"Essential oils as a control alternative of *Fusarium falciforme*, responsible of onion (*Allium cepa* L.) basal rot"

Orininal

Abstract

In Burkina Faso, onions (*Allium cepa* L.) are one of the main vegetable crops. It is a very important source of income for producers. However, its production is subject to loss, one of the main ones being disease caused by fungal pathogens, including *Fusarium falciforme*, which causes onions basal rot. This study aimed to contribute to the onion production improvement through agroecological methods by setting up a pathogenicity test and an antifungal test based on essential oils of local plants against *Fusarium falciforme*. For the pathogenicity test, a conidial suspension of each strain was produced at 1 x 10^6 conidia/ml using a haematimeter. It was carried out in the Biosciences Laboratory of Joseph KI-ZERBO University and in the greenhouse. A randomized block design with 04 replicates and 06 treatments was used. For antifungal test, the method by direct contact was used. The germination rate parameter was measured in the laboratory. The rate of emergence and damping-off were assessed in the greenhouse. The pathogenicity test results showed that the impact of *Fusarium falciforme* on onion seeds was relatively high in all pots. As regards the antifungal test, results showed a significant inhibitory effect of the essential oils used on the mycelial growth of *Fusarium falciforme*. All the essential oil concentrations of *Cymbopogon citratus* and *Cymbopogon nardus* were able to completely inhibit the fungus' growth. In contrast, the essential oil of *Eucalyptus camaldulensis* showed little mycelial growth at 1%, 2% and 3% concentrations of 1.05 to 2.85 cm, with an inhibition rate of 63.83% to 86.68%. Based on these results, biopesticide-based biocontrol of *Fusarium falciforme* is therefore possible for onions basal rot management.

Keywords:Fusariosis, biopesticide, Burkina Faso.

1. Introduction

Agriculture is a very important economic activity in Burkina Faso, providing 84% of the country's working population with a 30.3% share of gross domestic product (GDP) [1]. Vegetable production is one of the most important growth areas. Species grown include

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tomatoes, peppers, onions, and aubergines. Onion bulbs are the country's main vegetable crop. It is a very important source of income for producers and makes a significant contribution to the fight against poverty and food insecurity [2]. Its production does, however, face a host of biotic and abiotic constraints. One of the major constraints to onion production is disease caused by pathogens, mainly fungal [3]. Pathogens can cause yield losses of up to 80% [4]. Throughout the world, several species of *Fusarium* have been associated with this disease. These include *F. acuminatum*, *F. oxysporum*, *F. proliferatum*, *F. redolens*, *F. verticillioides*, *F. equiseti*, *F. solani*, *F. fujikuroi, F. culmorum, F. falciforme, F. brachygibbosum, F. acutatum, F. thapsinum* and *F. anthophilium* ([5],[6],[7],[8]). Seven species of the *Fusarium* genus are associated with this disease in Burkina Faso, including *F. falciforme*, the new agent responsible for onions basal rot.

Control methods to date have mainly involved good agricultural practices, use of resistant varieties and chemical pesticides [9]. Without resistant varieties, the use of persistent chemical pesticides is now widely criticized for its adverse effects on the environment, on our food and on consumers. Researchers are developing new, environmentally-friendly methods of control, such as the use of biopesticides and biofertilizers, to protect the environment. This study, is part of this approach. The aim of the study is to contribute to onion production improvement applying agro-ecological approaches

2. Materials et Methods

2.1. Plant Materials

The onion varieties used in the study were Violet de Galmifrom INRAN (Niger) and Prema 178 from East-West Seed company (Thaïlande).Seeds have been sold in seed shops and are widely used by producers.They were chosen because of their susceptibility to onion basal rot. They are varieties adapted to the climatic conditions of Burkina Faso, and are also produced and marketed in Burkina Faso.

2.2 Fungal materials

Two strains of *Fusarium falciforme*(*Fusarium falciforme* 29 and *Fusarium falciforme* 30) were used in this study.Strains were stored in Potato Dextrose Agar (PDA)at 4°C.These strains were supplied by the Phytopathology and Tropical Mycology Team of the Biosciences

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Laboratory at Joseph Ki-Zerbo University. They were isolated during research on onion from samples collected in the Sahelian and Sudano-Sahelian areas of Burkina Faso [8].

