



RESEARCH ARTICLE

EFFICACY OF FOUR ESSENTIAL OILS ON FUSARIUM HEAD BLIGHT CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *RADICIS LYCOPERSICI* (FORL), A FUNGAL TELLURIC PATHOGEN OF TOMATO GROWN UNDER COVER

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Manuscript Info

Manuscript History

Received: 30 September 2021

Final Accepted: 31 October 2021

Published: November 2021

Key words:

Antifungal Activity, Essential Oils, FORL, Lycopersiconesculentum, Korhogo

Abstract

Fusarium head blight caused by *Fusarium oxysporum* f. sp. *Radicis-lycopersici* (FORL) is a constraint to tomato cultivation in Korhogo, Côte d'Ivoire. To control this disease, the antifungal activity of essential oils extracted from certain plants was evaluated in comparison with that of a synthetic fungicide for use. *In vitro* tests were performed on the different life stages of FORL. The *in vivo* evaluations consisted in carrying out two modes of treatment (curative and preventive) on plants of a susceptible variety of tomato inoculated with FORL. The results showed that the essential oils significantly reduced the different life stages of *F.oxysporum* f. sp. *Radicis-lycopersici* (FORL) as did the synthetic product. Thus, the essential oil *Cymbopogon citratus* at concentrations of 4000 and 6000 ppm strongly inhibited *in vitro* the different stages of FORL. In preventive and curative treatment, the essential oils not only improved the growth parameters of tomato plants but also reduced the incidence and severity of diseases. Thus, it was found that *Cymbopogon citratus* (4000 ppm) in preventive treatment, and Banko plus (250 ppm) in curative treatment reduced the mortality rate of FORL up to 6.66% and improved the growth parameters and reduced the flowering time of the tomato plant. The essential oil of *C. citratus* could be used for biological control of *F. oxysporum* f. sp. *Radicis lycopersici*.

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Introduction:-

Vegetable crops occupy an important place in the human diet and contribute significantly to family incomes in West Africa and specifically in Côte d'Ivoire (Yarouet *et al.*, 2017). These crops are considered as sovereignty crops playing a primary role in most nutrition and poverty alleviation programs (James *et al.*, 2010; Yolouet *et al.*, 2015).

In the north of Côte d'Ivoire, specifically in the city of Korhogo, urban market gardening holds an important place. The market garden produce produced there supplies many cities in the country with fresh vegetables (Soroet *et al.*,

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2018). Among these vegetables, the tomato occupies a place of choice. Indeed, it is the most consumed vegetable and had been the subject of industrialization in Côte d'Ivoire through SODEFEL before being held today by the producers themselves (Doubouya *et al.*, 2012). National tomato production has increased from 34,734 tons in 2013 to about 44,078 tons in 2018 (Anonymous, 2019). Despite this increase, production remains insufficient and remains a concern for the whole country. This insufficiency is related to the huge losses caused by bioaggressors and especially fungal diseases. These diseases not only depreciate the quality but also contribute to the reduction of the yield. To increase their production and improve their yield, most market gardeners resort to the systematic use of a panoply 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 adverse effects on human, animal health and the environment. Various studies on essential oils from aromatic plants have shown their effectiveness in traditional medicine, cosmetics and especially in agriculture. Thus, the use of some extracts and essential oils of various plant species has yielded convincing results on bioaggressors and especially on fungal pathogens (Faye, 2015; Cissé, 2017; N'guessan *et al.*, 2020). In this context, essential oils could be used to reduce the incidence of fungal pathogens especially *Fusarium oxysporum* f. sp. *Radicis lycopersici* (FORL). The work carried out in this study consisted, firstly, in determining, *in vitro*, the effect of essential oils from *Cymbopogon citratus*, *Ocimum basilicum*, *Eucalyptus camaldulensis* and *Lippia multiflora* on the different life stages of *Fusarium oxysporum* f. sp. *Radicis lycopersici*. Secondly, the *in vivo* evaluation of the activity of essential oils of *Cymbopogon citratus* and *Ocimum basilicum* on the expression of *Fusarium oxysporum* f. sp. *Radicis lycopersici* symptoms, as a preventive and curative treatment was evaluated.

The objective of this work was to evaluate the activity of some essential oils on the incidence of fusarium head blight caused by *Fusarium oxysporum* f. sp. *Radicis lycopersici* (FORL).

Materials And Methods:-

Fungal strain

The strain of *Fusarium oxysporum* f. sp. *Radicis-lycopersici* was isolated from tomato plants showing symptoms of wilting and ascending crown rot. The symptomatic tomato plant was collected in the market garden lowland of the "Petit Paris" district (Korhogo), characterized by a diversity of vegetable crops.

Plant material

The tomato variety used was the UC 82 B cultivar. It is the tomato variety essentially cultivated by the market gardeners of the production zones of Korhogo. The seeds of this tomato variety were obtained from the seed company SEMIVOIRE.

