RESEARCH ARTICLE

In vivo assessment of fungicides and oils against *Macrophomina phaseolina* and *Fusarium equiseti*

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ABSTRACT

Fusarium equiseti and *Macrophomina phaseolina* are major fungal agents associated with soya. In response to the use of synthetic pesticides and their corollaries, the use of essential oils is an alternative in the fight against these pathogens. Isolates of *Fusarium equiseti* and *Macrophomina phaseolina* were used to inoculate soybean seedlings of the G196 variety. The experimental set-up used was a factorial block with five modalities and three levels (doses) repeated 3 times. After inoculation, foliar sprays containing copper hydroxide, mancozeb, essential oils of *Cymbopogon citratus*, *Lippia multiflora* and *Azarachdica indica* were applied every fortnight. The results showed that all infected plants showed disease symptoms with low and medium-low severity levels on plants treated with fungicides and essential oils compared with the untreated control (severity: 9). Treatments based on *Cymbopogon citratus* oil (5.66-1.16) and *Lippia multiflora* (7-1) recorded the lowest scores for average severity on *Fusarium equiseti*. Mancozeb (6.5-1.5) obtained the lowest mean severity scores on *Macrophomina phaseolina*. Mancozeb and *Cymbopogon citratus* were very effective as curative treatments at lower doses. Based on these results, *Cymbopogon citratus*, *Lippia multiflora, and mancozeb could be considered effective active ingredients for controlling Fusarium equiseti* and *Macrophomina phaseolina* on soybean under real conditions.

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INTRODUCTION

Soybn (*Glycine max* [L.] Merr.) is an important legume whose seeds are used as food and feed and are a source of edible oil (Hartman *et al.*, 2011). It plays a crucial role in sustainable agriculture thanks to its ability to enrich the soil with nitrogen through biological fixation, which reduces dependence on chemical fertilisers and reduces their impact on soil quality as well as the greenhouse gas emissions associated with their production and use (Stagnari *et al.*, 2017).In Burkina Faso, soya production is expanding rapidly and is the fourth most important cash crop after cotton, groundnuts and sesame. (MAAH, 2016). Over the past ten years, national soybean production has risen from 31,314 tonnes in 2018 to 129,225 tonnes in 2023 (DSSE/DGESS/MARAH, 2024). The area sown has increased from 28,206 ha in 2018 to 122,794 ha in 2023 (DSSE/DGESS/MARAH 2024). Despite its food, industrial and socio-economic importance, soya production faces various biotic and abiotic constraints. Pests and diseases are the most damaging and can cause significant production losses, in some cases amounting to more than 30%. (PDCA, 2019).

Soybean diseases reduce yields by 10 to 30% in most production areas and represent a real threat (Akem, 1992). The genera *Cercospora, Colletotrichum, Fusarium and Macrophomina* are the main causes of soybean fungal diseases (Akem, 1992). The genus Fusarium is one of the most important pathogenic fungi, as it contains not only numerous plant pathogens, but also mycotoxin producers and is considered to be an opportunistic pathogen of humans (Ma *et al.*, 2013). These aforementioned fungal genera generally cause vascular wilt, cankers and root and stem rots (Burgess and Bryden, 2012). Various control methods have been used to reduce the impact of these pathogens. The most widely used remains chemical control. However, the question of their lasting effectiveness and their negative impact on the adjacent environment remains. In view of this situation and the growing demand for soya throughout the world, particularly in Burkina Faso, an effective and environmentally friendly control alternative is needed. The use of essential oils to combat plant pathogens is expanding rapidly throughout the world (Yarou *et al.*, 2017 ; Toundou *et al.*, 2020 ; Sirima *et al.*, 2020). In soybean in particular, very few studies have focused on the efficacy of essential oils against pathogens. This study aims to test the efficacy of two

essential oils against the major fungal agents of soybean, namely *Fusarium equiseti* and *Macrophominaphaseolina* in Burkina Faso.

MATERIALS AND METHODS

Study site: This study was conducted at the phytopathology/mycology laboratory of the Farako-Bâ research station of the Institut de l'environnement et de recherchesagricoles (INERA). The station is located in western Burkina Faso, precisely 10 km south-west of the town of Bobo-Dioulasso on the Bobo-Banfora road. It lies between 04° 20' 00" West and 11° 60' 00" North (Figure 1).
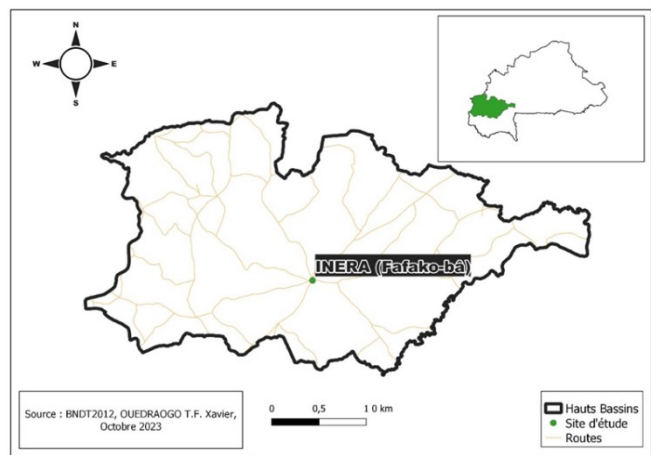

Figure 1. Map of the study area. Ouedraogo (2023)

Plant material: The plant material used was the Yellow G196 (SRA 26/72) variety, which comes from Senegal, and was popularised in Burkina Faso in 2005. It has a 105-115 day cycle and a potential yield of 2 to 2.5 t/ha.