2.3 Essential oils used in this study

Essential oils used in this study were obtained from local plants such as *Cymbopogon citratus* (DC) Stapf, *Cymbopogon nardus* L. and *Eucalyptus camaldulensis*Dehnh. These oils were obtained from the Natural Substances Department of the "*Institut de Recherche en Sciences Appliquées et Technologies* (DSN/IRSAT, CNRST-Burkina Faso)". These plants were all collected in the central area of Burkina Faso.

2.4. Germination test in the laboratory

The test consisted of the incubation of seeds of the two varieties in Petri dishes with blotting paper. To do this, 25 seeds were arranged in each Petri dish, three times over. Onion seeds were washed in distilled water and shaken for 10 min in 1% sodium hypochlorite. The seeds werethen rinsed with distilled water before being shaken again for 15 min.After the seeds were deposited, the dishes were incubated at room temperature in the laboratory (28±2°C).

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2.5. Inoculum formulation and inoculation

Ten-day old strains on PDA medium were used. A conidial suspension of each strain was obtained by adding 10 ml of distilled water to the Petri dishes contained the conidia. Tubes were used to adjust the suspension to 1 x 10^6 conidia/ml using a haematimeter. The seeds were then soaked in the conidia concentrate for one hour for inoculation.

2.6. Pathogenicity test on seeds

This test evaluated the germination capacity of seeds inoculated with *Fusarium falciforme*. To do this, 25 seeds of each seed variety inoculated with each strain of *Fusarium falciforme* were placed in Petri dishes with blotting paper on the bottom. The control was pure seed of each variety. Four replicates were established.

2.7. Growing pots and sowing preparation

Twenty-four (24) pots were used for the trial. Four fine holes about 0.5 cm in diameter were drilled in the bottom of each pot to prevent any possible asphyxiation of the plants. A mixture of sand and steam-sterilized compost was used to fill the pots. The control pots were not fertilized. The inoculated seeds were sown in the pots using sterile forceps in furrows approximately 1 cm deep and 5 cm apart. Twenty seeds were sown per pot. The pots were then gently sprayed with distilled water every day after sowing.

2.8. Experimental design and used treatments

A completely randomized block design with four replicates was used. The elementary plot was a single pot of 22 cm top diameter, 16 cm bottom diameter and 22 cm depth. There was a 50 cm gap between replicates and a 10 cm gap between pots. A total of six treatments were applied, including two controls: Tep (Prema 178 + Biofertiliser) and Tvg (Violet de Galmi + Biofertiliser). The pF29_biof treatment was a combination (Prema 178 + *F. falciforme* 29 + Biofertiliser), pF30_biof (Prema 178 + *F. falciforme* 30 + Biofertiliser), vgF29_biof (Violet de Galmi + *F. falciforme* 29 + Biofertiliser) and vgF29_biof (Violet de Galmi + *F. falciforme* 30 + Biofertiliser). The biofertilizer used was compost.

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2.9. Culture medium used to study antifungal activity

Three essential oils were used to formulate the culture medium. A micropipette was used to draw up the quantities of the oils, which were 0.45, 0.90 and 1.35 ml. These quantities were then made up with 44.55, 44.10 and 43.65 ml of PDA respectively to give 45 ml of culture medium. Each mixture was then stirred for 3 min to homogenize it. The mixture was then poured into Petri dishes at a rate of 15 ml per dish. Concentrations of 1%, 2% and 3% of each oil were obtained in the culture media. The simple PDA medium without added oils was used as a control [10].

2.10. Antifungal activity test

For this test, the direct contact method was used. It involved inoculating mycelial discs of a 4-millimeter diameter culture of seven-day old *Fusarium falciforme*30 in the middle of each Petri dish (1 disc/dish). Three replicates of each oil concentration were performed. The dishes were incubated at room temperature (28 ± 2° C) for ten (10) days, after which time the inner surface of the control dishes was completely filled with the mycelium of the fungus[10].

