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Antifungal activity of essential oil-based formulations used in corn preservation in Burkina Faso

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Fungi and their toxins have an impact on the hygienic standards and market value of corn (*Zea Mayas* L.) in Burkina Faso. Though effective, other pesticides have been shown to be harmful. This study aims to aid in the management of corn diseases by assessing the effectiveness of bio-fungicide formulations. In this study, the blotter method was used to assess the antifungal activity at doses of 0.5% (5 g/kg) and 1% (10 g/kg) of powdered formulations of *Cymbopogon giganteus* (F1) and *Eucalyptus camaldulensis* (F2) essential oils on the fungal flora of a corn sample. Untreated controls were also observed. The results of this study demonstrated that *Aspergillus flavus* and *Aspergillus parasiticus*, which could produce aflatoxin, were more susceptible to the 0.5% dose at F1 than at F2. Though *Aspergillus niger* and *Rhizopus* sp. were resistant to 0.5% of F1 and 0.5% of F2, while *Fusarium* sp. was resistant to 0.5% of F1. All of the above fungi were vulnerable to 1% of F1 and F2. Therefore, these two formulations could be utilized successfully to combat the decline in the hygienic, nutritional, and market value of corn in Burkina Faso caused by aflatoxin contamination and other toxins produced by these fungi. However, it would be important to evaluate the synergistic effect of these biofungicide formulations *in situ* and on a large scale.

Key words: Antifungal, *Cymbopogon giganteus, Eucalyptus camaldulensis*, essential oil, biofungal formulation, corn.

INTRODUCTION

In Burkina Faso, corn is the second most cultivated cereal after sorghum. Its production increased from 1,133,480 tonnes in 2011 to 1,170,898 tonnes in 2020 (FAO/OMS, 2020). Despite this production, food insecurity, amplified by the risks of uncontrolled food poisoning, remains a real problem in Burkina Faso.

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Indeed, cereal production is particularly affected by numerous ecological constraints (Waongo et al., 2013). In addition, the impact of rudimentary production methods and poor storage conditions increases the risk of contamination by toxins. Also, stored corn is subject to daily attacks by toxin-producing moulds. To protect crops and ensure their good phytosanitary quality, various crop management methods are used. Among them, those using synthetic chemicals have proved to be effective and have shown convincing results. For example, peroxydan induces a total inhibition (100%) of the mycelial growth of two fungi, *Botrytis cinerea* and *Penicillium digitatum* at a dose of 2.5% (Elbouchtaoui et al., 2015). Unfortunately, the use of these products has disastrous environmental, health, and economic repercussions. Environmental pollution leads to the contamination of surface water and groundwater, the contamination of food products from treated areas or surrounding areas, the contamination of certain herbaceous or medicinal plants, the gradual disappearance of certain species of useful herbs in animal feed, and some aquatic animal species (Tarnagda et al., 2017). This pollution also leads to a decrease in soil fertility, as well as a loss of biodiversity and the selection of strains of pests resistant to pesticides (Sala et al., 2000; Lehmann et al., 2018). Cases of acute poisoning by pesticides constitute a public health problem. Indeed, more than 385 million cases of unintentional acute poisoning worldwide each year (Boedeker et al., 2020) and more than 29% in the two major university hospitals, between the years 2006 and 2007, linked to the intensification of the use of pesticides by agriculture (Ouédraogo et al., 2011). Costs resulting from pesticide poisonings now exceed the total annual amount of development assistance given to the sub-Saharan African region for basic health care. The total cost of pesticide-related illnesses and injuries could reach $90 billion (USD) by 2020 (Heindel et al., 2013). In Burkina Faso, in particular, the cost of economic losses linked to the mismanagement of chemicals in the agricultural sector is estimated at around 4.2 billion CFA francs (Lankoande and Maradan, 2013). These synthetic products, of their exorbitant costs and their toxicity, also constitute an economic burden for farmers. Indeed, these pesticides are one of the ways to the impoverishment of the peasants, because the high residues of pesticides in agricultural products also raise food safety products and constitute a serious obstacle to exports. These ecological problems and their consequences on the environment, human and animal health have prompted the development of innovative post-harvest management strategies based in particular on the search for new substances that are effective, less toxic, less expensive, and respectful of the environment. In this context, several studies have already proven the effectiveness of natural substances in the fight against fungi. The essential oils of *Cymbopogon schoenanthus*, *Lippia multifliora* and *Ocimum americanum*, tested on cow pea seed-borne