Fungal material: Strains of *Macrophomina phaseolina* and *Fusarium equiseti* were used. They were isolated in 2021 from soybean organs by Ouedraogo *et al.* (2024).

Chemical fungicides: Two synthetic fungicides were used in the study. These were copper hydroxide (*65.5% Copper hydroxide, WP*) and mancozeb *(Mancozeb, 800 g/Kg, WP)* (Table I),with the characteristics given in Table I. These pesticides are on the Sahelian Pesticides Committee (CSP) list approved for the 2028 season.

Table I. Characteristics of the fungicides tested

N°	Composition	Dosage	Nature
1	65.5% Copper hydroxide, WP	3 kg /ha	Contact
2	Mancozeb, 800 g/Kg, WP	2 kg/ha	Contact

Legend: WP: wettablepowder

Essential oils: Three essential oils were used. The characteristics of the oils tested are given in Table II.

Table II. Chemical composition of essential oils tested

Essential oils	Chemical composition	Origin
*Cymbopogoncitratus*oil	Thymo (30%), Acetate (20.8%), γ-Terpinene (3.1%), Limonene	IRSAT
*Lippiamultiflora*oil	Citral, 1,8-Cineol, Linalol, α-terpineol,Thymol, phenol	IRSAT
*Azadirachta indica*oil	Azadirachtin, Nimbidinin, Meliantriol, Nimbidin, Solanine, fatty acids	IRSAT

Methods

Table III: Characteristics of the various treatments

Treatments	Product name	g or ml/L
TNT	Untreated control	0
T1HD1	Copper hydroxide	7.5 g
T2HD2	Copper hydroxide	10 g
T3HD3	Copper hydroxide	12.5 g
T1MD1	Mancozeb	5.1 g
T2MD2	Mancozeb	6.7 g
T3MD3	Mancozeb	8.4 g
T1CD1	*Cymbopogoncitratus*oil (CC)	2 ml
T2CD2	*Cymbopogoncitratus*oil (CC)	3 ml
T3CD3	*Cymbopogoncitratus*oil (CC)	4 ml
T1LD1	*Lippiamultiflora*oil (LM)	2 ml
T2LD2	*Lippiamultiflora*oil (LM)	3 ml
T3LD3	*Lippiamultiflora*oil (LM)	4 ml
T1AD1	*Azadirachta indica*oil (AI)	2 ml
T2AD2	*Azadirachta indica*oil (AI)	3 ml
T3AD3	*Azadirachta indica*oil (AI)	4 ml

Experimental set-up: The experimental setup used to assess the products' efficacy in vivo was a factorial block-split-plot design. The set-up consisted of products (fungicides and essential oils) with 5 modalities and strains of pathogenic fungi (*Macrophomina phaseolina* and *Fusarium equiseti*), all repeated 3 times. The characteristics of the different treatments are given in Table III.

Setting up and running the trial: To ensure rigorous asepsis, the soybeans were treated with a three-stage chemical disinfection process. First, the beans were immersed in a 75% hydroalcoholic solution for 30 seconds. Next, they were treated for 30 seconds in a 1% sodium hypochlorite solution to eliminate fungal spores. Finally, the seeds were rinsed thoroughly with sterile distilled water. The sterile seeds were sown to a depth of 5 cm in 15 cm high pots containing an autoclaved substrate. This substrate was composed of a homogeneous mixture of potting soil, garden soil and organic matter (compost). Each pot was sown with five seeds.

Preparation of inoculum and inoculation of soybean plants: Pure strains of *F. equiseti* and *M. phaseolina* were grown on PDA medium. These strains were purified on PDA medium. After a growth phase of 14 to 20 days, the conidia were recovered by washing, filtered to remove debris and then adjusted to a concentration of 10^5 conidia/ml in a 0.5% aqueous gelatin solution (Kassankogno *et al.*, 2021). Seedlings (3-4 leaf stage) were inoculated in the afternoon by foliar spraying using a 1-litre hand sprayer. The control treatment was sprayed with sterile distilled water only. (Kassankogno et al., 2021). A Levoit LV600 HH Hybrid Ultrasonic Humidifier was used to maintain high humidity (75-100%) in the culture chamber for the inoculated plants. The inoculated seedlings were placed under controlled conditions in a greenhouse equipped with a mosquito net to prevent any arthropod infestation. The plants were monitored regularly, starting 48 hours after inoculation. Symptoms were assessed visually and recorded at regular intervals.

Preparing the different treatment doses: To assess the effect of different concentrations, three doses were defined for each treatment. For synthetic fungicides, the doses were 75% (D1), 100% (D2, i.e. 10g/L) and 125% (D3) of the recommended dose. For essential oils, the doses corresponded to 67% (D1), 100% (D2, i.e., 3 ml/L), and 133% (D3) of the normal dose. Treatments were applied by foliar spray 48 hours after inoculation, using a laboratory micro-sprayer (1 litre capacity). Each pot received approximately 6.5 mL of fungicidal spray or essential oil, supplemented with sterile distilled water and Tween 20. A negative control was performed using sterile distilled water only.