2.11. Measured parameters

- Germination rate in the laboratory

The germination rate was evaluated at eight days. It was calculated as the ratio between the number of germinated seeds and the number of seeds placed in Petri dishes per variety and per treatment, based on 100(Formula 1).

$$\text{Germination rate} = \frac{\text{number of germinated seeds}}{\text{number of seeds placed in Petri dishes}} \times 100 \text{(Formula 1)}$$

- Emergence rate

The emergence rate was assessed at 7 and 14 days after sowing. It was calculated as the ratio between the number of germinated seeds and the number of seeds sown in the pots per treatment, expressed as a percentage, according to the formula 2:

$$\text{emergence rate} = \frac{\text{number of germinated seeds}}{\text{Number of seeds sow}} \times 100 \text{(Formula 2)}$$

- Damping-off rate

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Damping-off was calculated at 21 and 45 days after sowing. It was calculated by dividing the number of dead seedlings per pot by the number of emerged seeds per pot, based on 100 (Formula 3).

$$\text{Damping} - \text{off rate} = \frac{\text{Nombre de graines} \qquad -\text{nombre plantules} \qquad \text{entes}}{\text{Nombre de graines}} \times 100(\text{ Formula 3})$$

Radial growth inhibition

Measurements were taken every two days. Two perpendicular diameters of the mycelial mat were measured at each date. These measurements were used to determine the impact of the oils on the fungus.

2.12. Data analysis

All the data collected was recorded in an Excel 2016 spreadsheet. Averages and histograms were calculated using this spreadsheet. Analysis of variance was performed using R v 4.1.2 software. Means were compared using Dunnett's test at the 5% threshold.

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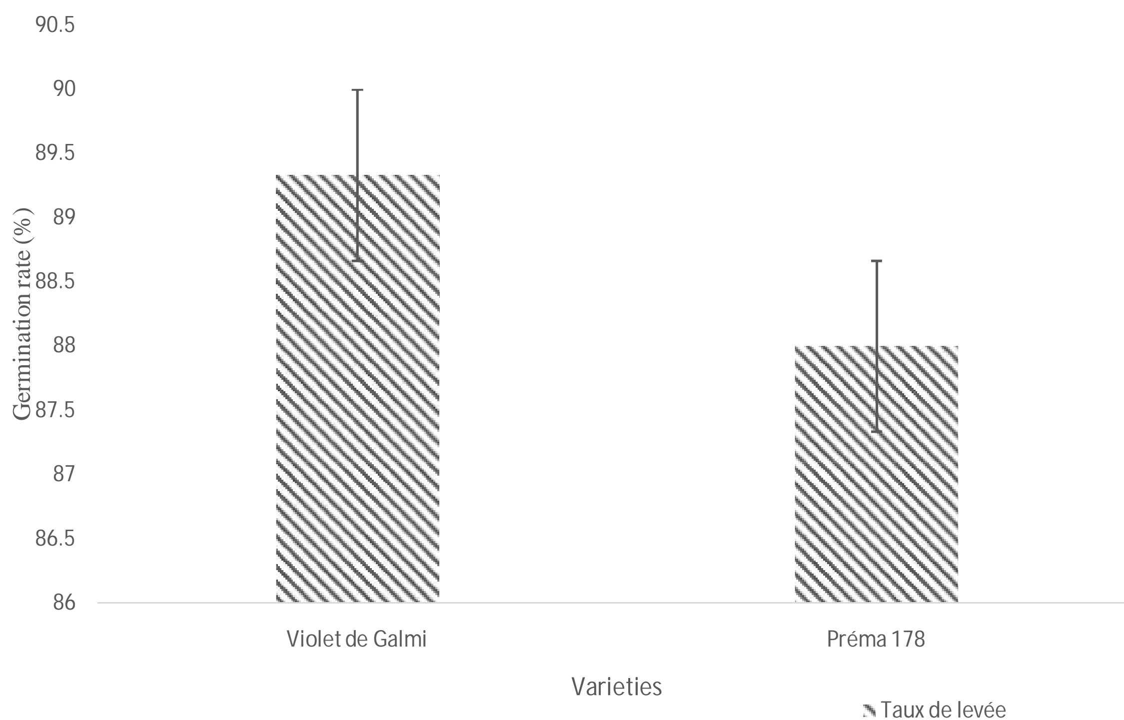
3. Results and discussion

3.1 Results

3.1.1.Germination rate

The germination rate of the seeds after 08 days is shown in Figure 1. Both varieties recorded successful germination rates. The results show that the Violet de Galmi variety had the highest



germination rate at 89.33% compared with 88% for the Préma 178 variety.

