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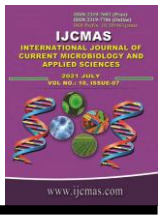

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Biological Control of *Helminthosporium mays* Responsible of Maize (*Zea mays* L.) Helminthosporiosis through the Application of Essential Oils of *Eucalyptus citriodora* and *Ocimum gratissimum* in Côte d'Ivoire

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To control helminthosporiosis of maize (*Zea mays* L.) in Côte d'Ivoire, farmers mainly use chemical fungicides. However, these products are very expensive and polluting. This study aimed to propose a biocontrol strategy against the fungal agent *Helminthosporium mays* using essential oils of two aromatic plants (*Eucalyptus citriodora* and *Ocimum gratissimum*). Strains of *Helminthosporium mays* were isolated from symptomatic leaves of maize. Two maize cultivars (Cultivar jaune de Anyama CJA and Cultivar jaune de Ferké CJF) were inoculated with these fungal strains in a complete randomised Fischer block design with two replicates. The effect of essential oils on the growth of the fungal strains was tested *in vitro* at different concentrations (500 ppm, 1000 ppm, 3000 ppm and 5000 ppm). A total of six (06) strains of the species *Helminthosporium mays* were isolated. Two strains SBA and SBI from Bassam and Bingerville were the most virulent. They induced a high mean number of symptoms on the two maize cultivars respectively 25.33 and 26.13 necrotic spots per leaf. The essential oil of the species *Ocimum gratissimum* was the most effective as it totally inhibited mycelial growth from a concentration of 1000 ppm. This study showed the potential fungicidal effect of essential oils on this phytopathogenic fungal agent.

Introduction

Maize (*Zea mays* L.) is one of the most widely grown grasses in the world (Mugisho *et al.,* 2019). In Côte d'Ivoire and most countries in West and Central Africa, this cereal forms the basis of the diet of rural populations (Deffan *et al.,* 2015). World maize production in 2013 was 839 million tonnes compared with 653 million tonnes for wheat (Deffan *et al.,* 2015). This crop has long been grown throughout Côte d'Ivoire (Kouakou *et al.,* 2010). The maize sector is capable of satisfying more than 95 percent of national consumption each year and exporting surpluses of several thousand tonnes to hinterland countries. However, this crop suffers from enormous abiotic and biotic constraints, leading to significant yield reductions (Moyal, 1991). Among the fungal diseases, helminthosporiosis responsible of corn-leaf blight disease, is one of the most devastating of maize crop (Pelche *et al.,* 1975; Moyal, 1991). It is caused by the fungal species *Helmithosporium mays* (Bervillé *et al.,* 1984). Under favorable conditions (high humidity and temperatures), early infections can cause yield losses estimated at more than 50 percent (Brink & Belay, 2006).

In Côte d'Ivoire, control of helminthosporiosis of maize crop is mainly through the use of chemical fungicides (Kobenan *et al.,* 2019). Other control methods such as the breeding of resistant varieties had also been used (Akanvou *et al.,* 2009; Deffan *et al.,* 2015; Mugisho *et al.,* 2019). But so far none of these had been fully effective against this disease (Kobenan *et al.,* 2019). Given the economic and ecological challenges of food security, the development of an environmentally safe, sustainable and effective biological control method for plant disease management is necessary (Harish *et al.,* 2008; Kassi *et al.,* 2014; Konate *et al.,* 2015). Various studies have highlighted the biological efficacy of medicinal plant extracts from the genera *Eucalyptus* and *Ocimum.* (Batish *et al.,* 2008; Camara *et al.,* 2010; Kassi *et al.,* 2014; Waghe *et al.,* 2015). This study aimed to propose a biological control strategy against the fungal agent *Helminthosporium mays* using essential oils from two species of aromatic plants (*Eucalyptus citriodora* and *Ocimum gratissimum*). The specifics aims were (i) to inventory and characterize the pathogenicity of some strains of the *Helminthosporium mays* species in the region of Abidjan and (ii) to evaluate *in vitro* the fungicidal effect of the essential oils of *Eucalyptus citriodora* and *Ocimum gratissimum* against *Helminthosporium mays*.

Materials and Methods

Fungal material

Helminthosporium mays strains were inventoried and used for inoculation. These strains were isolated from symptomatic leaves collected in areas under maize crop (Anyama, Bingerville, Bassam and Abidjan) and were designated SAN, SBI, SBA and SAB.

