IN VITRO AND *IN VIVO* EVALUATION OF ANTIFUNGAL ACTIVITY OF ESSENTIAL PLANT OILS AGAINST *FUSARIUM* WILT OF SESAME

ABSTRACT

Fusarium wilt, caused by the genus *Fusarium*, is a fungal disease that is a major threat to the health of sesame plants in Burkina Faso. To reduce the incidence of the disease, most farmers abuse synthetic pesticides. These pesticides have negative effects on the environment and human health, and confer resistance to pathogens. With the aim of finding alternatives to the use of synthetic pesticides as a means of control, essential oils of *Cymbopogon schoenanthus*, *Eucalyptus camaldulensis*, *Ocimum americanum* and *Lippia multiflora*, used alone or in combination, were evaluated *in vitro* and *in vivo* for their inhibitory activity against *Fusarium duofalcatisporum* and *F. proliferatum*, two pathogenic isolates of sesame. Each product was tested *in vitro* at the dose of 1% and in greenhouse under artificial seed contamination at the doses of 0.5%, 1%, 1.5% and 2%. The promising treatments were then tested in greenhouse under natural seed contamination at the dose of 0.5%. The experimental designs employed included a completely randomized design with Fisher's blocks for the *in vitro* tests and a split-plot design for the *in vivo* tests. All treatments used in vitro significantly reduced the growth of *F. duofalcatisporum* (62.16-89.97% growth reduction) and *F. proliferatum* (33.70-85.08%) compared to the untreated control; seven of these treatments reduced fungal growth more (73.33-89.97% growth reduction) than Calthio C (62.34-76.70% growth reduction). *In vivo*, among the applied doses, the 0.5% dose distinguished from the others by its high emergence rate (93.05-96.52%) and high rate of normal plants. The same dose applied to natural infected seeds by *Fusarium*, significantly reduced the infection rate (≤ 8.5%) compared to the control, which had an infection rate of 67%. A validation of this study on farm would be necessary to propose these essential oils as alternatives to chemical fungicides for the management of *Fusarium* wilt in sesame.

***Keywords**: Antifungal evaluation, essential oils, seed germination, Fusarium, sesame*

1. INTRODUCTION

The spread of *Fusarium* through seeds or other means presents a major challenge to sesame production. The genus Fusarium, recognized as the cause of *Fusarium* wilt in crops, is of significant agricultural and economic importance. It includes many plant pathogens and is a producer of mycotoxins that affect a wide range of crops (Smith et al., 1988). According to Macoumba (2002), agricultural losses in Africa are around 27%, resulting from insect attacks (15%) and diseases (12%) affecting seeds and other sesame organs.

Recent research in Burkina Faso has shown the importance of *Fusarium* species in sesame seeds. The work of Ouali et al. (2023) showed that 97% of 72 seed samples were contaminated by *Fusarium* fungi. According to Soalla et al. (2023), out of 149 samples of diseased sesame seedlings collected, 85% were infected by various Fusarium species, including *F. proliferatum*, *F. equiseti*, *F. penzigii*, *F. incarnatum*, *F.*

oxysporum, *F. fujikuroi* and *F. solani*. The pathogenicity and harmfulness of certain *Fusarium* species found in sesame has also been established. In Pakistan, Li et al. (2012) characterized the pathogenicity of 25 Fusarium isolates isolated from diseased sesame plants, and highlighted the highly pathogenic nature of five isolates belonging to the *F. oxysporum* species. In Uganda, *Fusarium* wilt has been reported as one of the diseases devastating sesame production. Its incidence ranges from 17.1% to 73.3% (Egonyu et al., 2005). In Burkina Faso, pathogenicity study of Ouali et al. (2024) showed that five Fusarium isolates including *Fusarium oxysporum*, *F. moniliforme*, *F. solani*, *F. equiseti* and *Fusarium sp*. were virulent on sesame. Additionally, the research by El-bramawy et al. (2009) indicated that Fusarium wilt of sesame can lead to yield losses ranging from 50% to 100%. Among the proposed methods to control fungal diseases and improve sesame production, synthetic pesticides are the most commonly used nowadays. However, this practice creates significant pollution risks and poses a threat to plant, animal and human health. Pest control through chemicals has negative health and environmental impacts (Kanda et al., 2009; Ahouangninou et al., 2011). The use of plant extracts is also being considered as an alternative to chemicals. Indeed, research today has documented the efficacy of biopesticides in controlling pathogenic fungi and their environmental benefits. Salim et al. (2023) evaluated the antifungal activity of aqueous extracts of nine medicinal plants including *Rhus coriaria*, *Boswellia carterii*, *Nigella sativa*, *Aloe vera* on *Fusarium* spp. of sesame *in vitro* and revealed that extracts of these plants have strong natural fungicidal potential. Several previous studies in Burkina Faso have also demonstrated the antifungal effects of plant extracts against fungi associated with pearl millet, sorghum and onion (Zida, 2009; Kintéga, 2022, Sogoba, 2023). However, little information is available on the effects of local plant extracts on *Fusarium* spp. associated with sesame in Burkina Faso. The aim of the present study is to evaluate the efficacy of extracts from four local plants against *Fusarium* wilt of sesame as a seed treatment.