Essential oils and synthetic products

Essential oils from the plant species *Ocimum gratissimum* L., *Cymbopogon citratus* (D.C.) Stapf, *Eucalyptus camaldulensis* Dehnh. and *Lippia multiflora* Moldenke were used.

Banko plus fungicide, a combination of chlorothalonil (550 g/L) and Carbendazim (100 g/L), which is known to be effective in treating fungal pathogens in vegetable crops, served as a positive control.

Obtaining the fungal strain

Isolation was made from the crown and stem of the plant symptomatic of Fusarium head blight. These parts were cut into small fragments that, after being disinfected, in sodium hypochlorite solution (4%), rinsed with sterile distilled water and then dried in sterile blotting paper. The sterilized explants were inoculated into Petri dishes containing PDA (Potato Dextrose Agar) culture medium. This culture medium was supplemented with an antibiotic, "Moxifloxacin hydrochloride" (50 mg: 1000 ml) in order to avoid the proliferation of bacterial colonies. Petri dishes containing the explants were incubated in the dark at a temperature of $26 \pm 1^\circ\text{C}$ for 3 to 4 days. Purification of the fungal colony from the explants was performed by removing the mycelial fragments from the growth front and subculturing onto new PDA medium. From this new colony, a monospore culture was performed. A spore suspension was spread on the PDA medium and incubated in the dark for 24 h at $26 \pm 1^\circ\text{C}$. A germinating spore was removed under a binocular magnifying glass and transplanted onto a new PDA medium. After two weeks of incubation in an oven at 28°C , the spore gave rise to a homogeneous clone of the fungus. Identification of the pathogen was carried out macroscopically and microscopically according to the determination key given by Nelson *et al.* (1983). Pathogenicity test on tomato seedlings was carried out according to the technique of Bachir (2017).

Obtaining the different concentrations of essential oils and synthetic product for the tests

The amendment of the PDA culture medium with the essential oils or the synthetic fungicide was performed according to the technique described by *Soroet al. (2011)*. The choice of concentrations was made according to previous studies (*Doumbouyaet al., 2012; Diagne, 2015 and Tiendrebeogo et al., 2017*). Thus, the concentrations of products (synthetic fungicide and essential oils) selected to be tested against the pathogen were 250 ppm, 500 ppm, 1000 ppm, 2000 ppm, 4000 ppm and 6000 ppm. The preparation of the stock solutions of each essential oil was carried out using DMSO. Thus, 1 ml of DMSO is mixed with 5 ml of essential oil and then homogenized by manual shaking for 5 minutes. This step makes the essential oils miscible in the culture medium. Each dose taken from the stock solution was added to 100 ml of PDA just before its distribution in the 90 mm diameter Petri dishes. For 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 culture medium (PDA) then homogenized and distributed in Petri dishes.

Mycelial growth inhibition test and mycelial disc recovery

A 5 mm diameter mycelial disc was collected and placed in the centre 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 three replicates per concentration. The controls were carried out under the same conditions and consisted only of PDA medium with added DMSO. The test was performed three times. The seeded plates were incubated in a photoperiod of 12 h at room temperature. Mycelial growth was measured every 24 h. For this purpose, two perpendicular diameters passing through the middle of the mycelial disc were traced 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 taken for seven (7) days. From the measurements taken, the rate of inhibition of mycelial growth of the two pathogens compared to the control was calculated using the formula below.

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

TIC = Mycelial Growth Inhibition Rate;

T = average growth of the fungus (mm) in the control;

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 explants that had not grown on the culture media amended with the different products were transplanted onto new PDA medium. The aim is to determine whether the product is fungicidal if the explant does not grow or fungistatic if it does grow.

Evaluation of the activity of essential oils and synthetic products on the production of spores of FORL

After seven (7) days of incubation, ten (10) 5 mm diameter discs were removed from the Petri dishes used for mycelial growth measurements and placed in a test tube containing 2 ml of sterile distilled water. The tubes were shaken in 15-second sequences for 2 min to detach the spores from the conidiophores. The resulting suspensions were filtered through wattman paper to remove mycelial fragments. The number of spores was counted using a Malassez slide. The sporulation inhibition rate was thus determined by the formula:

$$TIS = \frac{N_{So} - N_{Stc}}{N_{So}} * 100$$

N_{So}: Average number of spores in the control,

N_{Stc}: Average number of spores in the tubes at concentration C

Evaluation of the activity of the products on the germination of spores of FORL

From a 6-day culture on PDA medium, 10 mycelial washers were collected and placed in a tube containing 2 ml of sterile distilled water. The spores released after shaking were counted on a Malassez slide in order to calibrate the spore suspension to 10^5 spores. A volume of 100 μ L of this suspension was spread in petri dishes containing PDA medium only (control) or with the addition of different concentrations of essential oils and synthesis product. Three replicates per concentration were performed. After 48 h of incubation in 12 h photoperiod, the number of germinated

spores was counted on a total of 500 spores. The percentage of germination inhibition compared to the control was calculated.