fungi, were found to reduce the contamination rates of *Colletotrichum dematium* and *Fusarium* spp., *Cladosporium* sp. and *Macrophomina phaseolina*, respectively (Toé et al., 2022). In view of the antifungal potential of these essential oils on various pathogenic fungal species, they could be a promising alternative strategy for combating moulds. Given the results obtained, the use of these bio-pesticides could be an interesting alternative to the use of conventional pesticides, particularly chemical pesticides. This alternative based on the use of local natural resources at a lower cost and ecologically sustainable could contribute to reducing farmers' expenses, preserving the environment, sustainably managing soil fertility, guaranteeing the quality of crops, and respecting sanitary and phyto-sanitary standards for food products. All these aspects will contribute to developing the maize sector to sustainably improve its contribution to food security, quality and nutritional aspects, access to markets, poverty reduction, and accelerated growth of the national economy. It is in this context that the present study falls under the general objective of which is to evaluate the antifungal activities of two essential oil-based formulations with a view to developing a fungicide for the management of toxigenic moulds in corn in Burkina Faso.

MATERIALS AND METHODS

Plant material

For the study, the corn variety Kamboinsé Extra Precoce Jaune (KEJ) was collected in Dédougou (12°28′N 3°28′W) located in Burkina Faso using a sterile transparent bag during the period of September 2021 and kept at 5°C in the SAMSUNG RL4352LBASP refrigerator at the Laboratory of Phytopathology (CNRST/INERA), CREAF Kamboinsé for less than 24 h before analysis.

Biopesticides based on essential oils

Two ready-to-use formulations based on essential oils of *C. giganteus* (F1) and *E. camaldulensis* (F2) fixed on powdered starch were used.

The control pesticide

Calthio MIX 485 WS (CM) (Imidacloprid 350 g/kg, thiram 100 g/kg, Metalaxyl 35 g/kg), a systemic insecticide or fungicide used as a seed treatment for corn against soil-borne pests (CSP, 2020), was used in this study as a reference control. The dose used in the study was the manufacturer's recommended dose of 5 g of product per 1 kg of grain (or 0.5%).

Processing of corn grains

The treatment of corn grains with the fungicides F1, F2 and Calthio MIX 485 WS was done by coating. For each formulation, doses of 5 g fungicide per kg of corn (or 0.5% concentration) and 10 g fungicide per kg of corn (or 1% concentration) were tested. The

tests were carried out in the presence of an untreated control (negative control) and a treatment with a reference pesticide, Calthio MIX 485 WS (positive control). Thus, 4 treatments per formulation (that is, 8 samples in total) were tested:

1. 2 samples of untreated corn (NT) ;
2. 2 samples of corn treated with Calthio MIX 485 WS at a 0.5% dose (CM);
3. 2 samples of corn treated respectively with the bio-fungicide formulations based on *C. giganteus* and *E. camaldulensis* essential oil at a dose of 0.5% (F1-1 and F2-1);
4. 2 corn samples treated with the essential oil formulations of *C. giganteus* and *E. camaldulensis* at a 1% dose (F1-2 and F2-2).

Seed health analysis

The health analysis of corn was carried out using the blotting paper method (ISTA, 1999). Corn grains were placed equidistantly in 9 cm diameter Petri dishes (10 grains per Petri dish) containing 3 layers of blotting paper soaked in sterile distilled water. For each treatment, 200 seeds were used in 4 replicates of 50, following a Fischer block design. The seeded dishes were kept in incubation at $22 \pm 3°C$ and illuminated by an alternating cycle of 12 h per day of darkness and 12 h of near-ultraviolet light, for 7 days following the method described by Mathur and Kongsdal (2003). The incubated grains were removed and inspected individually with a Leica M210 stereomicroscope (zoom range 7.5 - 60x) for identification of the fungi by macroscopic characters (colour of mycelium, pycnidia, acervuli and conidiophores). A sample of the observed fungus was then mounted between slide and coverslip and observed with a Motic BA210 light microscope (N-WF 10 x/20 mm wide field eyepieces with dioptre adjustment on both tubes, 360° binocular rotating head, 2 CCIS EF-N PLANS 4x, 10x, 40x, and 100x immersion objectives). The identification of the fungus was confirmed by examining the microscopic characters (shape and size of the conidia, structure of the mycelium) with reference to the identification manual for fungi by Mathur and Kongsdal (2003). The species of fungi present on each grain were noted, and the percentage of grains infected by a given fungus was calculated for each seed treatment. The effect of each treatment in controlling the fungi was evaluated in comparison with the fungal infection indices obtained with untreated corn and corn treated with the reference fungicide.