Assessing the incidence and severity of disease and the effectiveness of products: The incidence of each disease was determined using the method of Kassankogno *et al* (2021) : I (%)= $\frac{(n1-n2)}{n1 \times 100}$ with n1: the total number of plants, n2: the number of

symptomless plants. The severity of each disease was assessed by assigning severity scores according to the severity scale of Vales (1992) rated from 1 to 9. Slight modifications were made to assess the products' efficacy (Table IV).

Evaluation of the air of disease progression according to treatments and data collection: The efficacy of each product was assessed using the area under the disease progress curve (AUDPC). This index represents the cumulative foliar severity of the disease over the observation period. It is used to assess the effectiveness of each dose and each product. The AUDPC was calculated using the formula of Shaner and Finney (1977) : $AUDPC = \sum \left[\left\{ \frac{(Yi+Y)}{2} \right\} x (ti + n - ti) \right]$ with : Yi and Yi+1 are the severity of each disease expressed as a percentage of diseased leaf area (DLA) and observed at times ti+1 and ti respectively; (ti+1 - ti) represents the time interval in days between two observations. *In vivo*, data were collected on the severity and incidence of each disease at $5^{\text{ème}}$, $8^{\text{ème}}$, $11^{\text{ème}}$ and $14^{\text{ème}}$ DAI.

Data analysis: The collected data were entered and processed in Excel 2016, and the graphs were produced. ANOVA was used to analyse variance using R 4.3.1 software. Means were compared using the Tukey test.

RESULTS

Evaluation of the efficacy of products on fungi *in vivo*: The results of this study revealed symptoms of *F. equiseti* and *M. phaseolina* on soybean leaves after inoculation (Figure 2). The appearance of symptoms shows that the fungus is pathogenic for soybeans. The strain caused yellowing, small yellowish and brownish spots, leaf lesions, and leaf drop. All infected plants showed disease symptoms, and by the third assessment, the incidence had progressively decreased across all doses except the control.

Figure 2. Symptoms of *Macrophomina phaséolina* (A) and *Fusarium equiseti*(B) on soybean

Comparison of the incidence and severity of *Fusarium equiseti* in the curative treatment of synthetic products: Comparison of the means for severity and symptom incidence induced by Fusarium equiseti at the $5^{\text{ème}}$ and $14^{\text{ème}}$ days after inoculation (DAI) showed a very highly significant difference at the 5% probability threshold between the treatments. (Table V). At dose 1 of each substance, all infected plants showed disease symptoms at the $5^{\text{ème}}$ JAI. At the $14^{\text{ème}}$ DAI, the lowest statistically significant incidence was observed with copper hydroxide (50%). As for severity, from $5^{\text{ème}}$ to 14 DAI, the lowest score was recorded with copper hydroxide (6.3 to 3.2). At dose 2 of each product tested, the incidence of symptoms at $5^{\text{ème}}$ DAI was 100%. However, at $14^{\text{ème}}$ DAI, the incidences varied statistically. The lowest incidence was recorded with copper hydroxide, at 44%. The severity assessed at $5^{\text{ème}}$, $8^{\text{ème}}$, $11^{\text{ème}}$ DAI, shows that copper hydroxide recorded the lowest score, which is 5.33, 4.33, 4.00 and 2.6, contrary to mancozeb. At dose 3 of each product tested, the incidence of symptoms at $5^{\text{ème}}$ DAI was 100%. On the other hand, the

incidences varied statistically at $14^{\text{ème}}$ DAI. The lowest rates were recorded with copper hydroxide, at 33 and 33% respectively. As for severity, from $5^{\text{ème}}$ to $14^{\text{ème}}$ DAI, the lowest score was recorded with copper hydroxide from 4.6 to 2.2.

Comparison of the incidence and severity of *Fusarium equiseti* in the curative treatment of essential oils: Comparison of the means for severity and symptom incidence induced by Fusarium equiseti at 5 and 14 days after inoculation (DAI) showed a very highly significant difference at the 5% probability threshold between the essential oil treatments. (Table VI). At dose 1 of each substance, the incidence of symptoms induced by the fungus was 100% at 5th DAI. At 14th DAI, the lowest statistically significant incidence was observed with the essential oils of *C. citratus* and *L. multiflora* (38.88 ± 9.62). As for severity, at 5th, 8th, and 11th days, the lowest score was recorded with *C. citratus* oil (5.66, 4.5, 3.6). Similarly, at 14th DAI, the lowest scores of 2.00 and 2.16 were obtained with *C. citratus* and, *L. multiflora*, respectively.

