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Abstract

Tomato (*Solanum lycopersicum* L.) is the world's most consumed vegetable. In Cameroon, it is the most cultivated vegetable and the second most produced fruit after ripe banana. However, a decline in yield is recorded in Cameroon due to bug infestations, such as *Tuta absoluta*, which has favored tomato rot caused by *Geotrichum candidum*. The most common method for pest management is the application of synthetic pesticides, which have toxic effects on humans and the environment. Therefore, alternative strategies such as those based on the use of natural products are needed. Essential oils have shown promise based on their efficiency, eco-safety and cost effectiveness. The purpose of this study was to determine the insecticidal and antifungal activities of essential oil from *Cymbopogon citratus*. Hydrodistillation was used to extract the oil. The insecticidal activity of the oil was determined by ingestion toxicity against *Tuta absoluta* larvae. *Geotrichum candidum* was isolated from rotten-infected fruits perforated by *Tuta absoluta,* and its morphological and molecular traits were characterized. Essential oil was tested for antifungal activity on conidia germination through the microdilution method. The results showed that oil exerted insecticidal efficiency with LD_{50} and KD_{50} values of 29.762 µL/mL and 27.499 µL/mL, respectively. *Cymbopogon citratus* oil lengthened pupal duration compared to the controls. Molecular identification confirmed the identity of the pathogens as isolates of *Geotrichum candidum.* Essential oil inhibited the germination of pathogen conidia with minimal inhibitory concentrations from 0.71 µL/mL to 5.67 µL/mL. This study reports for the first time the insecticidal potential of the ingestion route of *Cymbopogon citratus* essential oil against *Tuta absoluta*. The results demonstrate that *Cymbopogon citratus* oil could be used in the control of *Tuta absoluta* and *Geotrichum candidum;* thus, the insecticidal and antifungal activities of this oil should be investigated further under greenhouse and field conditions.

Keywords: Tomato, *Tuta absoluta*, *Geotrichum candidum, Cymbopogon citratus* oil, insecticidal and antifungal activities.

Introduction

Tomato (*Solanum lycopersicum* L.) is the most highly consumed vegetable due to the status of its fruits as a basic ingredient in a large variety of raw, processed or cooked foods (OECD, 2017). In Cameroon, tomato is the most cultivated vegetable and ranked as the second most produced fruit after ripe banana (MINADER, 2012; Heumou *et al*., 2015). Tomato yield has recently dropped in Cameroon, failing to supply the demands of national and neighboring countries. Severe bug infestations, such as *Tuta absoluta*, have favored tomato rot caused by *Geotrichum candidum*, which is one of the main reasons for decreased productivity. Indeed, *Tuta absoluta* (Meyrick 1917) was reported as a key pest of tomato since the 1950s, causing a drastic tomato yield of 100% owing to its leaf-mining activity mediated by larvae and through fruit infestation (Desneux *et al*., 2010; Ayalew, 2015; Biondi *et al*., 2018). Infested fruits present galleries of larvae and are characterized by holes that constitute penetration routes for pathogenic fungi such as *Geotrichum candidum*. In addition, *G. candidum* infections produce mycotoxins that affect the organoleptic, nutritional and market values of the fruits.

To manage *T. absoluta* and *G. candidum,* farmers desperately use a set of synthetic products such as Lynx (λ-cyhalothrin 15 g/L + acetamiprid 20 g/L), Doyen 62 EC (emamectin benzoate 12 g/l + imidacloprid 50 g/L) and Propiconazole Tilt 25 EC. However, drawbacks such as the build-up of pesticide residues on tomato fruits cause human toxicity and environmental pollution. Therefore, plant-based control strategies exhibiting insecticidal and antifungal activities have become extremely important since plants have physiological properties or defense mechanisms through active metabolites that protect against insects and prevent pathogen invasion (Bennett and Wallsgrove, 1994). Thus, alternative approaches to control *T. absoluta* and *G. candidum* include the utilization of plant-derived active metabolites with insecticidal and antifungal properties (Stappen *et al*., 2018; Debbabi *et al*., 2020; Ghasemi *et al*., 2020).