2. MATERIAL AND METHODES

2.1. Fungal material

In this study, two pathogenic Fusarium isolates were selected due to their high pathogenicity on sesame as reported in previous work by Ouali et al. (2023, 2024). These isolates, originated from Niangoloko and Matiacoali were initially identified morphologically as *Fusarium oxysporum* and *Fusarium moniliforme*, respectively. However, molecular analysis confirmed their identity as *Fusarium duofalcatisporum* for the Niangoloko isolate and *Fusarium proliferatum* for the Matiacoali isolate. On the 1-5 severity scale used in the study, *F. duofalcatisporum* scored 4.32 and *F. proliferatum* scored 3.07. These scores place these pathogens in the highly pathogenic category.

2.2. Plant extracts

The plant extracts studied included four essential oils (EO) derived from the following plant species: *Cymbopogon schoenanthus* (CS), *Eucalyptus camaldulensis* (EC), *Ocimum americanum* (OA), and *Lippia multiflora* (LM). These plants were selected based on their reported efficacy in managing crop pathogens (Zida, 2009).

2.3. Synthetic product used as reference treatment

The reference chemical fungicide for seed treatment is Calthio C, composed of 25% chlorpyrifos-ethyl and 25% thiram. Calthio C, a member of the organophosphate and carbamate family, has a dual function combining insecticidal and fungicidal activity for seed treatment. It is a contact product with a recommended application rate of 2.5 g / kg seed. In the in vitro study, this product was used at a dose of 2.5 g / liter of culture medium.

2.4. Sesame seed samples

Two sesame seed samples were utilized to assess the efficacy of the extracts under controlled conditions: one from the S42 variety sourced in Banfora and another from a local variety (T2) collected in Tenkodogo. The *Fusarium* spp. infection rates were 1% for the S42 variety and 32% for the local variety (Ouali et al., 2023).

2.5. Preparing essential oils

Essential oils were purchased from the Laboratoire des Technologies de l'Environnement et des Produits Naturels (LTEPN) of the Institut de Recherche en Sciences Appliquées et Technologies (IRSAT). They were extracted from the leaves of *C. schoenanthus*, *E. camaldulensis*, *O. americanum* and *L. multiflora* by hydrodistillation in a stainless steel still.

2.6. Assessment of the effect of essential oils on the radial growth of *Fusarium duofalcatisporum* and *Fusarium proliferatum*

Various potato dextrose agar (PDA) media containing essential oils were prepared for the in vitro culture of *Fusarium* isolates according to the methodology of Zida (2009). The essential oils were first filtered through millipore filters (Ø = 0.45 µm) to remove any spore contamination, and then incorporated into sterilized PDA medium at the 1% (v/v) concentration previously used by Kintéga (2022). The PDA medium in which Calthio C was incorporated at a rate of 2.5 g per liter of PDA was used as the reference control, while the plain, i.e. untreated, PDA medium served as the untreated control in the study. Combinations of essential oils were also evaluated. The treatments studied were:

T0 = PDA

T1 = PDA + Calthio C

T2 = PDA + *C. schoenanthus* EO

T3 = PDA + *E. camaldulensis* EO

T4 = PDA + *O. americanum* EO

T5 = PDA + *L. multiflora* EO

T6 = PDA + [*C. schoenanthus* EO + *E. camaldulensis* EO]

T7 = PDA + [*C. schoenanthus* EO + *O. americanum* EO]

T8 = PDA + [*C. schoenanthus* EO + *L. multiflora* EO]

T9= PDA + [*E. camaldulensis* EO + *O. americanum* EO]

T10 = PDA + [*E. camaldulensis* EO + *L. multiflora* EO]

T11 = PDA + [*O. americanum* EO + *L. multiflora* EO]

For each *Fusarium* isolate, a 4 mm diameter mycelial explant was aseptically harvested from an 8-day-old pure culture, then placed in the center of a Petri dish containing the treatment to be tested. The plates were sealed with adhesive parafilm and incubated at laboratory temperature. The experimental design used for each isolate was the Completely Randomised Design, comprising 21 treatments in four (4) replicates.

Colony diameters were measured in each dish on days 3 and 9 after explant incubation. The result, expressed in millimeters, was the average of two diameters measured perpendicularly to each other, minus 4 mm (explant diameter). The inhibition rate was calculated according to the formula of Kra et al. (2011). The products with the highest antifungal activity were selected for *in vivo* (laboratory) evaluation as sesame seed treatments.

T (%) = ((D-d)/D) x 100), With T: inhibition rate (%), D: Average diameter of mycelial growth in untreated Petri dishes, d: Average diameter of mycelial growth in the Petri dishes of the treatment in question.

2.3. Determination of optimal doses

At the end of the in vitro test, five promising treatments with essential oils were applied as seed treatments to the S42 sesame variety, along with a control (sterile distilled water), to assess the potential phytotoxicity of these products. For each treatment, seeds were pre-disinfected with sodium hypochlorite (1%) and dried for 1 h before being exposed to different doses of 0.5%, 1%, 1.5% and 2%. For each dose, 160 seeds (40 seeds per replication) were sown in plastic blister packs containing sterilized sand. The sterile sand was saturated with water prior to sowing. The seeded alveoli were placed in a plant growth chamber at a temperature of 25-30°C and watered regularly to maintain adequate humidity. The chamber was subjected to an alternating cycle of white light and darkness for 12 hours per day for 21 days. The experimental design was a split-plot with four replicates, with treatment as the main factor and doses as the sub-factor. Observations included counting the number of emerged plants at 10 days after sowing (DAS), followed by counting normal and dead plants, measuring plant height, and recording fresh plant weight at 21 DAS.