At dose 2 of each product tested, the incidence of symptoms at 5thDAI was 100%. However, at 14th DAI the incidences varied statistically. The lowest rates were recorded with *C. citratus* and *L. multiflora*, at 33, 3 and 33.3% respectively. As for severity, from 5 to 11th DAI, the lowest score was recorded with *C. citratus* (5.8, 3.6, and 2.7). By the 14th DAI, the essential oils of *C. citratus* and *L. multiflora* had the lowest scores of 1.6 and 1.66, respectively. At dose 3 of each product tested, the incidence of symptoms at 5thDAI was 100%. However, at 14th DAI the incidences varied statistically. The lowest rates were recorded for C. citratus and L. multiflora at 33, 3, and 33.3%, respectively. As for severity, from 5 to 8th DAI the lowest score was recorded with *C. citratus* (4.8; 3.2). From 11 to 14th DAI, the essential oils of *C. citratus* (2.16; 1.16) and *L. multiflora* (1.6; 1.00) obtained the lowest scores, respectively.

Effect of doses of synthetic fungicides on the incidence and severity of symptoms induced by *Macrophomina phaseolina*: Comparison of the means of the severity and incidence of symptoms induced by *Macrophomina phaseolina* at 5 and 14th days after inoculation (DAI) showed a very highly significant difference ($p < 2e^{-16}$) at the 5% probability threshold between treatments. (Table VII). At dose 1 of each substance, the incidence of fungus-induced symptoms was 100% at $5^{\text{ème}}$ DAI. At 14 days, the lowest statistically significant incidence was observed with mancozeb (50%). As for severity, from 5 to 14th DAI the lowest score was recorded with mancozeb (6.5 to 2.5). At dose 2 of each product tested, the incidence of symptoms at 5thDAI was 100%.

However, at 14th DAI, the incidences varied statistically. The lowest incidence was recorded with mancozeb, at 50%. The severity assessed at 5,8, 11 and 14th DAI, shows that copper hydroxide recorded the lowest score of 5.5, 5.0, 3.6; 2.00 in contrast to copper hydroxide. At dose 3 of each product tested, the incidence of symptoms at 5thDAI was 100%. However, at 14th DAI, the incidences varied statistically. The lowest incidences were recorded with mancozeb and copper hydroxide, at 50% respectively. As for severity, from 5th to 14th DAI the lowest score was recorded with mancozeb, from 5 to 1.5.

Effect of essential oil doses on the incidence and severity of symptoms induced by *Macrophomina phaseolina*: Comparison of the means for the severity and incidence of symptoms induced by *Macrophomina phaseolina* at the 5ème and 33ème days after inoculation (DAI) showed a very highly significant difference at the 5% probability threshold ($p < 2e^{-16}$) between the essential oil treatments. (Table IIX). At dose 1 of each substance, the incidence of symptoms induced by the fungus was 100% at 5th DAI. At 14 au 33th DAI, the lowest statistically different incidence was obtained with the essential oil of *C. citratus* and *L. multiflora* (50%). As for severity, at 5, 14 and 24th days, the lowest score was obtained with *C. citratus* oil (6.5, 5.00, 4.00). Similarly, at the 14th DAI, low scores of 4.6 and 4.5 were obtained with *C. citratus* and *L. multiflora* respectively. At dose 2 of each product tested, the incidence of symptoms at $5^{\text{ème}}$ DAI was 100%. However, at 14 and 33th DAI, the incidences varied

Table IV. Adapted scale for assessing the severity and efficacy of fungicides

Severity rating	Types of symptoms	Number of spots/Lesions	Induced reaction	Level of product efficacy
1	No symptoms	0	R	Very good efficiency (TBE)
2	b rare	<10	R	Very good efficiency (TBE)
3	bmany	≥10	R	Good efficiency (BE)
4	g rare	<10	MR	Averageefficiency (ME)
5	gmany	≥10	MS	Low efficiency (FE)
6	rarepbg	<10	MS	Low efficiency (FE)
7	Pbg/q many	≥10	S	Very lowefficiency (TFE)
8	pqbg rare	<10	TS	Very lowefficiency (TFE)
9	pqbgmany	≥10	TS	Very lowefficiency (TFE)

Table V. Comparison of the average severity and incidence of *Fusarium equiseti* when treated with synthetic fungicides