Choice of cultivars

Two yellow maize cultivars were used. One cultivar from Anyama called “Cultivar jaune de Anyama” and one from Ferké called “Cultivar jaune de Ferké”. These cultivar were designated CJA and CJF.

Sampling of aromatic plants leaves and extraction of essential oils

The leaves of *Eucalyptus citriodora* and *Ocimum gratissimum* L. (Lamiaceae) plants used for the extraction of essential oils were sampled in the region of Abidjan. These leaves were dried at room temperature. The two essential oils were extracted by hydrodistillation method with saturated steam using a Clevenger-type apparatus for 3 hours (Castro *et al.,* 2010; Santos *et al.,* 2013).

Assessment of the incidence of maize helminthosporiosis in field

The assessment of the incidence of maize helminthosporiosis in field was carried out in different production zones in the district of Abidjan (Abidjan, Anyama, Bingerville, Bassam). For each zone 2 or 3 plots were identified and visited. This assessment was based on the number of infected plants (showing the leaf symptoms of helminthosporiosis) on a randomly selected sample of 30 plants. In each plot visited, symptomatic leaves were collected and used for future isolation of the different strains of *Helminthosporium mays*.

Isolation of the different strains of *Helminthosporium mays*

The symptomatic leaves collected were carefully rinsed with tap water and cut into explants after drying. These explants were disinfected with 4 percent of sodium hypochlorite and then rinsed 3 times with sterile distilled water for 3 minutes each. These explants were then dried on sterile blotting paper. The explants were inoculated in Petri dishes containing sterilized PDA (Potato Dextrose Agar) culture medium. The plates were incubated at 27 °C and purifications were made by transferring the mycelium of each strain to new culture medium (Bhavani *et al.*, 2016).

Pathogenicity tests of different strains of *Helminthosporium mays*

The experimental design used was a complete randomised Fischer block design with two replications. All jars received a uniform application of 25 percent cattle manure. The seeds (2 or 3) of two cultivars of maize (Cultivar jaune de Anyama and Cultivar jaune de Ferké) were sown into pots at the same time. The seeds were disinfected by soaking in a 4 per cent sodium hypochlorite solution and then sown in plastic pots. Two weeks (14) days after sowing the young plants were unmarried and the most vigorous ones were preserved.

Both maize cultivars were inoculated with the 06 fungal strains of the species *Helminthosporium mays*. Twelve (12) plants per cultivar were used as a primary re plicate during the test. For each of the two cultivars, the study involved 168 maize plants whose seeds were sown in pots containing a sterilized mixture of soil and bovine manure. For each cultivar and each strain, 12 plants were inoculated and 12 additional plants per cultivar were used as controls. This experiment was repeated twice.

Preparation of fungal strains (inoculum) and mycelial suspension

Strains were grown in 90 mm diameter Petri dishes poured with PDA (Potato Dextrose Agar) culture medium. The seeded Petri dishes were kept at photoperiod of 12 hours at 27°C under continuous light. Half of a culture of each strain of *Helminthosporium mays* was collected in small fragments in test tubes containing a mixture of 12 mL sterile distilled water and 1 µL of Tween 20. This mixture was then vortexed for 5 minutes.

Inoculation of the maize plants

Inoculation of both cultivars was done at the leaf level at the 04-leaf stage. It consisted to drop 1 mL of the mycelial suspension (10^6 spores/mL) per plant on the upper and lower leaf surfaces (Bhavani *et al.*, 2016).

The first 3 young leaves of each plant were treated. Twelve (12) plants per strain and per cultivar were inoculated during the trial. The latency time corresponding to the period that the plant spends from its inoculation to the

appearance of the first characteristic symptoms of the pathogen. It was measured for each fungal strain and per cultivar.

Agronomic parameters

Various agronomic parameters were examined during this study, mainly: the number of leaves showing symptoms, the measurement of thatch height, the measurement of thatch diameter, the number of necrotic spots present on the leaves and the amount of dry matter.

Number of symptomatic leaves

The number of leaves showing symptoms of helminthosporiosis for each plant was estimated after the inoculation. This number was calculated for each strain of *Helminthosporium mays* per plant, per age and per cultivar.

Number of necrotic spots on leaves

The evaluation of this parameter consisted in counting all the necrotic spots present on the diseased leaves of each plant. For each cultivar, the average necrotic spots induced by each isolate of *Helminthosporium mays was* calculated per leaf, per age and per plant.