Essential oils, as one of the most reliable biological pesticide sources, have shown promise in the control of insect pests and fungal pathogens (Tak *et al*., 2015; Sameza *et al*., 2016; Samira and Habib, 2017; Tančinová *et al*., 2021). Essential from *Cymbopogon citratus* (DC.) Stapf. (Poaceae) plant used in Cameroon for nutritional, cosmetic and medicinal purposes, has shown insecticidal and antifungal properties against a wide range of insect pests and pathogenic fungi from tomato crops. For instance, Ngongang *et al*. (2021) reported the contact and fumigant activities of *C. citratus* and *T. vulgaris* essential oils against *T. absoluta* larvae. In addition, Gangavarapu and Palwai (2021) showed the activity of *C. citratus* oil on *A. solani, F. oxysporum, P. ultimum* and *R. solani* associated with tomato rot. Thus, the purpose of this study was to determine the insecticidal and antifungal activities of *C. citratus* essential oil against *T. absoluta* and *G. candidum* on tomato.

1. Material and methods

1.1. Plant collection and essential oil extraction

Fresh aerial parts of *C. citratus* were collected in August 2017 (8. AM) in Centre region (3°52′22.22″ N, 11°28′48.86″ E) of Cameroon. and was identified at the National Herbarium of Cameroon under reference number 48536/SRF/CAM. The hydrodistillation method was used for essential oil extraction using the Clevenger-type apparatus. The resulting oil was dried by passing through an anhydrous sodium sulfate column followed by storage in a dark glass bottle. The yield of extraction was determined as follows:

Yield (%) = [(Mass of essential oil (g)/Mass of vegetal material (g)) x 100].

1.2. Insecticidal activity assay of *Cymbopogon citratus* essential oil

1.2.1. *Tuta absoluta* and tomato plant rearing

Tuta absoluta was reared as described by Ngongang *et al*. (2021). Indeed, *T. absoluta* larvae were collected in a commercial tomato field located in the West Region of Cameroon (5º29'00.447" N, 10º40'45.959"E) and transferred individually into Petri dishes supplied with fresh tomato leaves. The leaves were collected from tomato (cv. Rio Grande) plants grown from seeds at the experimental field of the University of Yaoundé I, Cameroon. Once adults emerged from the larvae, they were released into an egg-laying cage for oviposition on potted tomato plants. The rearing conditions were 25 ± 2°C, 65 ± 5% RH, and a photoperiod of 16/8 hours light to dark cycles. After egg hatching, the first instar larvae were collected individually on fresh tomato leaves into Petri dishes and reared until the fourth larval stage used for ingestion toxicity.

1.2.2. Ingestion toxicity assay of *Cymbopogon citratus* essential oil

The ingestion activity of *C. citratus* essential oil was performed following the procedure of Moustafa *et al*. (2021). Briefly, the stock solution of the EO was prepared using Tween 80 (Sigma Aldrich, Munich, Germany). From the stock solution, twofold dilutions were performed to obtain test oil concentrations of 12.5 µL/mL, 25 µL/mL, 50 µL/mL, 100 µL/mL and 200 µL/mL based on preliminary tests. Petri-dishes were provided with filter paper to protect larvae from excessive humidity. Tomato leaves were dipped in each of the prepared concentrations for 1 min and then left to air dry before introducing in Petri-dishes, then 10 larvae were transferred. A negative control was performed using 0.9% Tween® 80 in distilled water, while Lynx® (λ-cyhalothrin 15 g/L + acetamiprid 20 g/L) (MINADER, 2019) served as a positive control and was prepared according to the manufacturer's instructions (Sun valley Hall Limited, Hong Kong) at 3.33 mL/L, which is the recommended dose for field application against *T. absoluta*. The Petri dishes were sealed and maintained under the previously mentioned rearing conditions. Larvae were left to feed for 4 hours. Experiments in quadruplicate were repeated twice on different days. The number of knocked down larvae was recorded and expressed as follows:

Knockdown (%) = [(Number of knocked down larvae/total number of larvae) x 100]

In addition, the doses required to knock down 50% and 90% (KD_{50} and KD_{90}) of larvae were determined. The mortality was evaluated as described by Ngongang *et al*. (2021) by transferring the knocked down larva on untreated tomato leaves**,** after 24 hours, the percentage of mortality and the doses killing 50% and 90% (LD_{50} and LD_{90}) of larvae were determined. Larval and pupal durations were evaluated following the procedure of Ngongang *et al*. (2021). Thus, surviving larvae were reared individually on fresh tomato leave in Petri dish and observed daily. Then, larval and pupal duration were calculated.