Doses	Valuation date	TNT (control)	Copper hydroxide	Mancozeb	Pr>F	Meaning
D1	Im1	100	100	100	0.479	NS
	Im2	100	100	100	0.479	NS
	Im3	100	83.49 ±16.673ab	72.55 ±19.53ab	0.0146	S
	Im4	100 ± 0.000a	50 ± 0.00e	83.33 ± 0.00b	1.7e-15	THS
	Sm1	8.5 ± 0.000a	6.33 ± 0.00e	7 ± 0.00c	<2e-16	THS
	Sm2	9 ± 0.000a	5.66 ± 0.00ef	6.66 ± 0.00d	<2e-16	THS
	Sm3	9 ± 0.000a	5 ± 0.00d	5.5 ± 0.00d	<2e-16	THS
	Sm4	9 ± 0.000	3.22 ±0.381h	4.67 ±0.289e	<2e-16	THS
	NE	-	**BE**	**EM**	-	-
D2	Im1	100	100	100	0.479	NS
	Im2	100	100	100	0.479	NS
	Im3	100	83.33 ±16.67ab	72.55 ±19.532ab	0.0146	S
	Im4	100	44.44 ±9.624e	66.66 ± 0.00cd	1.7e-15	THS
	Sm1	8.5 ± 0.00a	5.33 ± 0.00 h	6.66 ± 0.00d	<2e-16	THS
	Sm2	9 ± 0.00a	4.33 ± 0.00 gh	6 ± 0.00e	<2e-16	THS
	Sm3	9 ± 0.00a	4.00 ± 0.00 e	5 ± 0.00d	<2e-16	THS
	Sm4	9 ± 0.00a	2.667i ±0.289	4.22 ±0.381f	<2e-16	THS
	NE	-	**TBE**	**EM**	-	-
D3	Im1	100	100	100	0.479	NS
	Im2	100	100	100	0.479	NS
	Im3	100	72.383± 9.913ab	72.55 ± 19.532ab	0.0146	S
	Im4	100	33.33 ± 0.00 e	61.10 ± 9.619d	1.7e-15	THS
	Sm1	8.5 ± 0.00a	4.66 ± 0.00 j	6 ± 0.00f	<2e-16	THS
	Sm2	9 ± 0.00a	4.00 ± 0.00 hi	5.5 ± 0.00f	<2e-16	THS
	Sm3	9 ± 0.00a	3.66 ± 0.00 ef	5 ± 0.00d	<2e-16	THS
	Sm4	9 ± 0.00a	2.22 ± 0.381ij	3.77 ± 0.196g	<2e-16	THS
	NE	-	**TBE**	**BE**	-	-
NEP		-	**TBE**	**EM**	-	-

Legend: THS: Very Highly Significant; NS: Not Significant; DAI: Day After Infection; Im: Average Incidence; Sm: Average Severity; D: Dose. NE: Level of efficacy; NEP: Level of efficacy of the product; FE: Low efficacy; BE: Good efficacy; EM: Average efficacy; TFE: Very low efficacy; TBE: Very good efficacy; IM: Average incidence; Sm: Average severity. Means followed by the same letters in the same column are not significantly different according to the Tukey statistical test at the 5% threshold.

Table VI. Comparison of the average severity and incidence of *Fusarium equiseti* in essential oil treatments

Doses	Valuation date	*Cymbopogon citratus* oil	*Lippia multiflora* oil	*Azadirachta indica* oil	Pr>F	Significant
D1	Im1	100	100	100	0.479	NS
	Im2	100	100	100	0.479	NS
	Im3	55.55 ± 19.24ab	55.55 ± 9.61ab	78.10 ±9.91ab	0.0146 *	S
	Im4	38.87 ± 9.62e	38.87 ±9.62e	77.94 ±9.77bc	1.7e-15	THS
	Sm1	5.66 ± 0.00 g	7 ± 0.00c	8 ± 0.00b	<2e-16	THS
	Sm2	4.5 ± 0.00g	5.2 ±0.34f	7.33 ±0.28bc	<2e-16	THS
	Sm3	3.67 ±0.28ef	3.83 ±0.57ef	7.22 ±0.485b	<2e-16	THS
	Sm4	2 ± 0.00jk	2.16 ±0.28ijk	7 ± 0.00b	<2e-16	THS
	NE	**TBE**	**TBE**	**TFE**	-	-
D2	Im1	100	100	100	0.479	NS
	Im2	100	100	100	0.479	NS
	Im3	49.97 ±16.65b	55.55 ± 9.6ab	78.10 ±9.91ab	0.0146 *	S
	Im4	33.33 ± 0.00e	33.33 ± 0.00e	72.38 ±9.91bcd	1.7e-15	THS
	Sm1	5.83 ± 0.28f	6.887 ±0.19c	8.5 ± 0.00a	<2e-16	THS
	Sm1	3.67± 0.00i	5.5 ±0.500f	7.5 ± 0.00b	<2e-16	THS
	Sm2	2.77 ±0.47g	3.33 ±0.28f	6.87 ±0.19bc	<2e-16	THS
	Sm4	1.60 ±0.09k	1.67 ±0.289jk	6 ± 0.00c	<2e-16	THS
	NE	**TBE**	**TBE**	**FE**	-	-
D3	Im1	100	100	100	0.479	NS
	Im2	100	100	100	0.479	NS
	Im3	55.55 ±19.24ab	55.55 ± 9.61ab	72.55 ±19.53ab	0.0146*	S
	Im4	33.33 ± 0.00e	33.33 ± 0.00e	72.38 ±9.91bcd	1.7e-15	THS
	Sm1	4.87± 0.19i	6 ± 0.00f	8 ± 0.00b	<2e-16	THS
	Sm2	3.22 ±0.38j	5.16 ±0.28f	7 ± 0.00cd	<2e-16	THS
	Sm3	2.16 ±0.28h	2.16 ±0.28h	6.5 ± 0.00c	<2e-16	THS
	Sm4	1.16 ±0.28l	1 ± 0.00l	5.22 ±0.38d	<2e-16	THS
	NE	**TBE**	**TBE**	**FE**	-	-
NEP		**TBE**	**TBE**	**FE**		

Legend: THS: Very Highly Significant; NS: Not Significant; DAI: Day After Infection; Im: Average Incidence; Sm: Average Severity; D: Dose. NE: Level of efficacy; NEF: Level of product efficacy; FE: Low efficacy; BE: Good efficacy; EM: Average efficacy; TFE: Very low efficacy; TBE: Very good efficacy. Means followed by the same letters in the same column are not significantly different according to the Tukey statistical test at the 5% threshold.