Height of the thatch of maize plants

The work consisted of measuring the height of the thatch with a tape measure or double decimeter from the collar to the cigar formed by the last, less open leaves. The average was calculated for each strain of *Helminthosporium mays* by plant, age and cultivar.

Stubble diameter

The diameter of the stubble was measured with a manual caliper. The average was calculated for each strain of *Helminthosporium mays per* plant, age and cultivar.

Dry matter quantity

The quantity of dry matter represents the mass of dry matter produced by the plant. Plants dug up at flowering were dried in an oven for 10 days. The dry mass of the plants was then measured using a Roberval balance with a sensitivity of 1 g to determine the dry biomass. The average was calculated for each strain of *Helminthosporium mays* per plant and per cultivar.

In vitro fungicidal effect of essential oils on the strains of *Helminthosporium mays*

Preparation of different doses of essential oils

The doses of 500 ppm, 1000 ppm, 3000 ppm and 5000 ppm were prepared for each essential oil. These different doses were incorporated into the P.D.A. culture medium. The mixtures were then poured into Petri dishes (Camara *et al.*, 2007).

Choice of fungal strains

Strains of the species *Helminthosporium mays* previously isolated from maize leaves from Abidjan, Anyama, Bingerville and Bassam plots of land were tested for their pathogenicity and the most virulent were selected for essential oil tests.

Assessment of mycelial growth

Each of the two essential oils was incorporated into the P.D.A. (Potato Dextrose Agar) culture medium after autoclaving at 121°C, 1 bar for 30 min. The mixture was then poured at a rate of 17 ml into 9 cm diameter Petri dishes. For each concentration of essential oil, four Petri dishes were inoculated

with a 6 mm diameter explant from the margin of a 5-days-old young culture taken with a punch. Plates were incubated at 25°C under continuous light. The control was carried out under the same conditions but without the addition of essential oil. The mycelial growth was measured every 24 hours by averaging 2 perpendicular diameters passing through the middle of the disc. Three replicates were carried out for each concentration. The experiment lasted 21 days. The mycelial growth inhibition rate of the strains of *Helminthosporium mays* was calculated according to the following formula (Shahi *et al.,* 2003; Hamad *et al.,* 2015):

$$\text{T.I. (\%)} = \frac{\text{Do} - \text{Dc}}{\text{Do}} \times 100$$

T.I. (%) = inhibition rate; Do = average diameter of control of each fungal strain in the culture medium without essential oil (mm). Dc = average diameter of mycelial growth of each fungal strain in the culture medium with a dose of essential oil (mm).

Statistical analysis of the data

The results obtained were analysed using the STATISTICA 7.1 software. The comparison of the means of the study localities, treatments, strains and cultivars was carried out using the NewmanKeuls test at the 5 percent threshold.

Results and Discussion

Distribution and intensity of maize helminthosporiosis in the district of Abidjan

The survey was conducted on 10 agricultural plots divided into 4 zones: Abidjan, Anyama, Bingerville and Bassam. Maize helminthosporiosis was reported on all the surveyed sites. The intensity of the maize helminthosporiosis constraint varied from one study area to another. Thus, the Bingerville and Bassam areas showed 56.67 and 72.22 percent of diseased plants. These areas were less affected than those of Abidjan and Anyama, which showed 85.55 and 93.33 percent diseased feet, respectively.

Effect of the *Helminthosporium mays* strains on agronomic parameters of maize plants

Height and diameter of the maize plants at flowering

The height of inoculated maize plants was influenced by the effect of different strains of *Helminthosporium mays* species compared to control plants. Table II shows that the height of the inoculated plants of both cultivars (18.65 cm for CJA and 19.51 cm for CJF) was significantly slower than the control plants (22.10 cm for CJA and 22.87 cm for CJF); (Table I). The diametrical growth of inoculated plants of both cultivars was subjected to the action of different strains of *Helminthosporium mays* species. Plants of both cultivars inoculated with the different strains of *Helminthosporium mays* species showed significantly slower diametral growth (0.61 cm for CJA and 0.67 cm for CJF) compared to the controls (0.73 cm for CJA and 0.82 cm for CJF); (Table I).

