential oils *Ocimum gratissimum* and *Cymbopogon citratus*. The efficacy revealed *in vitro* with these products was thus evaluated *in vivo*. After transplanting plants of tomato variety UC 82 B into soil inoculated with *Rhizoctonia solani*, treatment with concentrations inhibiting mycelial growth of the fungus, with Banko plus (4000 ppm), *Cymbopogon citratus* (4000 ppm) and *Ocimum basilicum* (2000 ppm), resulted in a low attack of those plants. However, the degree of attack varied, depending on the mode of application of the products (preventive or curative). In general, the agronomic parameters of plants inoculated with *R. solani* and then treated with Banko plus or with the essential oils *O. basilicum* and *C. citratus* were improved. Among these products, Banko plus remains the best product against *R. solani* species in curative treatment. This synthetic product improved the height of the plants, the number of living leaves and the diameter at the stem collar. In addition, its efficacy was marked at the level of stem collar diameter, which limited the development of the disease. The fungitoxicity of one of the active ingredients of this synthetic fungicide (Carbendazim) lies in its systemic character (Davides, 1986) which allows an effective curative treatment at the beginning of infection. Studies done by Duamkhanmanee (2008) and Chand *et al.* (2013) also showed the efficacy of Carbendazim in reducing infections and improving growth parameters of tomato. Of the two essential oils, (*C. citratus* and *O. basilicum*), that of *O. basilicum* as a preventive treatment was found to be very effective on plants inoculated with *R. solani*. Treatments with *O. basilicum* essential oil improved the height, diameter and number of functional leaves of the tomato variety UC 82 B. The results obtained by this essential oil on *Rhizoctonia* disease reflect thus a certain efficacy similar to that produced by Banko plus in curative treatment. Compounds of this essential oil could therefore be easily assimilated by the plant and would act as an activator of the plant's defense system. This stimulation was essentially translated by a better growth in height of the stem, in diameter, in the number of leaves and by a reduction of the delay of flowering. Indeed, a study carried by Colpas *et al.* (2009) showed that the resistance of the plant following treatment with the essential oil of *O. gratissimum* would be explained by an activation of its defense system, an increase in the chitinase and peroxidase activity and an increase in the enzymatic activity in the leaves, thus inducing a systemic resistance in it. Moreover, according to Lateur (2002), a treatment with natural extracts can activate defense mechanisms in the plant, such as the synthesis of defense protein. The essential oil of *O. basilicum* induced the best activity against the development of *Rhizoctonia solani*, through a reduction of the incidence and severity of the disease. This activity reflects, in fact, the effectiveness of this product to totally inhibit, *in vitro*, the mycelial growth of this pathogen at 2000 ppm. These results are in agreement with those of Doumbouya *et al.* (2016) who demonstrated, *in vitro* and *in vivo*, the inhibitory effect of essential oils of *Ocimum gratissimum* and *Hyptis suaveolens* against *Sclerotium rolfsii* causal agent of sclerotinia disease of okra and which were effective at the dose of 2000 ppm

Conclusion

Apart from *Ocimum basilicum* essential oil (2000 ppm), the other essential oils (*Lippia multiflora*, *Eucalyptus camaldulensis* and *Cymbopogon citratus*) including Banko plus induced a MIC of 4000 ppm on mycelial growth of *Rhizoctonia solani*. The essential oil of *C. citratus* in curative treatment induced a severity index and a disease incidence of about 23%. That of *O. basilicum*, in preventive treatment, induced a lower severity index in preventive treatment (6.66%) than in curative treatment (16.66%). The synthetic fungicide Banko plus, as a preventive and curative treatment, and the essential oil of *O. basilicum* as a preventive treatment, did not record any mortality. These products also improved the agronomic parameters

of the inoculated tomato plants. Within the framework of an alternative strategy, the control of Rhizoctonia disease could thus be done by the use of the essential oil of *O. basilicum* in preventive treatment and that of *C. citratus* in curative treatment.

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