Table VII. Comparison of the average severity and incidence of *Macrophomina phaseolina* in the treatment of synthetic fungicides

Doses	Valuation date	*Macrophomina phaseolina*			Pr>F	Meaning
		TNT (control)	Copper hydroxide	Mancozeb		
D1	Im1	100	100	100	0.479	NS
	Im2	100	100	100	0.479	NS
	Im3	100	83.83 ±0.00	50 ±0.00	<2e-16	THS
	Im4	100	83.83 ±0.00	50 ±0.00	<2e-16	THS
	Sm1	8.33 ±0.00a	8 ±0.00c	6.5 ±0.00g	<2e-16	THS
	Sm2	9 ±0.00a	7 ±0.00b	5.66 ±0.00d	<2e-16	THS
	Sm3	9 ±0.00a	5 ±0.00d	4 ±0.00e	<2e-16	THS
	Sm4	9 ±0.00	6 ±0.00	2.5 ±0.00	<2e-16	THS
	NE	-	**FE**	**TBE**	-	-
D2	Im1	100	100	100	0.479	NS
	Im2	100	100	100	0.479	NS
	Im3	100	83.83 ±0.00	50 ±0.00	<2e-16	THS
	Im4	100	83.83 ±0.00	50 ±0.00	<2e-16	THS
	Sm1	8.33 ±0.00a	7.66 ±0.00d	5.5 ±0.00j	<2e-16	THS
	Sm2	9 ±0.00a	6 ±0.00c	5 ±0.00d	<2e-16	THS
	Sm3	9 ±0.00a	6 ±0.00c	3.66 ±0.00f	<2e-16	THS
	Sm4	9 ±0.00	5.66 ±0.00	2 ±0.00	<2e-16	THS
	NE	-	**FE**	**TBE**	-	-
D3	Im1	100	100	100	0.479	NS
	Im2	100	100	100	0.479	NS
	Im3	100	66.66 ±0.00	50 ±0.00	<2e-16	THS
	Im4	100	50 ±0.00	50 ±0.00	<2e-16	THS
	Sm1	8.33 ± 0.00a	6.66 ±0.00g	5 ±0.00k	<2e-16	THS
	Sm2	9 ±0.00a	6.66 ±0.00c	3.66 ±0.00e	<2e-16	THS
	Sm3	9 ±0.00a	6 ±0.00c	2 ±0.00g	<2e-16	THS
	Sm4	9 ±0.00a	4.66 ±0.00	1.5 ±0.00	<2e-16	THS
	NE	-	**EM**	**TBS**	-	-
NEP		-	**FE**	**TBE**	-	-

Legend: ***THS**: Very Highly Significant; **NS**: Not Significant; **DAI**: Day After Infection; **Im**: Average Incidence; **Sm**: Average Severity; **D**: Dose. **NE**: Level of efficacy; **NEF**: Level of product efficacy; **FE**: Low efficacy; **BE**: Good efficacy; **EM**: Average efficacy; **TFE**: Very low efficacy; **TBE**: Very good efficacy. Means followed by the same letters in the same column are not significantly different according to the Tukey statistical test at the 5% threshold.*

Table IIX. Comparison of average severity and incidence of *Macrophomina phaseolina* in essential oil treatments

Doses	Valuation date	*Cymbopogon citratus* oil	*Lippia multiflora* oil	*Azadirachta indica* oil	Pr>F	Meaning
D1	Im1	100	100	100	0.479	NS
	Im2	100	100	100	0.479	NS
	Im3	50 ±0.00	100	100	<2e-16	THS
	Im4	50 ±0.00	50 ±0.00	66.66 ±0.00	<2e-16	THS
	Sm1	6.5 ±0.00g	7 ±0.00f	8 ±0.00c	<2e-16	THS
	Sm2	5 ±0.00d	6 ±0.00c	7 ±0.00b	<2e-16	THS
	Sm3	4 ±0.00e	4.5 ±0.00e	6.66 ±0.00c	<2e-16	THS
	Sm4	4.6 ±0.00	4.5 ±0.00	7 ±0.00	<2e-16	THS
	NE	**EM**	**EM**	**TFE**	-	-
D2	Im1	100	100	100	0.479	NS
	Im2	100	100	100	0.479	NS
	Im3	100	100	100	<2e-16	THS
	Im4	66.66 ±0.00	66.66 ±0.00	50 ±0.00	<2e-16	THS
	Sm1	6.66 ±0.00g	6.66 ±0.00g	8.5 ±0.00a	<2e-16	THS
	Sm2	6 ±0.00c	5 ±0.00d	7.5 ±0.00b	<2e-16	THS
	Sm3	5.5 ±0.00d	3.5 ±0.00f	7 ±0.00b	<2e-16	THS
	Sm4	5 ±0.00	3 ±0.00	7 ±0.00	<2e-16	THS
	NE	**FE**	**BE**	**TFE**	-	-
D3	Im1	100	100	100	0.479	NS
	Im2	100	100	100	0.479	NS
	Im3	100	50 ±0.00	83.83 ±0.00	<2e-16	THS
	Im4	50 ±0.00	33.33 ±0.00	66.66 ±0.00	<2e-16	THS
	Sm1	5.66 ±0.00i	6 ±0.00h	7.50 ±0.00e	<2e-16	THS
	Sm2	5 ±0.00d	5.5 ±0.00d	7 ±0.00b	<2e-16	THS
	Sm3	4.66 ±0.00e	4 ±0.00b	7 ±0.00b	<2e-16	THS
	Sm4	3 ±0.00	2.5 ±0.00	6.66 ±0.00	<2e-16	THS
	NE	**BE**	**TBE**	**FE**	-	-
NEP		**EM**	**BE**	**FE**	-	-