Diseased leaves of maize plants at flowering

The number of diseased leaves on plants inoculated with the different strains was different from that of control plants. Indeed, inoculated plants showed an average number of 6.41 infected leaves per plant for the CJA cultivar and 5.85 leaves per plant for the CJF cultivar compared to the control plants (1.35 leaves per plant for CJA and 1.17 leaves per plant for CJF); (Table II). Leaf symptoms characterized by necrotic burns occurred with

highly significant frequency on leaves of both inoculated cultivars (24.23 necrotic spots per leaf on CJA and 22.02 necrotic spots per leaf on CJF) in contrast to control plants (4.77 necrotic spots per leaf on CJA and 2.37 necrotic spots per leaf on CJF); (Table II).

Quantity of dry matter at flowering

The results of this study showed that the different strains of *Helminthosporium mays* species influenced the amount of dry matter produced. Indeed, the amount of dry matter produced by the two cultivars after inoculation was significantly low (23.28 g/m^2 for CJA and 25.16 g/m^2 for CJF) compared to that produced by the control plants (31.71 g/m^2 and 32.59 g/m^2); (Table III).

The effect of helminthosporiosis on maize plant growth was variable depending on the fungal strain and maize cultivar. For all the parameters studied, there were significant differences between the inoculated plants and the controls of each of the two maize cultivars. Both cultivars were found to be susceptible to strains of *Helminthosporium mays*. The cultivar CJF was less susceptible compared to the cultivar CJA which showed more necrotic spots. A high number of necrotic spots would significantly affect the plants leaf system (where all photosynthetic activity takes place) which could induce a decrease in photosynthetic activity. Thus, more important the necrotic spots are, less photosynthetic activity will be. According to Moser *et al.,* (2006) and Cattivelli *et al.,* (2008), any foliar constraint of maize plant would reduce its photosynthetic capacity and consequently grain filling. The direct consequences of the dysfunction of the photosynthetic system under pathogenic stress conditions would be the significant disruption of growth, development and production of significantly low dry matter (Zelitch, 1982; Cattivelli *et al.,* 2008). Thus, the synthesis of organic matter would decrease with the reduction of photosynthetic activity (Cattivelli *et al.,* 2008).

The necrotic spots observed on the leaves of maize plants are due to the action of a specific toxin (helminthosporoside) produced by the species *Helminthosporium mays* (Chevaugeon, 1957). This toxin is thought to disrupt the growth and development of the maize plant by altering the membrane permeability of susceptible plants. This results in the disruption of the regulation of the passage of water and nutrient ions, particularly the K^+ ion. Hence a disturbance in the physiology and development of infected plants. This toxin blocks oxidative phosphorylation in the mitochondria of maize varieties carrying the Texas cytoplasm responsible for male sterility (Bervillé *et al.,* 1984). Moreover, the presence of helminthosporoside in leaf tissue would cause the destruction of mitochondrial tissues leading to a loss of their phosphorylation capacity and thus a low allocation of the assimilates synthesized in the infected leaves (Cattivelli *et al.,* 2008). This decrease in the allocation of synthesized assimilates would be significantly greater in the CJA than in the CJF.

Varietal sensitivity and leaf symptoms

The appearance and evolution of necrotic spots showed the susceptibility of both cultivars to *Helminthosporium mays*. Thus, up to the flowering stage, the "cultivar jaune de Anyama CJA" presented a high number of 25.15 necrotic spots per leaf in contrast to the "cultivar jaune de Ferké CJF" which recorded a mean of 23.28 necrotic spots. Thus, the "cultivar jaune de Anyama CJA" would be more susceptible than the "cultivar jaune de Ferké CJF". The action of the different strains studied in relation to the number of symptomatic spots made it possible to determine their degree of pathogenicity on the two maize cultivars.

Table.1 Effect of the 06 strains of the species *Helminthosporium mays* on the height and diameter of the maize plant at flowering

Strains	Height (cm)					Diameter (cm)			
	Cultivars					Cultivars			
	Cultivar Jaune Anyama		Cultivar Jaune Ferké			Cultivar Jaune Anyama		Cultivar Jaune Ferké	
	C JA	T0 C JA	CJF	T0 CJF		CJA	T0 CJA	CJF	T0 CJF
SAN1	19,51[a]	22,87[b]	18,35[a]	22,10 [b]		0,63[a]	0,73[b]	0,70[a]	0,82[b]
SAN2	21,18[a]	22,87[b]	21,65 [a]	22,10 [b]		0,60[a]	0,73[a]	0,78 [a]	0,82 [a]
SAB1	18,40[a]	22,87 [b]	20,80 [a]	22,10 [b]		0,64[a]	0,73[b]	0,68 [a]	0,82 [b]
SAB2	18,36[b]	22,87 [b]	20,83 [a]	22,10 [b]		0,57[a]	0,73[b]	0,66 [a]	0,82 [b]
SBA	17,49[a]	22,87 [b]	17,68 [a]	22,10 [b]		0,56[a]	0,73[b]	0,62 [a]	0,82 [b]
SBI	16,93[a]	22,87 [b]	17,68 [a]	22,10 [b]		0,55[a]	0,73[b]	0,61 [a]	0,82 [b]
Means	18,65[a]	22,87 [b]	19,51 [a]	22,10 [b]		0,61 [a]	0,73[b]	0,68 [a]	0,82 [b]
Sd	4,29	6,22	5,06	6,65		0,12	0,09	0,17	0,14