2.4. Assessment of the efficacy of the best essential oils against *Fusarium* spp. associated with sesame seeds

The seed sample taken from the local sesame variety (T2) with an initial Fusarium infection rate of 32% was used for this study. Four hundred (400) seeds from the sample were used for each of the five previously identified promising treatments. The seeds were treated according to the methodology of Kintéga (2022) with some modifications. They were mixed with distilled water and essential oil at the optimal dose, then kept away from light for about 3 hours before air drying for 1 hour. Seeds mixed with sterile distilled water were used as the untreated control and those treated with Calthio C (2.5 g/kg seed) as the reference treatment. Seeds treated in this way were evaluated for health using the blotting paper

method (ISTA, 1999). For each treatment, seeds were placed in Petri dishes lined with moistened blotting paper in a Fischer block design with four (4) replicates at a rate of 25 seeds per dish. Dishes were then incubated in a chamber at 20-25°C under alternating lighting of 12 hours of near-ultraviolet light and 12 hours of darkness per day for seven days. Incubated seeds were individually examined under a binocular magnifying glass for the presence of fungi, especially those belonging to the genus Fusarium, confirmed by microscopic observations with reference to the fungal identification key of Mathur and Kongsdal (2003). The *Fusarium* species present on each seed were noted and the percentage of seeds infected by Fusarium was calculated for each treatment.

2.5. Statistical analysis of data

The collected data were analyzed using SAS (Statistical Analysis System) software. Statistical analyses (ANOVA) were performed on fungal radial growth inhibition rates, emergence rates, normal and dead plant rates, plant height and fresh plant weight, and seed infection rates, followed by comparisons of means using the Duncan Range Multiple Test at 5% threshold for significant differences between treatments.

2. RESULTS

2. 1 Effect of plant extracts on fungal mycelial growth

Figure 1 shows the mycelial growth of *Fusarium duofalcatisporum* on different culture media 9 days after incubation of mycelial explants. Untreated PDA medium showed rapid growth of the fungal mycelium, while treated media, including Calthio C, showed high reduction of mycelial growth of the isolates.

Table 1 shows the results obtained in vitro on the mycelial growth of *F. duofalcatisporum* and *F. proliferatum* in the different culture media. The analysis of variance of the effect of the plant extracts on the mycelial growth of *F. duofalcatisporum* and *F. proliferatum* showed highly significant differences ($p < 0.0001$) between treatments at the 5% threshold, whatever the fungus and whatever the evaluation period. The inhibition rates induced by treatments such as EO_CS, HE_LM, EO_LM+EC, EO_OA+CS, EO_CS+LM, EO_CS+EC, EO_OA+CS, EO_OA+LM on the mycelial growth of *F. duofalcatisporum* and *F. proliferatum*, which were 53.39-60.05% and 71.73-73.51%, respectively, at 3 DAI, increased to 75.87-85.08% and 80.99-89.97%, respectively, at 9 DAI. These rates were also higher than those induced by the reference control (Calthio C) at both 3 DAI and 9 DAI. With the exception of EO_OA (74.29%), EO_OA+EC (66.59%) and EO_EC (62.16%), which caused lower inhibition rates than the reference treatment Calthio C (76.70%) at 9 DAI, all other oil-based treatments had higher inhibitory effects.