Statistical analysis

The data obtained per treatment and per replication, on grain mould infection rates were subjected to an analysis of variance (ANOVA) with the statistical software XLSTAT-Pro 7.5.2 version 2016 and the means were compared using the Student Newman Keuls (SNK) test at the probability threshold of $p = 0.05$.

RESULTS

The fungal species detected in the sanitary analysis of treated and untreated seeds were *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* sp., *Rhizopus* sp. and *Aspergillus parasiticus*. The results of the analysis of variance (Table 1) revealed that the seed treatments had a significant effect on the reduction of all these fungi. The lowest infection rates were noted on seeds treated with Calthio MIX 485 WS, while the highest infection rates

were obtained with untreated seeds (NT). Thus, Calthio MIX 485 WS showed high fungicidal activity compared to the untreated control. *Aspergillus niger, Aspergillus flavus, Fusarium* sp., *Rhizopus* sp. and *Aspergillus parasiticus* were reduced to 8.75±3.75, 21.75±9.25, 13.75±4.75, 3.25±3.37 and 3.12±1.41%, respectively, for Calthio MIX 485 WS compared to 83±8, 82.5±8, 59.5±10, 38.5±19 and 11.5±1.37% for untreated seeds.

Effects of F1 biopesticide on seed-borne fungi in corn

The results of the analysis of variance (Table 1) revealed that the corn treatment with the essential oil formulation of *C. giganteus* (F1) at dose 2 resulted in a significant reduction of the infection rates of all the exceptional fungi. Indeed, the contamination rates of the grains in *A. niger, A. flavus*, *Fusarium* sp., *Rhizopus* sp. and *A. parasiticus* decreased from 83 ± 8 to 44.25 ± 10.75%, from 82.5 ± 8 to 35.75 ± 7.37%, from 59.5 ± 10 to 23.75 ± 5.25%, from 38.50 ± 19 to 7 ± 8.37% and from 11.5 ± 1.37 to 5.5 ± 1.25% respectively. On the other hand, the contamination rate of the grains by *Fusarium* sp. was 59.5 ± 10 to 69 ± 8% with F1-1. Calthio MIX 485 WS also resulted in significant decreases in fungal infection levels compared to the untreated seeds treatment, although with slightly greater reductions than F1-2.

Effects of the biopesticide F2 on seed-borne fungi in corn

The results of the analysis of variance (Table 1) revealed that corn treatment with the essential oil formulation of *Eucalyptus camaldulensis* (F2) resulted in a significant reduction of fungi except for *Aspergillus niger* and *Rhyzopus* sp. for which the low dose biopesticide of 0.5% (F2-1) was ineffective. Indeed, the contamination rates of grains by *A. niger, A. flavus*, *Fusarium* sp., *Rhizopus* sp. and *A. parasiticus* went from 83 ± 8 to 73 ± 9 %, from 82.5 ± 8 to 52.5 ± 5.5 %, from 59.5 ± 10 to 33.5 ± 4.25 %, from 38.50 ± 19 to 34 ± 11 % and from 11.5 ± 1.37 to 7.75 ± 0.87 % respectively with F2. On the other hand, the contamination rate of the grains by *Aspergillus niger* and *Rhyzopus* sp. increased from 83 ± 8 to 88.5 ± 5.5 % and from 38.50 ± 19 to 49 ± 4 % respectively with F2-1.

Correlation of treatments and mycological profile

The principal component analysis (PCA) (Figures 1 and 2) showed a distribution of the data around two axes (*F*1 and *F*2: 91.37%). Thus, the 1st and 2nd dimensions of the PCA (F1 and F2) explained 91.37% of the information contained in the dataset. This means that if we summarize the 5 variables studied (*A. niger, A. flavus, Fusarium* sp.,

Table 1. Analysis of variance of the data from the sanitary analysis of treated and untreated corn grains.