In vivo evaluation of the activity of essential oils and synthetic fungicide

The essential oils *C. citratus* and *O. basilicum* were used in addition to the synthetic fungicide Banko plus for testing under semi-controlled conditions. This choice was based on their in vitro efficacy. For each product tested, the lowest concentration that gave total inhibition of mycelial growth or MIC was used for the in vivo tests.

Setting up the nursery

Seeds of the tomato cultivar to be tested were superficially disinfected by soaking in a sodium hypochlorite solution (4%) for 3 minutes and then rinsed thoroughly with sterile distilled water to remove chlorine and residues of pesticides used in seed treatment. After drying, the seeds were sown in three honeycomb plates containing sterile commercial potting soil. Watering was done with tap water daily and the plants were transplanted one month after sowing.

Preparation of the inoculum

The FORL inoculum was prepared from a six (6) day culture on PDA culture medium. Dipping ten (10) mycelial washers in a tube containing 2 ml of sterile distilled water obtained it. After manual shaking to loosen the spores, the resulting solution was filtered through 4-wattman paper to remove residues. The concentration was assessed using the Malassezhematimeter and adjusted to 10^6 spores/ml.

Transplanting substrate and soil inoculation

The transplanting substrate is a mixture of sand and sterilized soil. The sand was taken from a lowland market garden plot in the 'disaster' district of Korhogo (Côte d'Ivoire). The sampling was done randomly, in different places on the plot. The proportion of the sand/soil mixture is 2/1. The mixed substrate is distributed in the transplanting pots pierced at the base to allow drainage.

The inoculation technique consisted in spraying 1ml of the inoculum 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 favour the development of the mushrooms. After three days of incubation, the 21-day-old nursery plants were transplanted into the pots of inoculated or uninoculated substrate (control).

Antifungal treatments of plants

MICs of 4000 ppm, 4000 ppm and 250 ppm for *O. basilicum* essential oil, *C. citratus* essential oil and Banko plus fungicide were used. For a final volume of 125 ml, 0.5 ml of *C. citratus* and *O. basilicum* essential oils were diluted with 4.5 ml of tween 20 and added to 120 ml of sterile distilled water. With the Banko plus, a dilution was made using a volume of 0.03 ml added to 125 ml of sterile distilled water. The effect of essential oils was compared to that of the synthetic fungicide (Banko plus) through two treatments.

As a preventive treatment, the products were applied before transplanting the plants. It consisted in spraying 2 ml of the product in question into the inoculated transplanting hole. The seedlings were then transplanted into the pots under glass. Two types of control were made. The first type consists of plants transplanted into inoculated holes and sprayed with 2 ml of an aqueous solution of tween 20 (0.5 ml of tween for 12 ml of sterile distilled water). The second type consists of plants transplanted into uninoculated holes and sprayed with 2 ml of sterile distilled water.

The curative treatment procedure consisted in spraying a volume of 2 ml of the different products from the stem to the leaves 3 days after transplanting the plants into the inoculated holes. Two types of control were also carried out. The first type of plants were transplanted into holes inoculated with the fungus and sprayed with 2 ml of an aqueous solution of tween 20 (0.5 ml per 12 ml of sterile distilled water). The second type consisted of seedlings transplanted into holes not inoculated with the fungus and sprayed with 2 ml of sterile distilled water.

Experimental device

The transplanted plants were watered twice a day throughout the experiment. The pots were placed under the greenhouse in a split-plot design with two (2) factors: treatments (product-dose), mode of application (preventive-curative). Ten (10) plants per treatment were used. The experiment was repeated three times.

Monitoring the development of the disease

Monitoring of growth parameters and disease symptoms was done every two days starting from the first week after transplanting. The growth parameters measured were plant height, stem neck diameter, number of leaves and flowering time. These parameters were measured until flowering. The flowering time variable was assessed in number of days after sowing (Djidjiet *et al.*, 2010). For each parameter, the mean was calculated.

Concerning the indicators of disease symptom expression, the incidence and severity of the disease were assessed. The severity was assessed using a symptom rating scale proposed by Vakalounakis and Fragakiadakis (1999).

0: healthy plant;

1: slight yellowing, slight pivot and secondary root rot and crown rot;

2: yellowing of leaves and stems with or without wilting or stunting of plants;

3: death of the plant.

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

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

With **Ni** being the number of plants having received the same score; **Zi**: score (0; 1; 2; 3); **Nt**: total number of plants used for each treatment; **Z**: highest score (3).

Statistical analysis

These data were analyzed using Statistica version 7.1 software. An Analysis of Variance (ANOVA I) was performed and in case of significant difference between the means, the separation of the means was done by the NewmanKeuls test at the 5% threshold.