For each cultivar, on the same line, means followed by the same letter indicate non-significant differences at the 5 percent level (Newman-Keuls test).

Table.2 Effect of the 06 strains of the species *Helminthosporium mays* on the leaves of the maize plant at flowering

Souches	Number of infected leaves per plant					Number of necrotic spots per leaf			
	Cultivar Jaune Anyama		Cultivar Jaune Ferké			Cultivar Jaune Anyama		Cultivar Jaune Ferké	
	CJA	T0 CJA	CJF	T0 CJF		CJA	T0 CJA	CJF	T0 CJF
SAN1	6,152	1,35[b]	5,62 [a]	1,17 [b]		22,28[a]	4,78[b]	19,29 [a]	2,37 [b]
SAN2	5,79[a]	1,35 [b]	5,55 [a]	1,17 [b]		22,97 [a]	4,78 [b]	20,97 [a]	2,37 [b]
SAB1	6,14 [a]	1,35 [b]	5,90 [a]	1,17 [b]		23,05 [a]	4,78 [b]	21,25 [a]	2,37 [b]
SAB2	6,27 [a]	1,35 [b]	5,79 [a]	1,17 [b]		23,25 [a]	4,78 [b]	21,59 [a]	2,37 [b]
SBA	6,43 [a]	1,35 [b]	5,94 [a]	1,17 [b]		26,74 [a]	4,78 [b]	23,92 [a]	2,375[b]
SBI	7,67 [a]	1,35 [b]	6,32 [a]	1,17 [b]		27,12 [a]	4,78 [b]	25,13 [a]	2,37 [b]
Mean	6,41 [a]	1,35 [b]	5,85 [a]	1,17 [b]		24,23 [a]	4,78[b]	22,03 [a]	2,37 [b]
Sd	3,08	2,29	3,22	3,72		17,16	11,63	17,18	7,76

For each cultivar, on the same line, means followed by the same letter indicate non-significant differences at the 5 percent level (Newman-Keuls test).

Table.3 Effect of the 06 strains of the species *Helminthosporium mays* on the quantity of dry matter produced at flowering

			Amount of dry matter (g /m^2)	
Strains	Cultivars Jaune Anyama		Cultivars Jaune Ferké	
	CJA	T0 CJA	CJF	T0 CJF
SAN1	23,09[a]	31,71[b]	24,91 [a]	32,59 [b]
SAN2	29.21 [a]	31.71 [b]	30.17 [b]	32.59 [b]
SAB1	22,52 [a]	31,71 [b]	26,33 [a]	32,59 [b]
SAB2	22,64 [a]	31,71 [b]	27,44 [a]	32,59 [b]
SBA	22,11 [a]	31,71 [b]	23,26 [a]	32,59 [b]
SBI	20,12 [a]	31,71 [b]	18,83 [a]	32,59 [b]
Mean	23,28 [a]	31,71 [b]	25,16 [a]	32,59 [b]
Sd	4,38	2,96	5,23	7,23

For each cultivar, on the same line, means followed by the same letter indicate non-significant differences at the 5 percent level (Newman-Keuls test).