Legend: VHS: Very Highly Significant; NS: Not Significant; DAI: Day After Infection; Im: Average Incidence; Sm: Average Severity; D: Dose. NE: Level of efficacy; NEF: Level of product efficacy; FE: Low efficacy; BE: Good efficacy; EM: Average efficacy; TFE: Very low efficacy; TBE: Very good efficacy. Means followed by the same letters in the same column are not significantly different according to the Tukey statistical test at the 5% threshold.

statistically. The lowest incidence was recorded with *Azadirachta indica* at 50%, followed by *C. citratus* and *L. multiflora* at 66.66%. As for severity, from 5 to 33th DAI, the lowest score was recorded with *L. multiflora* (6.6, 5.00, 3.5 and 3.00). At dose 3 of each product tested, the incidence of symptoms at 5ème DAI was 100%. However, at the 14th DAI, the incidences varied statistically. The lowest incidence was recorded with *L. multiflora*, at 33.3%. As for severity, from 5 to 14th DAI, the lowest score was recorded with *C. citratus* (5.6; 5). From 14 to 33rd DAI, the essential oil of L. multiflora (2.16; 1.16) and *L. multiflora* (1.6; 1.00) obtained the lowest scores of 4 and 2.5, respectively.

Assessment of the evolution of symptoms induced by *Fusarium equiseti*: The efficacy of the products was assessed through changes in the area under the disease progression curve (AUDPC). The evolution of symptoms was a function of the product doses tested (Figure 3). The untreated control and the *A. indica* oil treatment recorded the highest AUDPC values among the products. The *C. citratus* oil treatment recorded the lowest values compared with the other products. It obtained AUDPC values of 45.52, 40.66, and 33.93 for the D1, D2, and D3 doses, respectively. Copper hydroxide and *L. multiflora* oil showed almost identical levels of control, with values ranging from 56.54 to 40.79. The copper hydroxide treatment obtained values of 56.54, 45.59 and 40.79, corresponding to the D1, D2 and D3 doses, respectively. The *A. indica* oil-based treatment obtained values of 53.03, 51.76 and 44.02, corresponding to the D1, D2 and D3 doses, respectively. In addition, the Mancozeb-based treatment recorded values of 65.46, 60.3 and 55.71 for doses D1, D2 and D3 respectively.
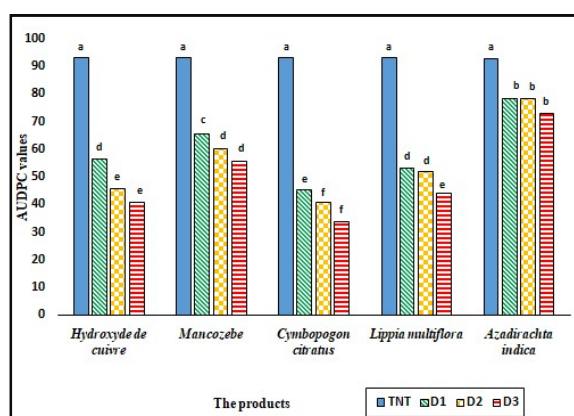

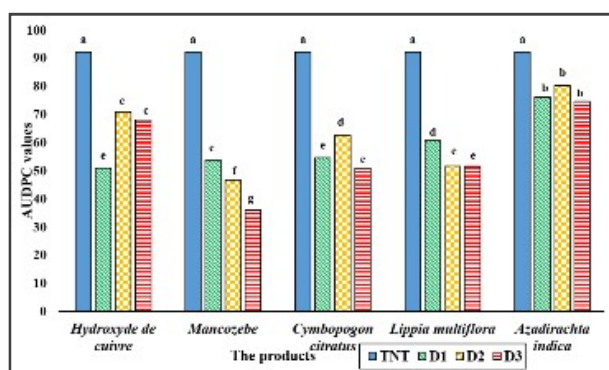**Figure 3. AUDPC value of symptoms induced by *Fusarium equiseti* as a function of product treatment doses**

Figure4: Comparison of AUDPC for anthrax depending on products and doses

Comparison of the area of progression of disease in curative treatment: A comparison of disease evolution showed variation by product and dose level. The AUDPC of the disease was higher in the untreated control (AUDPC = 92.23) than in the other treatments (Figure 4). Among the treatments, the one with the highest AUDPC values was the *A. indica* oil-based treatment. These values were 76.11, 80.25 and 74.6 respectively for doses D1, D2 and D3. However, the Mancozeb-based treatment recorded the lowest AUDPC values. These values were 53.79 ; 46.67 and 35.9 for doses D1, D2

and D3 respectively. In general, AUDPC was higher at dose D2 across all products than at other doses.