Results:-

Effect of the different products on the mycelial growth of FORL

Inhibition of FORL was product and concentration dependent (Figure 1). The synthetic fungicide (Banko plus) completely inhibited the pathogen at all concentrations used. As for the essential oils, total inhibition was observed at concentrations of 4000 ppm for the oils of *C. citratus*, *O. basilicum* and 6000 ppm for the essential oil of *L. multiflora*, respectively. The essential oil of *E. camaldulensis* showed the lowest inhibition rates. However, its significant antifungal activity is around 85% from 4000 ppm. The highest inhibitory activity was observed with the essential oil of *C. citratus*. The differences between the inhibition rates of mycelial growth of FORL are significant ($P < 0.001$) at the 5% threshold.

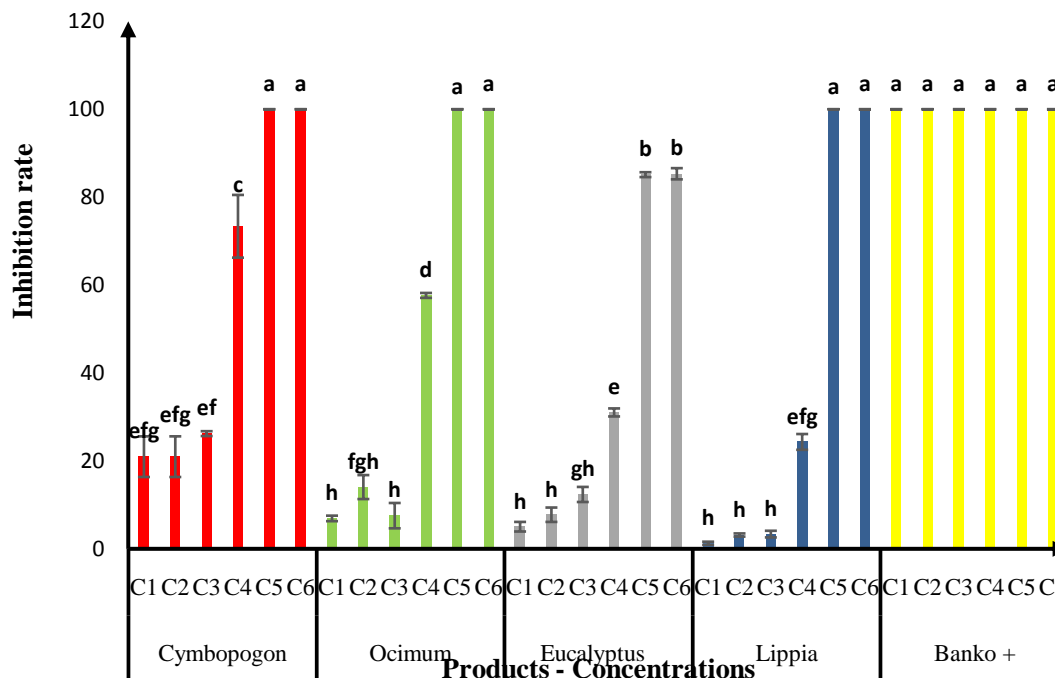


Figure 1:- Inhibition rate of mycelial growth of *F. oxysporum* f. sp. *Radicis-lycopersici* as a function of products and concentrations.

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

Fungicidal and fungistatic activity of essential oils and synthetic fungicide

From the concentration of 4000 ppm of the culture media amended with essential oils of *E. camaldulensis* and *O. basilicum*, it was observed a resumption of the growth of mycelial explants in the culture media amended with the oils of *L. multiflora* and *C. citratus*, which were fungicidal. With Banko plus, the fungistatic activity on FORL was obtained with the concentration of 250 ppm.

Effect of products on *F. oxysporum* f. sp. *Radicis-lycopersici* sporulation

Total inhibition of FORL sporulation was achieved with the fungicide Banko plus. As for the essential oils, *E. camaldulensis* showed the highest inhibition rates (above 70%), from 500 ppm. Conversely, the essential oil of *L. multiflora*, with the highest sporulation inhibition rate (37.29%) obtained at 4000 ppm, showed the lowest activity overall (Table 1).

Effect of products on germination of *F. oxysporum* f. sp. *Radicis-lycopersici*

All oils induced a strong inhibitory activity on spore germination (more than 85%) from 1000 ppm (Table 2). However, the essential oil of *L. multiflora* and the synthetic product Banko plus completely inhibited FORL spore germination from 1000 ppm. Complete inhibition (100%) was obtained at 4000 ppm with the essential oils of *C. citratus* and *E. camaldulensis*.

Effect of product application on disease severity index

Analysis of Figure 2 shows that the percentages of severity index were higher with inoculated and untreated plants (41.66%) compared to all treated plants. The severity index varied according to the treatment and the mode of application. It was noticed that curative treatments recorded the lowest severity indices except for the synthetic product Banko plus. Among the applied products, the highest index was obtained with the essential oil of *O. basilicum* in preventive treatment (21.73%) and the lowest value with that of *C. citratus* in curative treatment ($P < 0.001$).