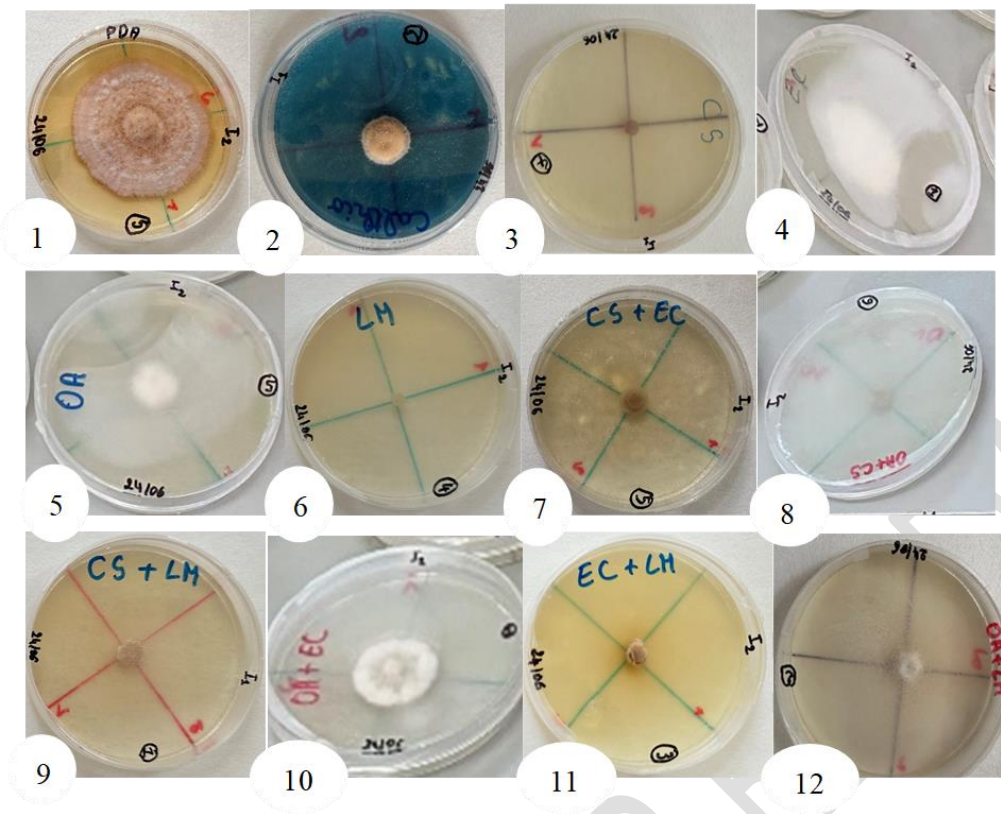

Figure 1 : Growth levels of *F. duofalcatisporum* on different culture media, 9 days after incubation of mycelial explants

(1: PDA, 2: PDA + Calthio C, 3: PDA + *C. schoenanthus* EO, 4: PDA + *E. camaldulensis* EO, 5: PDA + *O. americanum* EO, 6: PDA + *L. multiflora* EO, 7: PDA + [*C. schoenanthus* EO + *E. camaldulensis* EO], 8: PDA + [*C. schoenanthus* EO + *O. americanum* EO], 9: PDA + [*C. schoenanthus* EO + *L. multiflora* EO], 10: PDA + [*C. schoenanthus* + *O. americanum* EO], 9: PDA + [*C. schoenanthus* EO + *L. multiflora* EO], 10: PDA + [E. *camaldulensis* EO + *O. americanum* EO], 11: PDA + [*E. camaldulensis* EO + *L. multiflora* EO], 12: PDA + [*O. americanum* EO + *L. multiflora* EO])

Table 1 : Effects of plant extracts on mycelial growth of *F. duofalcatisporum* and *F. proliferatum* 3 and 9 days after incubation of mycelial explants

Treatments	Mycelial growth inhibition rate (%)			
	F. duofalcatisporum		*F. proliferatum*	
	3 DAI	**9 DAI**	**3 DAI**	**9 DAI**
CS_EO	60.05a	89.97a	73.51a	85.08a
LM_EO	59.05a	88.30ab	70.54ab	84.87a
LM+CS_EO	57.49a	85.18ab	66.87b	80.10a
LM+EC_EO	58.05a	83.28c	65.75bc	79.96a
CS+EC_EO	53.39a	80.99c	71.73ab	75.87a
CS+OA_EO	60.05a	89.97a	68.70ab	74.78a
OA+LM_EO	59.22a	81.41c	68.82ab	73.33a
OA_EO	53.39a	74.29d	67.60ab	70.96ab
CALTHIO	57.43a	76.70d	61,46c	62.34bc
OA+EC_EO	53.39a	66.59e	68.24ab	58.38b

EC_EO	36.98b	62.16f	39.25d	33.70c
PDA	0.00c	0.00g	0.00e	0.00d
Mean	**50.71**	**73.12**	**60.16**	**66.22**
P (5%)	**<0.0001**	**<0.0001**	**<0,0001**	**<0.0001**

Numbers in the same column assigned to the same alphabetical letter do not differ significantly at the 5% threshold according to Duncan's test. ***Legend****: EO: Essential Oils, CS: C. schoenanthus, EC: E. camaldulensis, OA: O. americanum, LM: L. multiflora, DAI: Days After Incubation.*

2. 2 Effect of different doses of essential oils on seedling growth and fresh plant weight

The results of in vivo evaluation of five promising treatments (LM_EO, CS_EO, LM+CS_EO, LM+EC_EO and CS+OA_EO) applied to cleaned seeds of sesame at different doses showed different effects on seedling growth. These treatments, identified as effective against mycelial growth of *Fusarium duofalcatisporum* and *Fusarium proliferatum* in in vitro tests, were evaluated for their effect on germination and seedling growth in the greenhouse. Figure 2 shows the appearance of seedlings 21 days after sowing. At 21 days after sowing (21DAS), only seedlings from seeds treated with the 0.5% dose of essential oils showed an appearance similar to that of untreated controls. Higher doses (1.5% and 2%) induced signs of phytotoxicity such as leaf color changes and increased emergence mortality.