Sample	*A. niger* (%)	*A. flavus* (%)	*Fusarium sp.* (%)	*Rhizopus sp.* (%)	*A. parasiticus* (%)
NT	83 ± 8[a]	82.5 ± 8[a]	59.5 ± 10[ab]	38.50 ± 19[a]	11.5 ± 1.37[a]
F2-1	88.5 ± 5.5[a]	74 ± 7[ab]	47.5 ± 6.75[bc]	49 ± 4[a]	11 ± 1[a]
F2-2	73 ± 9[a]	52.5 ± 5.5[bc]	33.5 ± 4.25[cd]	34 ± 11[ab]	7.75 ± 0.87[ab]
F1-1	76 ± 3[a]	42.5 ± 9.5[c]	69 ± 8[a]	29.75 ± 5[abc]	6.25 ± 1.25[bc]
F1-2	44.25 ± 10.75[b]	35.75 ± 7.37[cd]	23.75 ± 5.25[d]e	7 ± 8.37[bc]	5.5 ± 1.25[bc]
CM	8.75 ± 3.75[c]	21.75 ± 9.25[d]	13.75 ± 4.75[e]	3.25 ± 3.37[c]	3.12 ± 1.41[c]
Pr > F	0.0000	0.0000	0.0000	0.0000	0.0000
Significant	Yes	Yes	Yes	Yes	Yes

In the same column, the means assigned to the same alphabetical letter are not significantly different at the 5% threshold, according to the Student-Newman-Keuls multiple classification test; NT = Not treated; F1-1: *Cymbopogon* at the 0.5 % dose; F1-2: *Cymbopogon* at the 1 % dose; F2-1: *Eucalyptus* at the 0.5 % dose; F2-2: *Eucalyptus at* the 1 % dose; CM: Calthio MIX.
Source : Authors

Rhizopus sp. and *A. parasiticus*) by the two dimensions, then we recover 98% of the information contained in this dataset. In other words, we have an excellent summary that synthesizes these 5 variable almost perfectly. The first dimension of the PCA (*F*1) allowed the different treatments to be ranked in decreasing order of their antifungal activity (Figures 1 and 2). From left to right, the points furthest from untreated seeds correspond to the most fungicidal treatments. Thus, the biopesticides F1 and F2 were less effective than the synthetic pesticide Calthio MIX 485 WS, but F1 was more fungicidal than F2. The PCA shows that the fungus rate decreases with increasing treatment dose. The *F*1 dimension of the PCA (Figures 1 and 2) also informs us of the relationship between these fungi. Thus, there is a close correlation between these different fungi, especially between *A. flavus* and *A. parasiticus*, due to the proximity of their eigenvector values. The *F*2 dimension of the PCA (Figures 1 and 2) provides information on the level of resistance of these fungi to the different treatments. *Fusarium* sp. is most resistant to the F1 biopesticide, while *Rhizopus* sp. is most resistant to the F2 biopesticide.

DISCUSSION

In the present study, the fungal analysis of maize grains of the Kamboinsé Extra Précoce Jaune (KEJ) variety made it possible to identify a diversity of fungi such as *A. niger*, *A. flavus*, *Fusarium* sp., *Rhizopus* sp. and *A. parasiticus*. Previous studies conducted outside of Burkina Faso have found similar results. Indeed, *A. flavus*, *A. niger*, *Fusarium oxysporum*, *Penicillium* sp. and *Rhizopus* sp. were detected on corn with yellow grains, not disinfected, with an average temperature and relative humidity of 25°C and 40% respectively, harvested in Côte d'Ivoire (Ky and Diomandé, 2017). *A. flavus*, *A. nige*r, *R. stolonifer* and *Penicillium* sp. have been identified in corn-

based samples produced in Benin (Agassounon et al., 2020). The presence of these moulds in corn implies that the contamination likely occurs either before field harvest or during improper drying, storage and processing of corn grains into finished products. The presence of these moulds in corn implies that the contamination likely occurs either before field harvest or during improper drying, and storage of corn grains (Czerwiecki et al., 2010). Corn exposed to the air in large basins and without adequate protection during sale is likely to be contaminated with fungal and bacterial spores (Toffa et al., 2013). Mould growth in corn could also be favoured by inadequate physico-chemical parameters, including the amount of free water (A_w), temperature, presence of oxygen, nature of the substrate and pH (Siramon et al., 2013).

The results of the analysis of variance revealed that the seed treatments with the biopesticides F1, F2 and the synthetic pesticide Calthio MIX 485 WS had a significant effect on the reduction of all these fungi compared to the untreated control.

Calthio MIX 485 WS showed the highest fungicidal activity. In fact, the lowest infection rates were noted on seeds treated with Calthio MIX 485 WS. *A. niger, A. flavus, Fusarium* sp., *Rhizopus* sp. and *A. parasiticus* were strongly reduced by CM (3.12-1.75%) compared to NT (11.50-83%). Similar effects of Calthio MIX 485 WS have been described in several previous studies applied to corn (Szwejkowska et al., 2008), cow peas and groundnut seeds (Toé et al., 2022). The efficacy of Calthio MIX 485 WS against these fungi is likely to be due to the fungicidal activity of one of its active molecules (thiram), which acts through direct contact with the seed against numerous seed-borne pathogenic fungi, reduces the incidence of seed-borne rots and damping-off on seedlings (Séguy, 1999).