Effects of products on the incidence of *F. oxysporum* f. sp. *Radicis-lycopersici*

Sixty (60) days after transplanting, seedlings transplanted to soil inoculated with FORL and untreated (T+ Fus) showed high mortality rates (25%) (Figure 3). Depending on the product and the method of application, incidences

were low, less than or equal to 10%. The synthetic fungicide Banko plus as a preventive treatment induced the lowest mortality rate (3.84%).

Table 1:- Inhibition rate of sporulation of *F. oxysporum* f. sp. *Radicis-lycopersici*.

Products	Concentrations (ppm)	Inhibition rate (p.c)
<i>C. citratus</i>	250	- 2,04h
	500	38,78 ^e
	1000	44,90 ^e
	2000	55,10d
	4000	59,18c
<i>O. basilicum</i>	250	32,20 ^e
	500	49,15d
	1000	50,85d
	2000	61,02c
	4000	62,71c
<i>E. camaldulensis</i>	250	44,90 ^e
	500	71,43b
	1000	75,51b
	2000	77,55b
	4000	79,59b
<i>L. multiflora</i>	250	13,56g
	500	15,25g
	1000	20,34g
	2000	28,81f
	4000	37,29 ^e
Banko plus		100a

Table 2:- Germination inhibition rates of *F. oxysporum* f. sp. *Radicis-lycopersici*.

Concentrations (ppm)	Products				
	<i>C. citratus</i>	<i>O. basilicum</i>	<i>E. camaldulensis</i>	<i>L. multiflora</i>	Banko plus
1000	93,6	85,2	92,8	100	100
2000	98,2	87	96,8	100	100
4000	100	88	100	100	100

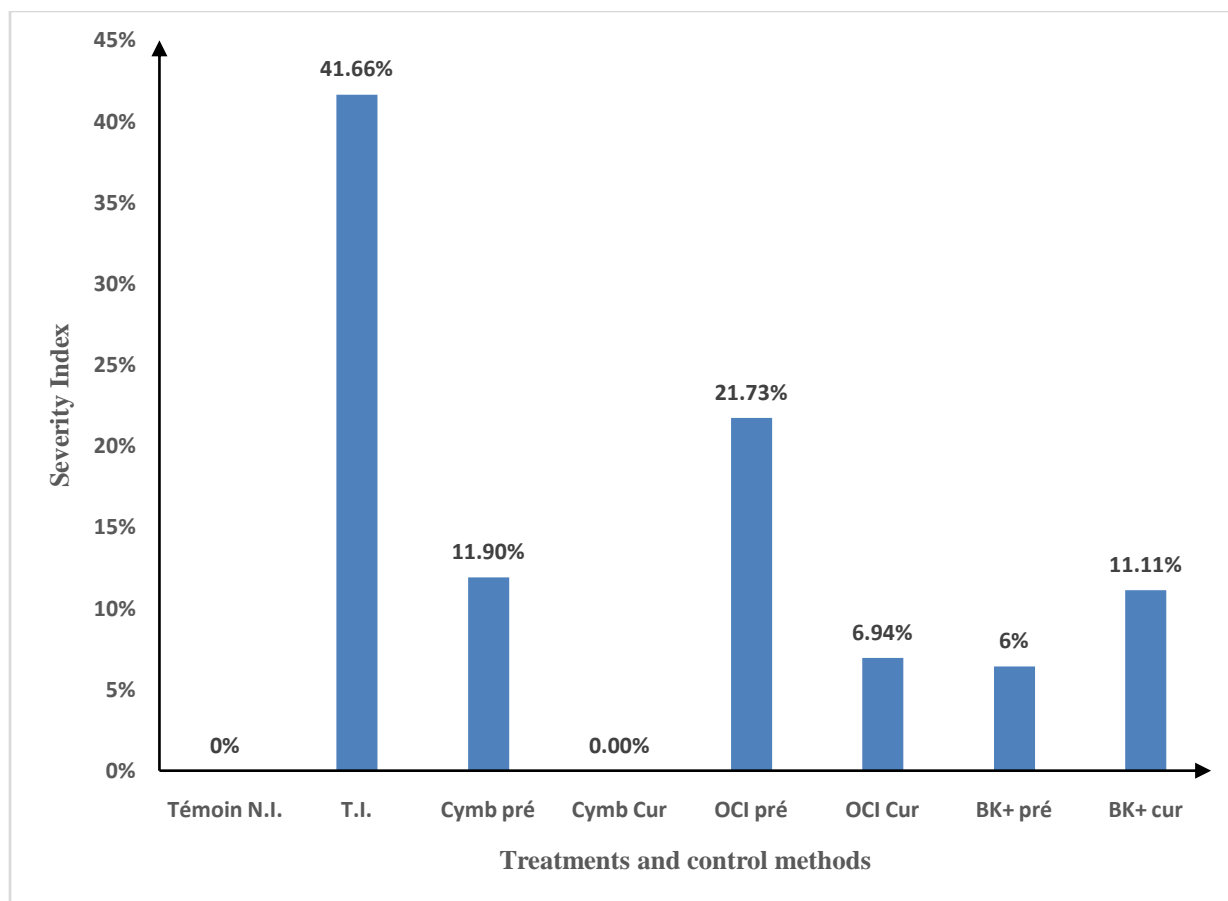


Figure 2:- Fusarium root and crown disease severity index in tomato according to products and application methods.