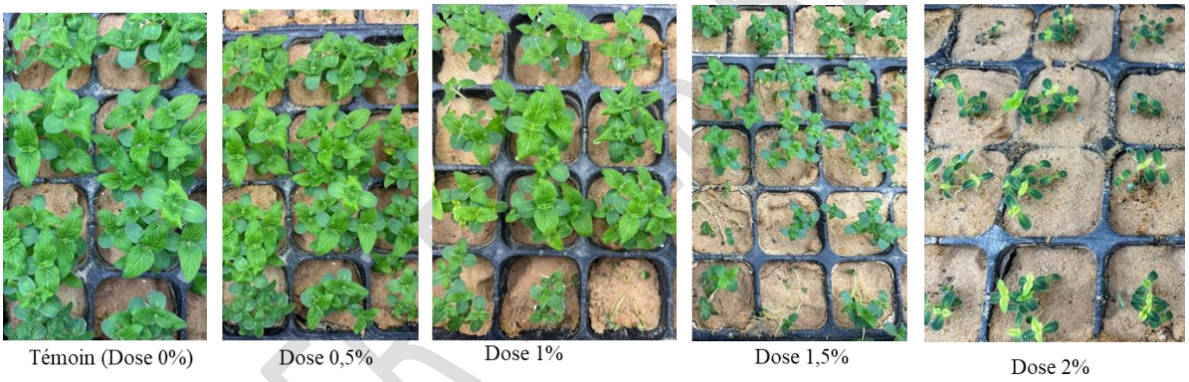

Figure 2: effects of essential oils at different application doses on seedlings

2.2.1. Effect of essential oils on plant emergence 14 days after sowing (DAS)

The different application rates of LM_EO, CS_EO, LM+CS_EO, LM+EC_EO and CS+OA_EO had different effects on seedling emergence (Table 2). For LM_EO and LM+EC_EO, doses of 0.5% and 1% significantly improved seedling emergence rates with 84.02-96.52% and 94.44-95.83% of emerged plants, respectively, compared to the doses of 1.5% (64.58-88.88%) and 2% (58.33-88.88%). CS_EO and CS+OA_EO applied at 0.5% also boosted emergence rates (93.05-95.13%) compared to the other doses (53.47-79.86%). At 0.5%, LM+CS_EO increased emergence by 90.97%, while the other doses induced low emergence. Regardless of the treatment used, the application of the 0.5% dose resulted in an emergence rate (90.97 to 96.52%) quite close to the 0% untreated control (95.14-100%) and higher than Calthio C (73.61%).

Table 2: Effect of different application doses of five promising sesame seed treatment products on plant emergence 14 DAS

Doses	Plant emergence rate (%) 14 DAS, depending on product					
	CS_EO	LM_EO	LM+CS_EO	CS+OA_EO	EC+LM_EO	Calthio C
D0%	99.30a	100a	95.14a	97.22a	98.61a	98.61a
D0.5%	95.13ab	96.52ab	90.97a	93.05a	95.83a	73.61b
D1%	79.86bc	84.02b	63.19b	79.86ab	94.44a	49.30c
D1.5%	73.61c	64.58c	41.67b	66.66bc	88.88a	45.13c
D2%	65.97c	58.33c	39.58b	53.47c	88.88a	35.41c
Mean	**82.77**	**80.69**	**66,11**	**78.05**	**93.33**	**60.41**
P (5%)	**<0.0023**	**<0.0001**	**0,0005**	**0.0005**	**0.1865**	**<0.0001**

Numbers in the same column assigned to the same alphabetical letter do not differ significantly at the 5% threshold according to Duncan's test. ***Legend****: EO: Essential Oils, CS: C. schoenanthus, EC: E. camaldulensis, OA: O. americanum, LM: L. multiflora, DAS: days after sowing; D0%: Controls (distilled water)*

2.2.2. Effect of essential oils on plant mortality 21 JAS

With the exception of CS_EO, significant differences in plant mortality were noted between application rates for the other products (Table 3). In general, plant mortality was lower for the dose 0.5% (4.92-16.71%) than the other doses (15.14 to 53.61%).

Table 3: Effect of different application doses of five promising sesame seed treatment products on plant mortality 21 DAS

Doses	Rate of dead plants (%) 21 JAS, depending on product					
	CS_EO	LM_EO	LM+CS_EO	CS+OA_EO	EC+LM_EO	Calthio C
D0%	3.57a	00b	5.25b	00b	0.69b	00c
D0.5%	4.92a	7.85ab	12.80b	16.71ab	10.42ab	28.44b
D1%	15.14a	16.85a	25.69ab	37.09a	23.98ab	18.18bc
D1.5%	19.37a	16.22a	26.35ab	45.55a	21.19ab	35.91b
D2%	25.27a	19.28a	53.61a	39.82a	38.75a	63.10a
Mean	**13,65**	**12.04**	**24.73**	**27.83**	**19**	**29.12**
P (5%)	**0,2409**	**0.019**	**0.0053**	**0.0251**	**0.0079**	**0.0004**

Numbers in the same column assigned to the same alphabetical letter do not differ significantly at the 5% threshold according to Duncan's test. ***Legend****: EO: Essential Oils, CS: C. schoenanthus, EC: E. camaldulensis, OA: O. americanum, LM: L. multiflora, DAS: days after sowing; D0%: Controls (distilled water)*

2.2.3. Effect on percentage of normal plants 21 JAS

The rates of normal plants obtained after seed treatment with the products at the different doses are shown in Table 4. Significant differences were noted between the doses with LM+CS_EO (p=0.0088) and CS+OA (p=0.0001), in contrast to the other products (LM_EO, CS_EO and LM+EC_EO). The 0.5% dose produced the highest percentage of normal plants (82.05-95.99%) compared to the 1% (60.90-91.43%), 1.5% (61.69-89.93%) and 2% (47.42-84.30%) doses. However, LM+CS_EO, LM_EO and CS_EO gave high rates of normal plants (83.59-90.78% on average), while CS+OA_EO and LM+EC_EO gave low rates of normal plants (73.81-81.64% on average). For all application rates, it was

noted that the percentage of normal plants recorded at the dose 0.5% was close to that of the untreated control (0%).