Corn treatment with the essential oil formulation of *C. giganteus* (F1) was also effective at both low and high doses in controlling the fungi identified in corn, resulting
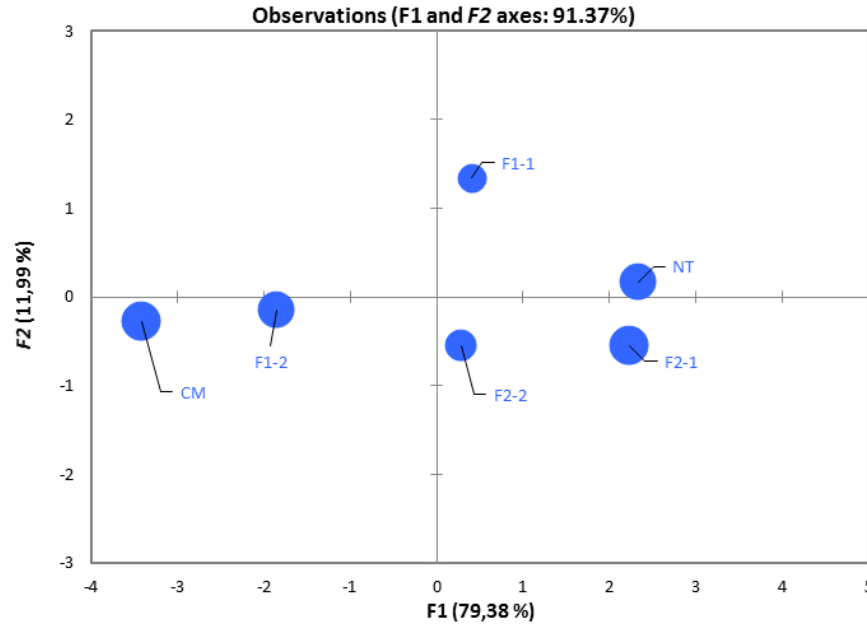
Figure 1. Classification of treatments in descending order of effectiveness.
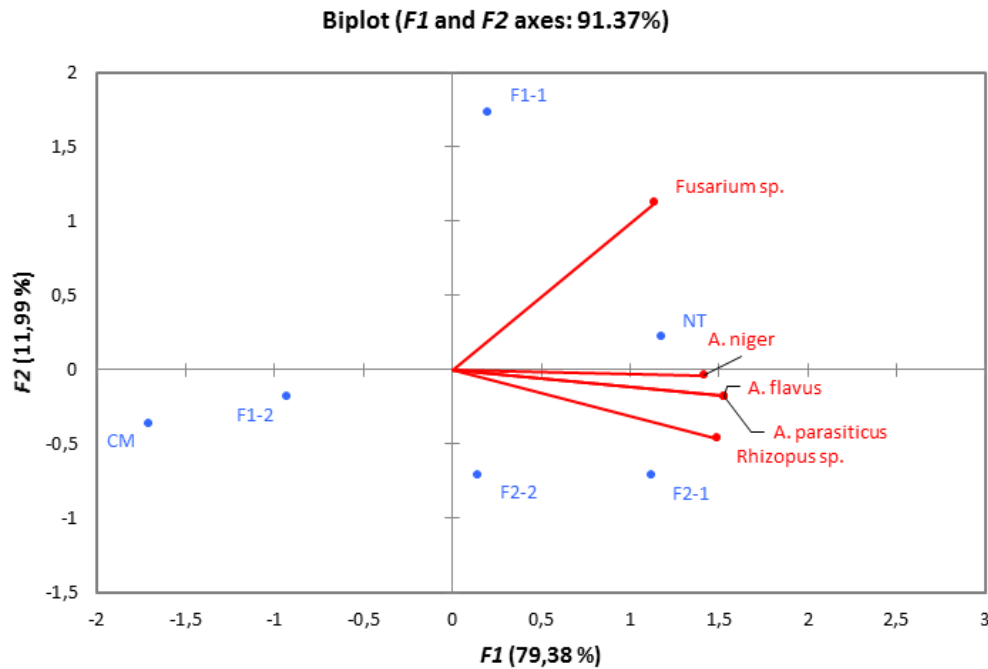Source : Authors