Fig.1 Reduction of mycelial growth of the strain SBA of the species *Helminthosporium mays* by the essential oil of the species *Eucalyptus citriodora*

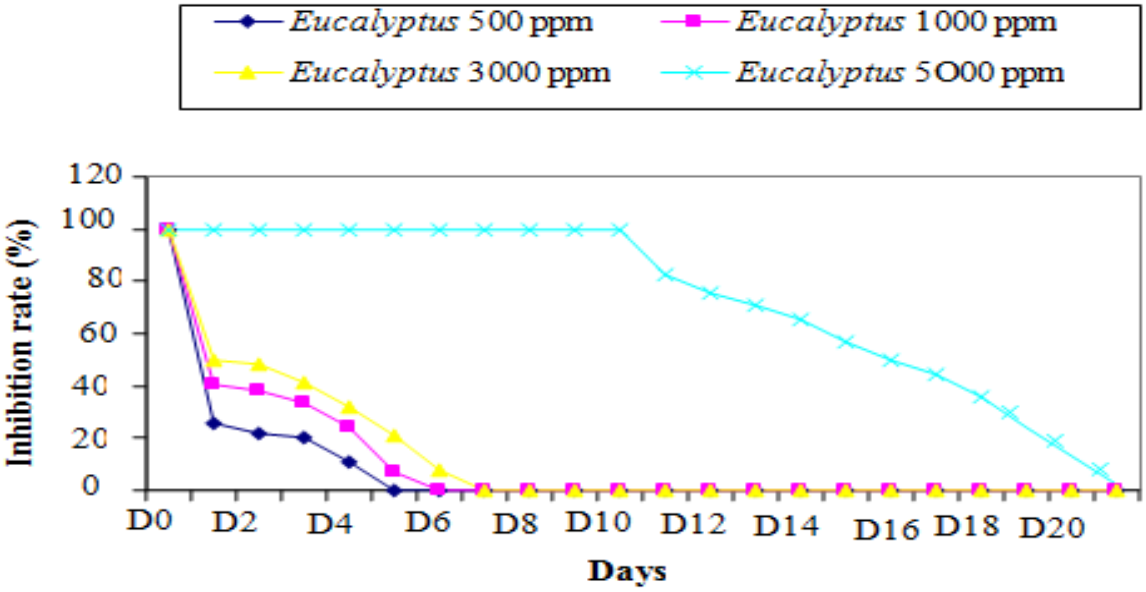


Fig.2 Reduction of mycelial growth of the strain SBI of *Helminthosporium mays* by the essential oil of *Eucalyptus citriodora species*

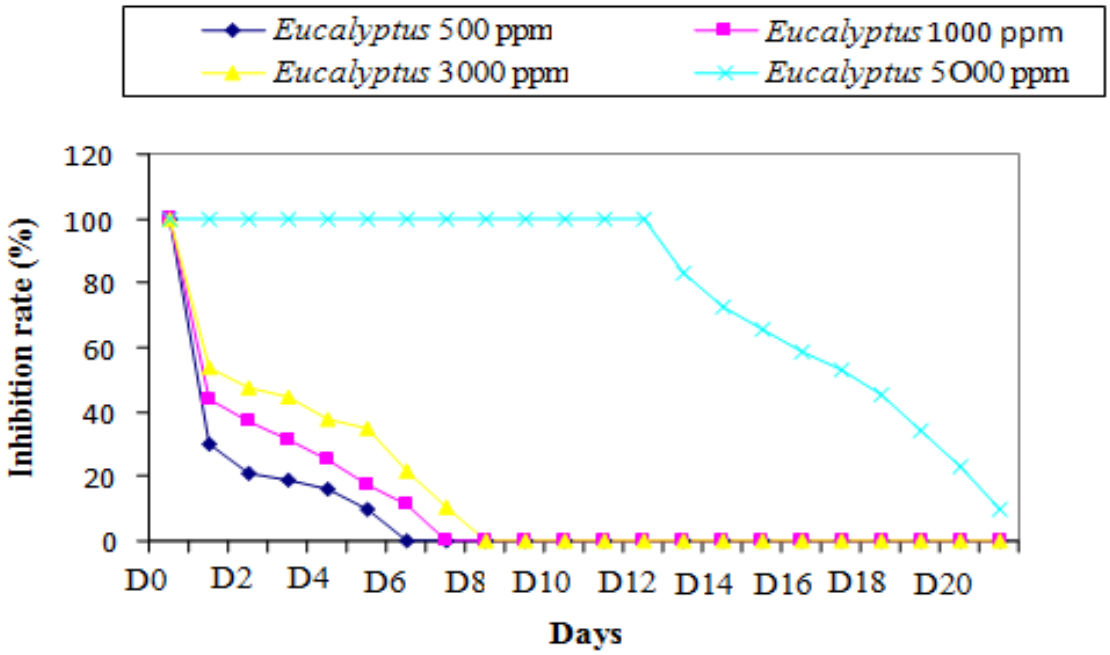


Table.4 Varietal susceptibility and leaf symptoms

Cultivars	Fungal strains					
	SAN1	SAN2	SAB1	SAB2	SBA	SBI
CJA	22,28[a]	22,97[a]	23,05[a]	23 ,25[a]	26,74[b]	27,12[b]
CJF	19,29[a]	20,97[a]	21,25[a]	21,59[a]	23,92[b]	25,13[b]
Mean	20,78[a]	21,97[a]	22,15[a]	22,42[a]	25,33[b]	26,13[b]
Sd	17,59	17,13	18,27	17,69	17,49	20,07