Table 4: Effect of different application rates of five promising sesame seed treatments on the rate of normal plants 21 JAS

	Normal plant rate (%) 21 DAS, by product					
Doses	**CS_EO**	**LM_EO**	**LM+CS_EO**	**CS+OA_EO**	**LM+EC_EO**	**Calthio C**
D0%	100a	99.30a	97.74a	100a	100a	100a
D0.5%	92.88a	92.16a	95.99a	80.76b	82.05a	77.96a
D1%	91.43a	90.92a	90.90ab	60.90c	76.65a	62.23ab
D1.5%	89.93a	90.14a	61.69c	79.96b	68.90a	85.20a
D2%	79.67a	84.30a	71.67bc	47.42d	80.60a	40.63b
Mean	**90.78**	**91.36**	**83.59**	**73.81**	**81.64**	**73.2**
P (5%)	**0.2892**	**0.2981**	**0.0088**	**<0.0001**	**0.0699**	**0.0262**

Numbers in the same column assigned to the same alphabetical letter do not differ significantly at the 5% threshold according to Duncan's test. ***Legend****: EO: Essential Oils, CS: C. schoenanthus, EC: E. camaldulensis, OA: O. americanum, LM: L. multiflora, DAS: days after sowing; D0%: Controls (distilled water)*

2.2.4. Effect of seed treatment with essential oils on plant height 21 JAS

Plant heights recorded as a function of application doses for the different products are shown in Table 5. Significant differences in plant height were observed between the doses used for all products. Compared to the sizes of the untreated control (D0%), the 0.5% dose stood out from the other doses with a larger plant height for each product. The use of this dose resulted in larger plant heights for the test products LM_EO (4.13 cm), CS_EO (4.87 cm), LM+CS_EO (4.49 cm), LM+EC_EO (4.19 cm) and CS+OA_EO (3.23 cm) than the other application doses (<3.85 cm). However, relatively small size plants were observed with the CS+OA_EO treatment using doses of 1% (2.24cm), 1.5% (2.85cm) and 2% (1.80cm).

Table 5: Effect of different application doses of five promising sesame seed treatment products on plant height 21 JAS

	Plant height 21DAS (cm)					
Doses	**CS_EO**	**LM_EO**	**LM+CS_EO**	**CS+OA_EO**	**LM+EC_EO**	**Calthio C**
D0%	4.32ab	4.53a	4.35a	3.90a	4.35a	4.32a
D0.5%	4.87a	4.13ab	4.49a	3.44a	4.22a	2.55bc
D1%	3.45c	3.59bc	3.85ab	2.17c	3.47b	2.24c
D1.5%	3.39c	3.12c	3.07b	2.83b	3.60b	2.85b
D2%	3.72bc	3.56bc	3.08b	1.80c	3.68b	2.90b
Mean	**3,96**	**3,79**	**3.77**	**2.83**	**3.86**	**2.99**
P (5%)	**0,0014**	**0,001**	**0.003**	**<0.0001**	**0.001**	**<0.0001**

Numbers in the same column assigned to the same alphabetical letter do not differ significantly at the 5% threshold according to Duncan's test. ***Legend****: EO: Essential Oils, CS: C. schoenanthus, EC: E. camaldulensis, OA: O. americanum, LM: L. multiflora, DAS: days after sowing; D0%: Controls (distilled water)*

2.2.5. Effect of essential oils on fresh plant weight 21 JAS

The fresh plant weights evaluated at 21 JAS according to the application doses of the different products are summarized in Table 6. Among the five (05) products used, LM+CS_EO ($p<0.0001$) and CS+OA_EO ($p<0.0371$) were the only products whose plant weights varied between doses. In fact, the 0.5% rate produced the heaviest weights (1.64-1.97g) compared to the other rates (<1.15g). For the three products (LM_EO, CS_EO and EC+ LM_EO), plant weights were invariant regardless of the dose. It should also be noted that the weights obtained with LM+EC_EO (1.06 to 1.53g) are also similar to those of the untreated (2.09 to 3.43g) for all doses.

Table 6 : Effect of different application doses of five promising sesame seed treatment products on fresh plant weight 21 JAS

	Fresh plant weight (g) 21 JAS, depending on product					
Doses	**CS_EO**	**LM_EO**	**CS+LM_EO**	**CS+OA_EO**	**LM+EC_EO**	**Calthio C**
D0%	2.51a	2.20a	2.58a	2.13a	2.77a	2.95a
D0.5%	1.00a	1.44a	1.97a	1.64ab	1.55a	0.62b
D1%	1.65a	1.15a	1.09b	1.15ab	1.79a	1.48ab
D1.5%	1.24a	1.16a	0.27b	0.43b	1.53a	0.26b
D2%	1.09a	1.12a	0.21b	0.13b	1.36a	0.32b
Mean	**1.5**	**1.42**	**1.22**	**1.13**	**1.8**	**1.13**
P (5%)	**0.3047**	**0.1477**	**<0.0001**	**0.0371**	**0.4675**	**0.0125**

Numbers in the same column assigned to the same alphabetical letter do not differ significantly at the 5% threshold according to Duncan's test. ***Legend****: EO: Essential Oils, CS: C. schoenanthus, EC: E. camaldulensis, OA: O. americanum, LM: L. multiflora, DAS: days after sowing; D0%: Controls (distilled water)*

2.3. Effects of seed treatments with essential oils on sesame seed-borne *Fusarium* spp.

Statistical analysis revealed that seed treatments with essential oils had a significant effect on the percentage of seeds infected with *Fusarium* spp. The rates of *Fusarium* spp.-infected seeds recorded on seeds treated or not with essential oils were shown in figure 2. The lowest infection rates were noted on seeds treated with Calthio C (0%), LM+CS_EO (3.5%), CS+OA_EO (5%), CS_EO (5.75%), LM_EO (6%) and LM+EC_EO (8.75%), while the highest infection rates were obtained with seeds treated with sterile distilled water (63.75%). Used at a dose of 0.5%, these essential oils were statistically as effective against *Fusarium* spp. as Calthio C, the reference control (0%).