Figure 1.Germination rate of the varieties used

3.1.2. Pathogenicity test on seeds

The results obtained on the emergence of the seeds of the varieties Violet de Galmi (vg) and Préma 178 (p) are presented in Table 1. Overall, emergence varied from 1.75 to 21.25. Analysis of variance showed a highly significant difference between treatments ($p \leq 0.0001$). Mean emergence ranged from 10.25 to 18.25 for seeds treated with the different strains of *Fusarium* on Violet de Galmi, compared with 20.75 for the control. In contrast, on the Préma variety, it varied from 1.75 to 3.5 for seeds treated with the different strains of *Fusarium*, compared with 21.25 for the control. The vgF29 treatment did not show a significant difference compared with the controls (Tep, Tevg) which were not exposed to the strains. The

Violet de Galmi variety was more resistant to the strains than the Préma 178 variety, which proved to be highly susceptible to both strains of *Fusarium falciforme*.

Table 1. Seed pathogenicity testing in the laboratory

Treatments	Averagegerminatedseeds	Groups
pF29	1,75 ± 0,06	c
pF30	3,5 ± 0,38	c
vgF30	10,25 ± 1,36	b
vgF29	18,25 ± 1,77	a
Tevg	20,75 ± 1,26	a
Tep	21,25 ± 2,06	a
Probability	≤ 0,0001 VHS	

Te: control,**VHS**: very highly significant, **p**: Préma 178, **vg**: Violet de Galmi, **F29**: *Fusarium falciforme* 29, **F30**: *Fusarium falciforme* 30.

Mean ± standard deviation. On the same line, means followed by the same letter are not significantly different at the 5% threshold according to Dunnett's test.

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3.1.3. Greenhouse emergence

Comparative analysis of emergence at DAS 7 and DAS 14 is shown in Table 2. At DAS 7, the average number of seeds emerged ranged from 3.75 to 4 for the Préma 178 variety inoculated with *Fusarium falciforme* strains, compared with 7.5 for the control; and from 4 to 6 for the Violet de Galmi variety, compared with 9.75 for the control. A highly significant difference between treatments was recorded ($p \leq 0.019$). At JAS 14, variance analysis revealed a highly significant difference ($p \leq 0.0001$) between treatments. It showed a variation in the average number of emerged seeds from 4.75 to 6.25 for the Préma 178 variety compared with 15.75 for the control; and from 7 to 8.75 for the Violet de Galmi variety compared with 18.75 for the control out of 20 seeds sown. Both varieties were susceptible to greenhouse strains.

Table 2. Emergence observed on the two varieties used in the greenhouse

Treatments	Emergence 7DAS	Groups	Emergence 14DAS	Groups
pF29_biof	3,75 ± 0,22	b	4,75 ± 1,5	d
pF30_biof	4 ± 0,83	b	6,25 ± 0,96	cd
vgF29_biof	4 ± 0,16	b	7 ± 1,63	cd
vgF30_biof	6 ± 1,83	ab	8,75 ± 1,71	c
Tep	7,5 ± 3	ab	15,75 ± 0,96	b
Tevg	9,75 ± 1,5	a	18,75 ± 1,71	a
Probability	≤ 0,019 HS		≤ 0,0001 VHS	

Te: témoin ;**HS**: highly significant ; **VHS**: very highly significant, **p**: Préma 178, **vg**: Violet de Galmi, **F29**: *Fusarium falciforme* 29, **F30**: *Fusarium falciforme* 30, **biof**: biofertilizer, **DAS**: Day after sowing

Mean ± standard deviation. On the same line, means followed by the same letter are not significantly different at the 5% threshold according to Dunnett's test.

3.1.4. Sowing damping-off rate

The rate of seed damping off as a function of treatments over time is shown in Figure 2. It was assessed at two dates, 21 DAS and 45 DAS. These results show that the damping-off rate ranged from 57.97 to 100% between treatments, comparedwith 5.35 to 6% for the controls at 21 days after planting. At 45 days, the rate ranged from 97.22 to 100% between treatments, in compared with 6.25 to 9.22% for the controls. These results show that the damping-off rate was nearlyidentical between treatments at the 45th DAS.