For each cultivar, on the same line, means followed by the same letter indicate non-significant differences at the 5 percent level (Newman-Keuls test)

Fig.3 Reduction of mycelial growth of the strain SBA of *Helminthosporium mays* by the essential oil of *Ocimum gratissimum*

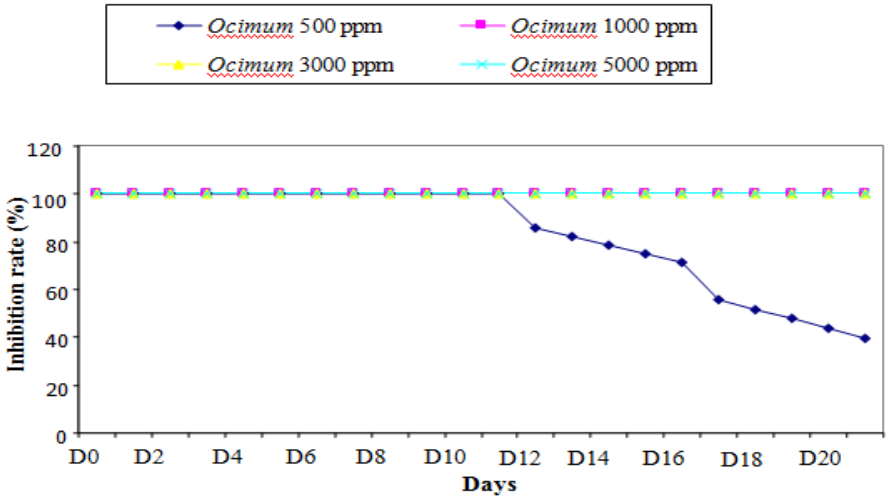
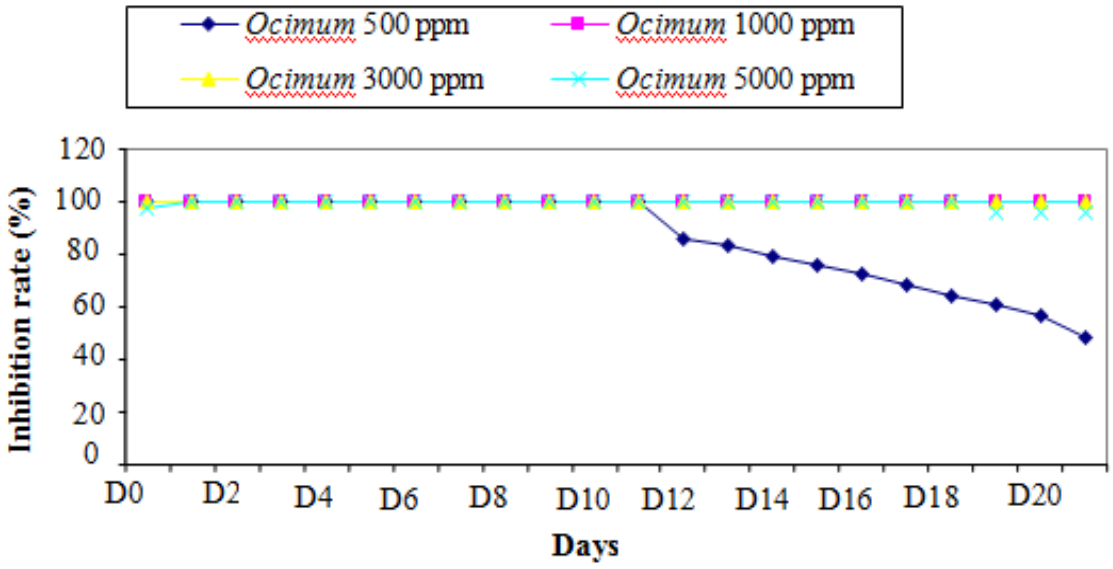


Fig.4 Reduction of mycelial growth of the strain SBI of *Helminthosporium mays* by the essential oil of *Ocimum gratissimum*



Thus, four strains (SAN1, SAN2, SAB1 and SAB2) induced the appearance of a high number of necrotic spots respectively (20.78; 21.97; 22.15; 22.43 necrotic spots per leaf). But, no difference was noted between these strains. Compared to the former, the SBA and SBI strains caused a significantly higher number of symptoms (25.33 and 26.13 necrotic spots per leaf), respectively (Table IV). Thus, more virulent is a strain, more important the leaf symptoms will be.