Figure 2. Relationships between treatment and frequency of contamination of corn.
Source: Authors

in a significant reduction in these fungi. Indeed, with F1, the level of contamination of treated corn was significantly reduced for all fungi except for *Fusarium* sp. when the biopesticide was used at the low dose of 0.5 % (F1-1). The rate of grain contamination by *Fusarium* sp.

increased from 59.5 ± 10 to 69 ± 8 % with F1-1. This effect was similarly observed in earlier studies where *C. citratus* essential oil was also only effective at high doses (1%). Similarly, *A. indica* seed oil accelerated the growth of *F. verticillioides* on stored corn (Fandohan et al.,

2004). The fungicidal effect of F1 would then increase with the dose of the treatment applied and more precisely with the dose of its constituents such as limonene, Z-Mentha-1(7),8-dien-2-ol, E-Mentha-1(7),8-dien-2-ol, E-*p*-Mentha-2,8-diene-ol and Z-*p*-Mentha-2,8-diene-1-ol (Bossou et al., 2020). This hypothesis corroborates the results of another study where it was shown that 1.0 µL/mL of *C. citratus* essential oil with 79% citral was more fungicidal than that with 68.4% citral (Martinazzo et al., 2019).

Seed treatment with the high-dose formulation of *E. camaldulensis* (F2) essential oil was also effective in controlling the fungi identified in corn, resulting in a significant reduction of these fungi, albeit with lower reduction rates. In addition, some fungi such as *A. niger* and *Rhyzopus* sp. were resistant to the low dose (0.5 %) of this biopesticide; the rate of contamination of grain by these fungi increased from 83 ± 8 to 88.5 ± 5.5% and from 38.50 ± 19 to 49 ± 4% respectively. Other studies also proved the effectiveness of *E. camaldulensis* essential oil against *A. flavus* (Abo Elgat et al., 2020) A. *parasiticus* NRRL2999 *in-vitro* (Rasooli et al., 2009) *A. niger* (Siramon et al., 2013) against five species of *Fusarium* at 10 ml/ml (Gakuubi et al., 2017) and *Rhizopus stolonifer* (Nasr et al., 2018). However, application of essential oil from *E. camaldulensis* leaves to tree wood showed no inhibition of *A. niger* at low concentrations (156.25 ppm) (Salem et al., 2016). The efficacy of F2 would therefore increase with the treatment dose and more specifically in synergy with the high oxygen content (at least 60%) of the essential oil (Barra et al., 2010) namely *α*-phellandrene, 1,8-cineole, *p*-cymene, α-pinene and γ-terpinene (Samaté et al., 2011).

The Principal Component Analysis (PCA) allowed us to draw a partial conclusion to our analysis by classifying in decreasing order of their effectiveness, (i) the type of pesticide (ii) the dose of the pesticide (iii) the type of relationship existing between the pesticide and these fungi and (iv) the level of resistance of these fungi to different treatments. Thus, the synthetic pesticide (Calcio MIX 485 WS), used as a reference control, was the most effective, followed by the formulation based *C. giganteus* essential oil (F1), and *E. camaldulenssis* (F2) respectively. The fungicidal efficacy of these two formulations increased with the prescribed dose. The aflatoxigenic pathogens *A. flavus* and *A. parasiticus are* thought to share close links at the ecological and sensitivity levels to the different pesticides. Specific resistance of *Fusarium* sp. and *Rhizopus* sp. to low doses of F1 and F2, respectively, was reported. However, the resistance of these fungi was attributed to the low content of oxygenated compounds present in essential oils (Barra et al., 2010). Although the resistance of these fungi was attributed to the low content of oxygenated compounds in the essential oils, this phenomenon of resistance could be further clarified by in-depth toxicological studies. These studies will allow us to

have a better knowledge of the mechanism of action of these essential oils towards fungi.

Conclusion

From the study of the effect of bio-fungicides based on essential oils of *C. giganteus* and *E. camaldulensis*, it appears that these formulations are effective against the mycelial growth of potentially aflatoxinogenic pathogens, *A. flavus* and *A. parasiticus*, of *A. niger*, of the genera *Fusarium* and *Rhizopus*. This study, therefore, shows that these two formulations could effectively replace synthetic pesticides in the fight against phytopathogenic and toxigenic fungi. On the one hand, this would contribute by improving the health of plants, animals, consumers and the environment. On the other hand, this would make it possible to ensure that corn produced complies with international standards, guarantee its competitiveness and consequently promote its speculation on national and international markets.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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