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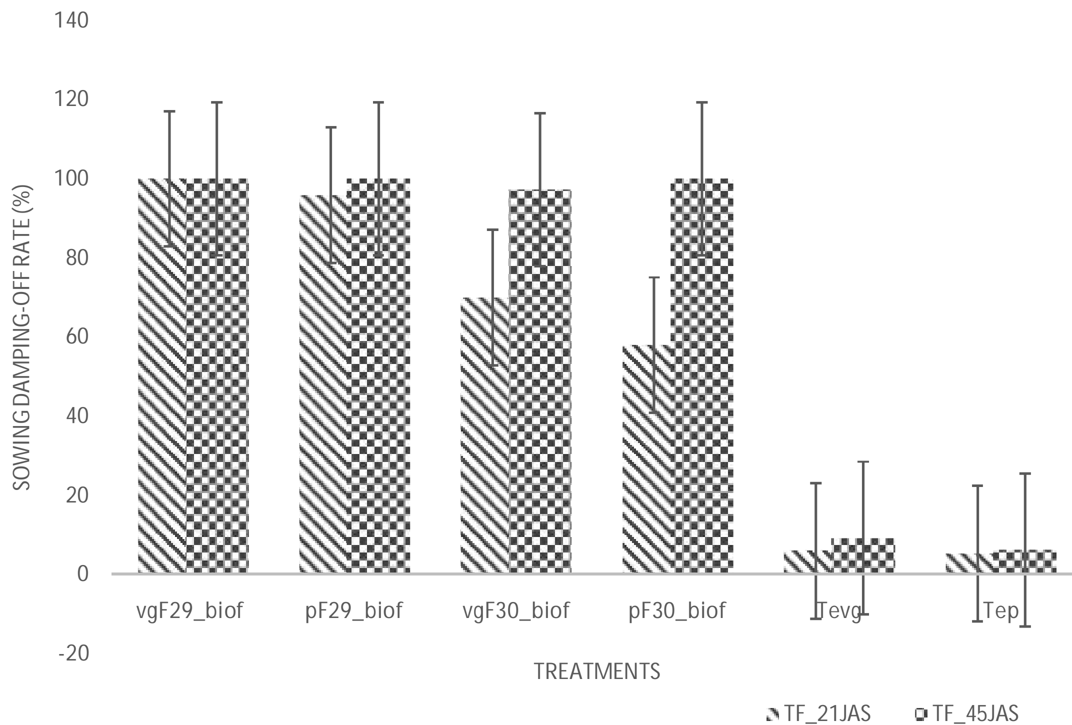

Figure2. Histogram of damping-off rates according to treatments

p: Préma 178, **vg**: Violet de Galmi, **F29**: *Fusarium falciforme* 29, **F30**: *Fusarium falciforme* 30, **biof**: biofertilizer, **DAS**: Day after sowing

3.1.5. Growth inhibition test for *Fusarium falciforme* strains

The average mycelial diameter of *Fusarium falciforme* 29 on 10 day after incubation (DAI) is shown in Figure 3. Average strain growth diameters ranged from 0 to 2.85 cm for the essential oil treatments, compared to 7.88 mm for the control. A highly significant difference was recorded ($p \leq 0.001$). All the concentrations of essential oils of *Cymbopogon citratus* Stapf and *Cymbopogon nardus* were able to completely inhibit the development of the fungus throughout the incubation period. On the other hand, weak mycelial growth of the strain was recorded at doses of 1%, 2% and 3%, with diameters of 2.85 cm, 1.83 cm and 1.05 cm respectively at 10 days old in the treatment composed of essential oil of *Eucalyptus camaldulensis*