T.N.I: Non-inoculated control; **T.I:** Inoculated control; **Cymb pré:** *Cymbopogon citratus* as a preventive treatment; **Cymb Cur:** *Cymbopogon citratus* as a curative treatment; **Bank+ pré:** Banko plus as a preventive treatment; **Bank+ Cur:** Banko plus as a curative treatment; **Oci pré:** *Ocimum basilicum* as a preventive treatment; **Oci Cur:** *Ocimum basilicum* as a curative treatment

The figures with 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 method.

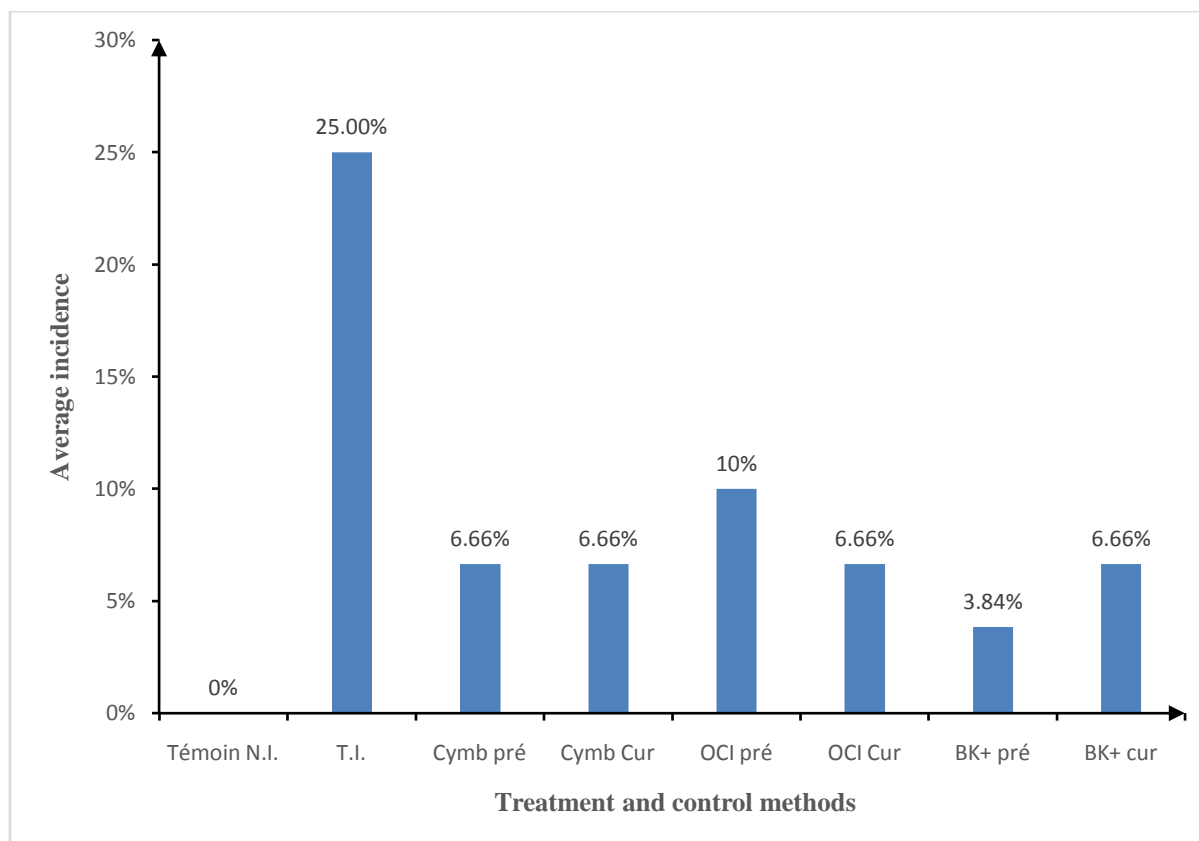


Figure 4:- Incidence of Fusarium root and crown blight in tomato according to products and application methods.

T.N.I: Non-inoculated control; **T.I:** Inoculated control; **Cymb pré:** *Cymbopogon citratus* as a preventive treatment; **Cymb Cur:** *Cymbopogon citratus* as a curative treatment; **Bank+ pré:** Banko plus as a preventive treatment; **Bank+ Cur:** Banko plus as a curative treatment; **Oci pré:** *Ocimum basilicum* as a preventive treatment; **Oci Cur:** *Ocimum basilicum* as a curative treatment

Effects of products on growth parameters of inoculated tomato plants

Following the treatments, the highest average heights were obtained with *C. citratus* essential oil in preventive (18.35 cm) and curative (16.47 cm) treatments. Plant height was significantly better in curative treatment (15.27 cm) than in preventive treatment (11.56 cm) with the synthetic fungicide Banko plus (**Table 3**).