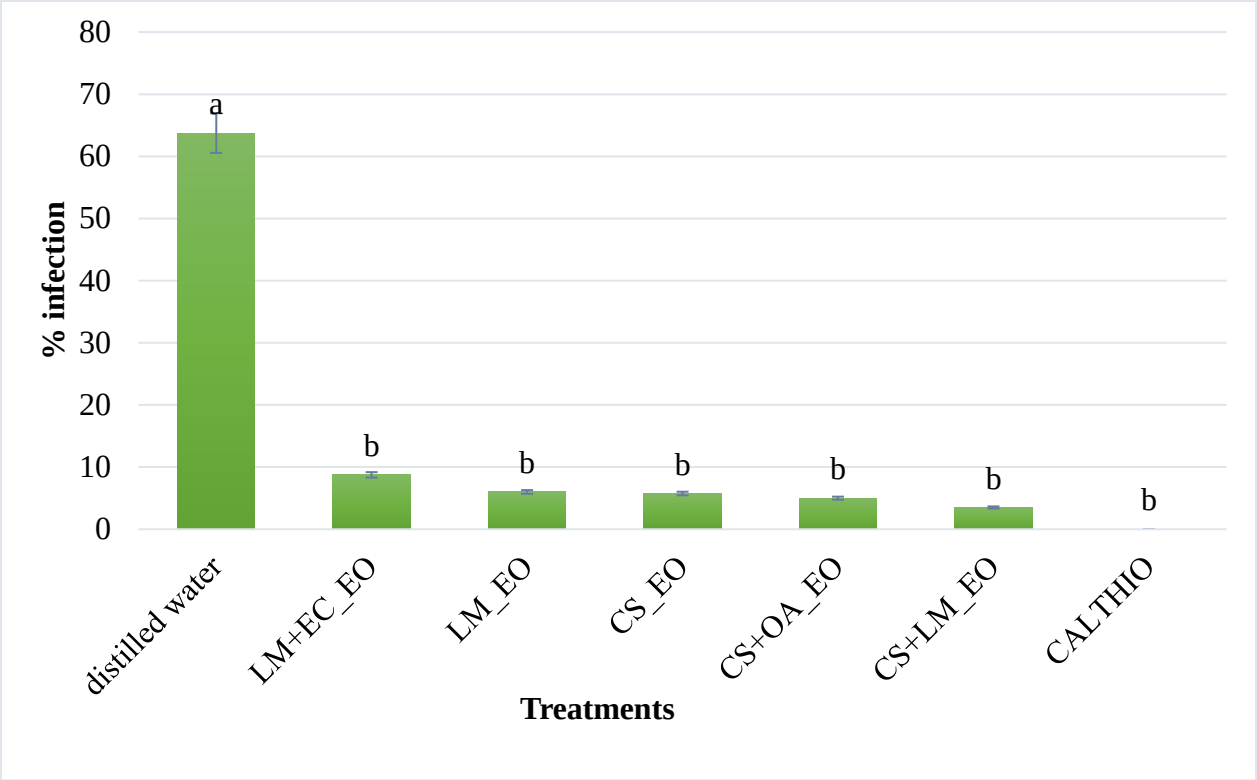

Figure 3 : Effects of essential oils on Fusarium spp. associated with sesame seeds

***Legend**: LM+EC_EO: essential oils of E. camadulensis + L. multiflora, LM_EO: essential oils of L. multiflora, CS_EO: essential oils of C. schoenanthus, CS+OA_EO: essential oils of C. schoenanthus + O. americanum, CS+LM_EO: essential oils of C. schoenanthus + L. multiflora*

3. DISCUSSION

This study was carried out to identify the best treatments based on essential oils from local plants against two fungal pathogens (*F. duofalcatisporum* and *F. proliferatum*) of sesame. The various tests carried out revealed significant differences in the antifungal activity of the extracts and the effects of promising seed treatment products.

The study demonstrated variable efficacy of the extracts in inhibiting radial growth of *Fusarium duofalcatisporum* and *Fusarium proliferatum*, highlighting significant differences in inhibitory potency. These variations could be attributed to several factors such as the variability of the active compounds contained in the extracts, synergistic interactions between different compounds or the chemical composition of each extract. These observations corroborate the findings of Lhoste et al (1993), Pamo et al (2003) and Ling et al (2003) who reported that plant extracts from a number of plants contain compounds such as tannins, flavonoids and alkaloids which have fungicidal properties. These compounds often act by disrupting fungal cell membranes, inhibiting essential enzymes or disrupting pathogen metabolism.