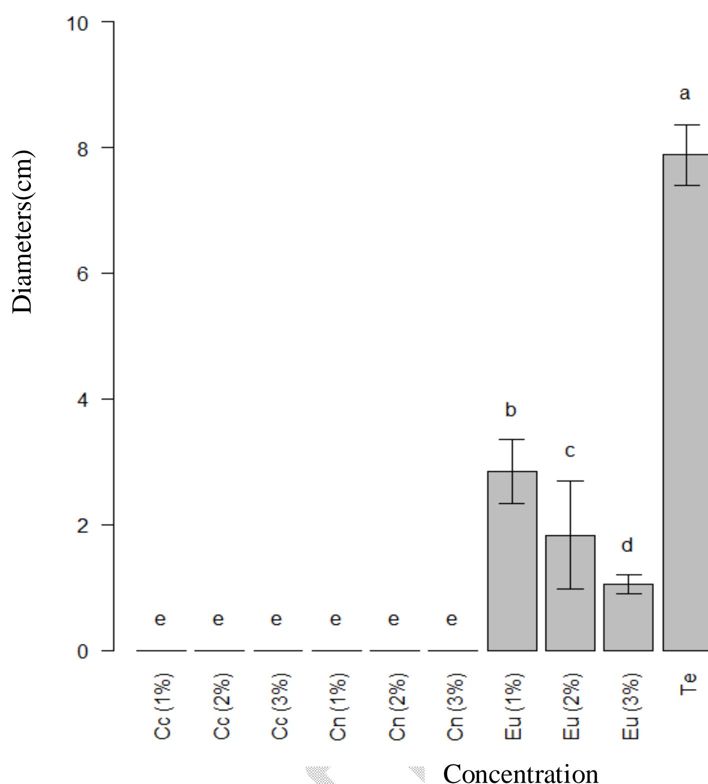

Figure 3. Average mycelial diameter of *Fusarium falciforme* 29 at 10 days old in the presence of essential oils

DAI = dayafter incubation,**Cc**:*Cymbopogoncitratus,* **Cn**: *Cymbopogonnardus*,**Eu**: *Eucalyptus camaldulensis,* **Te**: control

Histograms followed by different letters are statistically different at the 5% threshold according to Dunnett's test.

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3.2. Discussion

The results showed that the two strains of *Fusarium falciforme* identified had an inhibitory effect on onion seed emergence. The effect was observed both in the laboratory and in the greenhouse. This would confirm the pathogenicity of the species identified in this study. The pathogenicity test carried out on the two onion varieties enabled the *Fusarium* strains to be differentiated and their pathogenicity to be compared with controls not inoculated with the fungal strains. Violet de Galmi appears to be more resistant than Préma 178. This can be explained by the fact that this variety has resistance genes that make it more resistant than the Préma 178 variety. The use of biofertilizer in the greenhouse had no effect on seed emergence. This confirmed the pathogenic power of the strains inoculated during this study. In fact, several species of the *Fusarium* genus are known to be pathogenic fungi of onions. Our results are in agreement with those of Tirado-Ramirez et al.,[11] and Sogoba et al.,[12] who showed in their work that *Fusarium falciforme* can inhibit germination of onion seeds. As the pathogen was inoculated onto the seeds, it would have had time to activate its pathogenic ability.

At 45 days after sowing, damping-off was observed in all treated pots despite the presence of biofertilizer. Several authors have highlighted the action of fungi of the genus *Fusarium*, which may be responsible for damping-off, either individually or in combination([11], [13]). Damping-off was also observed in the controls, possibly reflecting contamination. These contaminations could be linked to the maintenance work carried out during the experiment.

Antifungal activity results from in-vitro tests revealed the efficacy of the essential oils of *Cymbopogon citratus*, *Cymbopogon nardus* and *Eucalyptus camaldulensis*. All treatments based on the essential oils of *C. citratus* and *C. nardus* tested at different concentrations showed inhibition of the mycelial growth of *Fusarium falciforme*. Previous work by Irkin and Mihriban [14], Dabiré et al [15] and Kaboré[16] showed that the essential oils of *Cymbopogon citratus*, *Cymbopogon nardus* and *Lippia multiflora* inhibited the development of species of the genus *Fusarium*. In addition, previous studies by Koïta, [17]; Tiendrebeogo et al., [18]; Sirima et al., [19]; Kaboré, [16]reported the inhibition efficacy of extracts of these plants (essential oil or aqueous extraction) on several species of fungi, including the genus *Fusarium*. The inhibition of pathogen growth by essential oils is thought to be due to the fact that these oils contain natural organic compounds with recognized antifungal activities, as reported in other plants ([20]; [21]). However, the results of antifungal test using *Eucalyptus camaldulensis*. essential oil showed low mycelial growth. Research by Gakuubiet al.,[22]

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confirmed the antifungal properties of *Eucalyptus camaldulensis*. essential oil and its potential use in the management of *Fusarium* fungi. In fact, this mycelial development could be due to the ability of these fungal species to adapt to *Eucalyptus camaldulensis*. essential oil and develop.

4. Conclusion

Onion production is an important activity given its socio-economic and nutritional importance. However, its production is subject to parasitic damage, including fungal diseases such as onion basal rot. At the end of the research, the results showed that *Fusarium falciforme* has a pathogenic effect on onion seeds. In this study, the antifungal test showed that the essential oils identified were effective against the growth of *Fusarium falciforme*. Based on these results, biopesticide-based biocontrol of *Fusarium falciforme* is therefore possible for onions basal rot management.

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