The highest diameter was obtained in preventive treatment with *C. citratus* essential oil compared to the two types of control. The results also revealed that the mean diameters of plants in preventive and curative treatment of Banko plus (3.68 mm and 3.48 mm), *O. basilicum* (3.56 mm and 3.48 mm) and inoculated control (3.56 mm) are statistically identical to the diameter of plants treated preventively with *C. citratus* (3.96 mm). The average diameter of the plants with *C. citratus* in curative treatment (3.15 mm) is statistically identical to that of the non-inoculated control plants (3.26 mm). As a result of the treatments carried out, the average number of living leaves of the plants was statically identical to that of the two types of controls. Nevertheless, the highest value was obtained with the essential oil of *C. citratus* in preventive treatment (10.23 leaves).

Table 3:- Effects of products and application methods on growth parameters of tomato plants inoculated with FORL

Treatments	Height	Diameter	Sheets
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60 days after transplanting

CYMB-Pre	18,35a	3,96a	10,23a
BK+Cur	15.27abc	3.68ab	9,16a
OCI-Pre	13.16cd	3.48ab	9,4a
Witness	17,92a	3,26b	9a
Control-In	14bcd	3.56ab	9a
CYMB-Cur	16.47ab	3,15b	8,9a
OCI-Cur	12.56cd	3.56ab	8,63a
BK+Pre	11,56d	3.48ab	8,43a

The numbers with the same letter are not significantly different at the 5% threshold.

Effect of essential oils and synthetic product on the average flowering time of inoculated tomato plants

The analysis of variance showed that there was no significant difference between the flowering time of the treated plants and that of the non-inoculated control plants. However, among the treatments carried out, the shortest flowering time was obtained following the preventive treatment with *O. basilicum* essential oil (Table 4).

Table 4:- Effect of essential oils and the synthetic product Banko plus on the average flowering time of tomato plants inoculated with *F. oxysporum* f. sp. *Radiciis-lycopersici*.

Treatments	Flowering time	Standard Error	Groups
OCI Cur	55,6667	2,9722	A
CymbPré	54,6667	2,9722	A
BK+ Cur	53,5000	2,5740	A
BK+ Pre	53,4444	2,9722	A
Cymb Cur	52,0000	2,9722	A
OCI Pré	51,6667	2,9722	A
Control	47,6667	2,9722	A

The numbers with 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 treatment associated with the control method.

Discussion:-

This study showed that all the products tested at different concentrations have an effect on *F. oxysporum* f. sp. *Radiciis-lycopersici*. This activity of the products, especially the essential oils would be due to the fact that these extracts possess natural organic compounds with antimicrobial activities recognized in several aromatic plants. These results are in agreement with the work of Oyedejiet al.(1999), Cimangaet al.(2002), Ling et al.(2003) and Tiendrebeogo et al.(2017) who showed that essential oils have antimicrobial, insecticidal, fungicidal and bactericidal activities. However, the sensitivity of the fungus is variable depending on the product and its life stage. This differential activity of products according to FORL life stage was also reported by Doumbouyaet al.(2012) with *Melaleucaquinquenervia* and *Ocimumgratissimum* essential oils. From their work, it was found that the tested essential oil of *O. gratissimum* induced much higher efficacy on the different life stages of *F. oxysporum* f. sp. *Radiciis-lycopersici* compared to the synthetic products Callicuivre and Banko plus with total inhibition of all life stages at 150 ppm. Instead, the work of Soroet al. (2011) indicates a threshold efficacy (50%) of mycelial growth inhibition of *F. oxysporum* f. sp. *Radiciis-lycopersici* at 250 ppm.

In vitro tests on FORL revealed a maximum inhibition threshold (100%) of mycelial growth at concentrations of 250 ppm for the synthetic fungicide Banko Plus and 4000 ppm for the essential oils of *C. citratus*, *O. basilicum* and *L. multiflora*, respectively. According to Carlton et al.(1992), fungi do not react in the same way to biopesticides. This could explain the behaviour of FORL towards the different biofungicides and the concentrations used. In addition, the active ingredients of these products are different.

Furthermore, the analysis revealed that the FORL strain tested is more sensitive to the synthetic fungicide Banko plus and Cymbopogon citratus essential oil compared to the other products. The good activity of Cymbopogon citratus essential oil was also evidenced in the work of Tiendrebeogo et al.(2017) which indicated that this oil totally inhibited the mycelial growth of *Bipolaris oryzae* at the concentration of 400 ppm and that of *Pyricularia oryzae* from 200 ppm. Testing the effect of two synthetic fungicides and two essential oils on mycelial growth of *F. oxysporum* f. sp. *Radiciis-lycopersici*, Doumbouyaet al. (2012) showed that Banko plus (Carbendazim +

Chlorothalonil) and *Ocimum gratissimum* essential oil completely inhibited mycelial growth at 600 ppm and 150 ppm concentrations, respectively, reflecting a better efficacy of this essential oil. The relative efficiency obtained in this work with *Ocimum basilicum* essential oil on FORL mycelial growth (total inhibition at 4000 ppm) would be due to a difference in chemotype but also in sensitivity of the fungal strains.