Although all the extracts showed significant antifungal activity *in vitro*, it is noted that the CS_EO, LM_EO, LM+CS_EO, LM+EC_EO, CS+EC_EO, CS+OA_EO and OA+LM_EO treatments had a stronger inhibitory effect on *F. duofalcatisporum* (80.99-89.97%) than on *F. proliferatum* (73.33-85.08%) at 9 JIA. Compared to Calthio C, the treatments LM_EO (essential oil of *Lippia multiflora*), CS_EO

(essential oil of *Cymbopogon schoenanthus*), LM+CS_EO (combination of essential oils of *L. multiflora* and *C. schoenanthus*), EC+LM_EO (combination of essential oils of *Eucalyptus camaldulensis* and *L. multiflora*) and CS+LM_EO (combination of essential oils of *C. schoenanthus* and *L. multiflora*) showed superior or comparable inhibition rates, with variations depending on the treatment. These results highlight the potential efficacy of essential oils, alone or in combination, as natural alternatives to Calthio C for the control of fungal pathogens. However, the differences in inhibition rates indicate a dependence on the specific compositions and synergistic interactions of the essential oils used. Therefore, numerous studies have shown that the activity of an essential oil is related to the majority of compounds and the possible synergistic effects between the constituents (OUSSOU, 2009; OUSSOU et al., 2010). In line with this study, similar results have been observed by Zida (2009), Koïta et al. (2017), Sirima et al. (2020), Kintega (2022) and Sogoba (2023). These authors reported that the essential oils of *L. multiflora*, *O. americanum* and *C. schoenantus* can completely or partially inhibit the mycelial growth of several fungal species *in vitro.*

In the case of OA_EO, OA+EC_EO and EC_EO treatments, the inhibitory effect was weaker than that of Calthio C. It could be said that the inhibiting molecules were probably less concentrated to reduce the mycelial growth of the fungal isolates. Certain factors, especially the hydrodistillation conditions of the organs combined with the thermal conditions and the low doses of active compounds, could explain the low rate of inhibition of fungal mycelial growth. AMADIOHA (2000) reports that thermal factors can modify the antifungal properties of plant extracts.

The results obtained from the phytotoxicity test highlighted the importance of finding a balance between the efficacy of essential oil-based treatments against pathogens such as *Fusarium* spp. and their safety for sesame seedlings. Although the tested treatments showed *in vitro* efficacy against the mycelial growth of *F. duofalcatisporum* and *F. proliferatum*, their application under *in vivo* conditions showed limitations related to their phytotoxicity, especially at doses higher than 1%. Indeed, the use of essential oils at doses of 0.5% and 1% resulted in a seed emergence rate close to the control, a high normal plant rate and low plant mortality at 21JAS. On the other hand, the 1.5% and 2% application rates of the products resulted in high mortality at 21JAS coupled with low normal plant rates. It was found that the 0.5% application rate would be the best and economic application dose regardless of the product for all the variables noted. Similar results have been reported by Zida (2009) and Kintéga (2022). These authors showed that emergence was good for onion, pearl millet and sorghum seeds at doses ranging from 0.5 to 2%. The high application doses seemed to induce some phytotoxicity in the plants, in contrast to the low rate of 0.5%. These results could be explained by the fact that high doses contain a higher concentration of molecules harmful to seedling health than low doses. However, it is important to consider appropriate doses for crop protection. For example, the application of EO to naturally contaminated sesame seeds at a dose of 0.5% showed a reduction in the contamination of these seeds after incubation. The highest infection rate among the five (05) best seed treatments was 8% and the lowest was 3%, while the contamination rate for the control was 63% and zero for Calthio C. These results indicate HE-induced fungistatic activity on seeds. These results are in line with those obtained by Nebié (2006) and Tiendrébeogo et al. (2017), who attest the efficacy of these EOs on fungal development thanks to the antifungal properties they contain.

The various tests carried out in this study with essential oils alone or in combination, and the doses used, made it possible to verify their efficacy against sesame fungi and to determine appropriate doses for seed treatment.

CONCLUSION

This study highlighted the value of using local plant extracts to control the Fusarium genus associated with sesame seeds. During in vitro analysis, all extracts showed fungicidal activity by inhibiting mycelial growth of *F. duofalcatisporum* and *F. proliferatum* in PDA culture medium. Five essential oil treatments consisting of *C. schoenanthus*, *L. multiflora*, *C. schoenanthus* combined with *L. multiflora*, *E. camaldulensis* combined with *L. multiflora* and *C. schoenanthus* combined with *E. camaldulensis* were most effective against sesame pathogenic fungi. These treatments significantly inhibited radial mycelial growth of fungal isolates and also reduced contamination by *Fusarium* spp. in seed treatments. However, their use at different doses to assess phytotoxicity on sesame seeds showed that the 0.5% dose of these EOs was suitable for seed viability and even good physiological plant growth. Application of these promising products to naturally contaminated sesame seeds showed that these oils were able to exert their antifungal activity against several fungi present. Given the efficacy of these products in treating sesame seeds, which resulted in a reduction in the effects of *Fusarium* spp. and an antifungal effect superior to that of Calthio C, it would be essential to evaluate the efficacy of these essential oils in a real environment in order to consider this alternative as a means of reducing the use of synthetic chemical pesticides.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author

Disclaimer (Artificial intelligence)

Option 1:

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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