The susceptibility of the two maize cultivars to infection induced by the fungal species *Helminthosporium mays* would be explained by the fact that they would probably both be carriers of the Texas cytoplasm which induces male sterility of the plants (Bervillé *et al.*, 1984). The infection induced by the different strains tested in this study, although variable from one strain to another, showed that they contain a particularly virulent "T" biotype on cultivars carrying the male sterility gene (Bervillé *et al.*, 1984). CJA (early cultivar) was found to be significantly susceptible to helminthosporiosis compared to CJF (semi-late cultivar). Similar observations reported by Bijlmakers and Verhoek (1995), showed that early cultivars were most susceptible to infection induced by *Helminthosporium mays*.

Effect *in vitro* of essential oils on mycelial growth of *Helminthosporium mays* strains

According to Figures 1, 2, 3 and 4, both essential oils showed antifungal activity against strains (SBA and SBI) of *Helminthosporium mays* species. The essential oil of *Eucalyptus citriodora* species was found to be less fungitoxic at concentrations of 500 to 5000 ppm. The rate of 50 percent reduction of mycelial growth was achieved at 11^{th} and 13^{th} day for the strains SBA and SBI, respectively. With, essential oil of *Ocimum gratissimum* species, mycelial growth of both strains of *Helminthosporium mays* species was inhibited to 100 percent at concentrations greater than or equal to 1000 ppm (Figures 3 and 4). The rate of 50 percent of mycelial growth inhibition was achieved for strains SBA and SBI, respectively, on the 10^{th} and 11^{th} days of the test. Both strains of *Helminthosporium mays* species were similarly inhibited by the essential oil of *Ocimum gratissimum* species. Mycelial fragments treated with *Ocimum gratissimum* species essential oil at doses above 500 ppm showed no recovery after transfer to neutral (no essential oil) PDA-based medium. However, a slowed growth was observed for the 500 ppm concentration.

These two essential oils reduced the mycelial growth of these strains at different concentrations. This study highlights the antifungal properties of these essential oils. The fungitoxicity of the essential oils of *Eucalyptus citriodora* and *Ocimum gratissimum* was also revealed by Dubey *et al.*, (2000) and Ramezani *et al.*, (2002). The essential oil of the species *Ocimum gratissimum* was found to be the most effective for the strains of *Helminthosporium mays*. It inhibited mycelial growth at concentrations above 500 ppm. It acted as some synthetic fungicides such as pyrazophos which is used at lower rates than synthetic fungicides. This essential oil could therefore be used in the context of safeguarding ecological biodiversity (Batish *et al.*, 2008).

The essential oil of the species *Ocimum gratissimum*, used at concentrations of 500 ppm or less, exerted a fungistatic effect. It became fungitoxic at concentrations above 500 ppm compared to the essential oil of *Eucalyptus citriodora*, which only exerted fungistatic activity at a dose of 5000 ppm. According to Camara *et al.*, (2007), the different activities (fungistatic and fungitoxic) of the essential oil of the species *Ocimum gratissimum* were fundamentally due to a

chemical constituent (eugenol) present at 93.9 percent. And eugenol was reported to have antimicrobial activities (Serghat *et al.,* 2004; Shama *et al.,* 2011).

This study showed the fungicidal properties of the plants species *Ocimum gratissimum* and *Eucalyptus citriodora*. Their essential oils inhibited *in vitro* the mycelial growth of *Helminthosporium mays* species. The essential oil of *Ocimum gratissimum* was found to be fungitoxic, because it inhibited totally mycelial growth of *Helminthosporium mays* at doses above 500 ppm. The essential oil of *Eucalyptus citriodora* was less effective for a brief period of 11 or 13 days. Of the two essential oils of the two aromatic plants, that of *Ocimum gratissimum* was the most effective and could be formulated as a biofungicide in the control of helminthosporiosis in maize.

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