The FORL strain on the other hand was susceptible to *C. citratus* compared to the other essential oils. This could be explained by the quality, quantity and chemical structure of the antifungal molecules contained in each essential oil (Zarithet *et al.*, 2018).

A reduction of more than 50% of the sporulation of *F. oxysporum* f. sp. *Radicis-lycopersici* was obtained with the essential oils *Eucalyptus camaldulensis*, *Ocimum basilicum* and *Cymbopogon citratus* from the concentrations 500, 1000 and 2000 ppm respectively. The essential oil *Eucalyptus camaldulensis* proved to be effective on germination with an inhibition rate higher than 90% at concentrations 1000, 2000 and 4000 ppm. On the other hand, Ondet and Gomes (2011) demonstrated the ineffectiveness of *Eucalyptus* sp. essential oil on the fungus *Monilia laxa*. As for the essential oil of *Lippia multiflora*, it was ineffective on sporulation but inhibited germination of *F. oxysporum* f. sp. *Radicis-lycopersici* 100% from 1000 ppm. These results are identical to those obtained with the synthetic fungicide Banko plus. Our study is in agreement with those of Koïta *et al.* (2012) who, while studying the antifungal activity of local plant extracts against groundnut rust, demonstrated that the aqueous extract of *Lippia multiflora* at the dose of 40 g/l induces the inhibition of germ tube elongation of *Puccinia arachidis*. As for the essential oils *C. citratus* and *O. basilicum*, they significantly reduced FORL germination with respectively inhibition rates exceeding 90% and 85% from 1000 ppm. Our results are in agreement with those of Doumbouya *et al.* (2012) who showed the effectiveness of *Ocimum gratissimum* essential oil on FORL germination and sporulation. The essential oils *C. citratus* and *O. basilicum* could also be used in preventive control as contact biopesticide. Their efficiency on the different life stages of FORL justifies their choice for the *in vivo* study.

Studies carried out during the *in vivo* experiments determined the pathogenic activity of the strain *F. oxysporum* f. sp. *Radicis-lycopersici*. This strain actually caused the appearance of symptoms on the collars of inoculated tomato plants. This confirms not only the infectivity of this fungal strain but also the susceptibility of the tomato variety used to the pathogen. Thus, the treatment of Fusarium head and root blight of tomato was carried out with Banko plus, *Ocimum gratissimum* and *Cymbopogon citratus*. The efficacy revealed *in vitro* with these products was observed *in vivo*. After transplanting plants of the tomato variety UC 82 B into soil inoculated with *F. oxysporum* f. sp. *Radicis-lycopersici*, treatment with the products at concentrations inhibiting the mycelial growth of the fungus resulted in a low attack of the fungus. However, the degree of attack varied depending on whether the products were applied as a preventive or curative measure.

As a curative treatment, the synthetic product improved plant height, number of live leaves and stem collar diameter. Its efficacy was marked in stem neck diameter. The fungitoxicity of one of the active ingredients (Carbendazim) lies in its systemic nature (Davides, 1986), which allows effective curative treatment at the onset of infection. The 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.

The essential oils of *Ocimum basilicum* and *Cymbopogon citratus*, when applied to plants inoculated with FORL, showed some efficacy on disease expression and on plant growth and development parameters. The essential oil *C. citratus* showed the best activity in preventive treatment. This essential oil stimulated the growth in height of the variety UC 82 B, induced the greatest diameter and number of functional leaves. The inhibition of FORL by this oil in controlled and semi-controlled conditions would be due to the fact that aromatic vegetable species would possess in a natural way compounds being able to inhibit the development of the microorganisms. Thus, a study conducted by Cabral *et al.* (2013) in relation to the effectiveness of the essential oil *Cymbopogon citratus* on the agent *Fusarium oxysporum* f. sp. *Radicis-lycopersici*, *in vitro* and *in vivo*, showed that it has bioactive compounds such as phytoalexins. These phytoalexins are known to have antimicrobial activity for plant protection including alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glycosides, terpenes, phenylpropanes and organic acids.

Conclusion:-

At the end of this study, it was found that, like the synthetic product, the essential oils significantly reduced the different life stages of *Fusarium oxysporum* f. sp. *Radicis-lycopersici* (FORL). Thus, the essential oil *C. citratus* at concentrations of 4000 and 6000 ppm strongly inhibited *in vitro* the different stages of FORL. In preventive and

curative treatment, the essential oils not only improved the growth parameters of tomato plants but also reduced the incidence and severity of diseases. Thus, for management of Fusarium root and crown blight, *C. citratus* essential oil in preventive treatment and Banko plus in curative treatment could be used.

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