DISCUSSION

Effect of substances on the incidence and severity of *Fusarium equiseti* and *Macrophomina phaseolina*: The results of this study revealed the presence of symptoms of F. equisetiand M. phaseolinaon soybean leaves after inoculation. The appearance of symptoms shows that the fungus is pathogenic for soya. The strain caused yellowing, small yellowish-brown spots, leaf lesions, and leaf drop. All infected plants showed symptoms of the disease and then, from the third evaluation onwards, the incidence progressively decreased for all doses except the control. The average severity of fusariosis and anthrax varied from 5ème to 14ème JAI. The average severity scores for fusariosis ranged from 6.33 to 2.22 for treatments based on copper hydroxide, from 7 to 3.77 for Mancozeb, from 5.66 to 1.16 for treatments based on *C. citatus* oil, from 7 to 1 for *L. multiflora* oil, from 8 to 5.22 for *A. indica* oil. The average anthrax severity scores were 8 to 4.66 for copper hydroxide treatments, 6.5 to 1.5 for Mancozeb, 6.5 to 3 for *C. citratus* oil, 7 to 2.5 for *L. multiflora* oil and 8 to 6.66 for *A. indica* oil. These results show that *C. citratus* and *L. multiflora* oil considerably reduced the severity of both diseases. In contrast, *A. indica* oil showed little reduction in the severity of these diseases. Copper hydroxide and Mancozeb reduced the severity of the diseases. These results suggest that *C. citratus* and *L. multiflora* oil are effective biofungicides because of their ability to considerably slow the development of these diseases by acting on the dynamics of the fungi concerned. Indeed, Montel (2005) showed that 1% essential oil of *C. citratus* completely prevented the development of *B. oryzae* and that 1% essential oil of *Lippia multiflora* completely prevented the development of *B. oryzae*. 1.5% inhibited all mycelial production by *B. oryzae* and *F. moniliforme*. In addition, Kabore *et al.* (2007) found that the essential oils of *L. multiflora, C. citratus, C giganteus* and *Ocimum bassilicum* could inhibit 100% of the mycelial growth of *Pyricularia oryzae, Bipolaris oryzae* and *Fusarium moniliforme*. Somda and Leth (2007) also showed that the essential oil of *C. citratus* was effective against *P. sorghina* and *F. moniliforme* as a sorghum seed treatment. The results also showed that copper hydroxide and Mancozeb are effective active ingredients for controlling these diseases in semi-controlled environments. Our results are similar to those of Kassankogno *et al.* (2022) who found that Mancozeb was effective from its lower dose as a preventive and curative treatment. It caused a low severity (1.33 - 2.66) of curvulariosis, one of the main fungal diseases of rice in Burkina Faso. Indeed, Tonon *et al.* (2017) also showed that the application of Mancozeb reduced the incidence and severity (by 86% and 90.4%) of anthracnose compared with untreated cashew leaves in Benin. Our results are similar to those of Bouet *et al.* (2020) who showed that treatment with COGA 80 WP (Mancozeb) maintained the severity of helminthosporiosis in rice at an average rating of 2. The results of our work show that the AUDPC for fusariosis and anthrax varied according to dose and product. It remained high for the control for each disease, with values of 92.81 and 92.23 for *Fusarium equiseti* and *Macrophomina phaseolina* respectively. At dose D3, *C. citratus* oil recorded the lowest AUDPC value (33) for control of *Fusarium equiseti*. Mancozeb recorded the lowest AUDPC value (35.9) in the control of *Macrophomina phaseolina* compared with the other products. These data show that *C. citratus*oil and Mancozeb were the most effective in controlling *Fusarium equiseti* head blight and *Macrophomina phaseolina*, respectively. They were effective at the high dose. Our results are in agreement with those of Hassikou *et al* (2002) who showed that the application of a high dose of Tricyclazole increased the control of curvulariosis in rice. Copper hydroxide and *L. multiflora* oil showed an almost similar level of control of fusarium wilt with values ranging from 56.54 to 40.79. These data show that these products are equally effective against fusariosis. Our results also show that *L. multiflora* oil obtained the same AUDPC index at dose D2 (51.72) and D3 (51.56). Both doses of this oil were equally effective in controlling *Macrophomina phaseolina*. *A. indica* oil recorded the highest AUDPC value for both diseases. At all doses, this oil was unable to effectively halt the progression of either disease.

Taken together, these results confirm that AUDPC is a good indicator of the effectiveness with which each product can impact the development of these two diseases.

CONCLUSION

The general objective was to contribute to the management of two major fungi, *F. equiseti* and *M. phaseolina*, of soya in Burkina Faso. The study results provided information on the efficacy of the tested products and doses. *In vivo* tests revealed that all the plants inoculated showed symptoms. After applying the substances at different doses to the inoculated plants, it was found that Mancozeb was the most effective against *M. phaseolina* at all doses. *C. ciratus*oil (doses: 2, 3, and 4ml), L. multiflora oïl (doses: 2, 3 and 4ml) and copper hydroxide (doses: 10g and 12g/L) were the most effective on *F. equiseti*. The study should continue by assessing the effectiveness of these products on seed yield and quality.

ACKNOWLEDGMENTS

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