1.3. Antifungal activity of *Cymbopogon citratus* essential oil against *Geotrichum candidum*

1.3.1. Fungal isolation

Tomato fruits infested by *T. absoluta* and presenting typical sour rot characteristics, such as a soft tissue-smelling sour odor, whitish mycelia and liquid secretion on crack points, were collected from commercial tomato farms in the western region of Cameroon and subjected to isolation using the protocol described by Lemma *et al.* (2014). Thus, small pieces (~ 4 mm diameters) were cut at rotting edges with a forcep. The pieces were sterilized by dipping in 2% sodium hypochloride for 3 min and rinsed three times with sterile distilled water. The air-drying fragments were inoculated on PDA medium supplemented with chloramphenicol (500 mg/L) and incubated at 25±2°C for 3 days. The emerging fungi were subcultured into fresh PDA medium under the same above conditions until pure colonies were obtained for the pathogenicity test.

1.3.2. Pathogenicity test

The pathogenicity test of the isolated fungi was performed using the procedure of Moline (1984). Briefly, apparently healthy fresh tomato fruits (cv. Rio grande), were washed under tap water and sterilized. The air-drying fruits were punctured with a sterile needle to a depth of 3 mm at the equatorial side and placed in a plastic box lined with wet, sterilized filter paper. Thus, a conidial suspension of suspected pathogens was prepared by scraping the mycelium from a 5-day-old culture in physiologic water to obtain 10^6 conidia/ml. Twenty microliters of inoculum was inoculated on the wounded surface, which was covered with humidified cotton wool. Fruits inoculated with physiological water served a negative control. Another control was prepared without conidia suspension and physiologic water. Fruits were stored at 25±2°C for 5 days. Three replications were prepared, and the experiment was repeated twice. The fruits were observed daily for rot development. After incubation, disease incidence was determined as the percent ratio between the numbers of rotten wounds per total number of wounds (Talibi *et al*., 2012). The section of each fruit was cut along the plane of inoculation, and the diameter of the rotted area of suspected pathogens was measured from inoculation points (Oladiran and Iwu, 1993). The virulence status was determined following the scale described by Chehri (2015) as follows:

a = no visible symptom (nonvirulent);
b = 5–10 mm^2 of rotted area (hypovirulent);
c = 11–20 mm^2 of rotted area (moderately virulent); and
d ≥ 21 mm^2 of rotted area (high virulence). Reisolations were made by culturing diseased tissues onto PDA, and the resulting colonies were identified to complete Koch's postulates.

1.3.3. Identification of pathogens

1.3.3.1. Morphological identification

The morphology was observed by using a special key for identification (De Hoog *et al*., 1986). Macroscopic observation was based on the coloration of colonies on the right and reverse sides of Petri dishes and the aspect and colony diameter after 10 days of growth on PDA medium. Microscopic observation was focused on the coloration, aspect and width of hypha, conidiogenesis, and aspect and shape of conidia.

1.3.3.2. Molecular identification

Sequencing of the ITS1-5.8S rDNA-ITS4 gene region was used to confirm identity from morphological tools. Molecular identification was performed through DNA extraction, DNA amplification and sequencing.

1.3.3.3. Genomic DNA extraction

Desoxyribonucleic acid (DNA) was extracted from mycelial fragments scraped from the surface of 7-day-old culture plates at 25±2°C. The mycelia were frozen at -21°C and lyophilized for 24 hours. Thereafter, a commercial kit (NucleoSpin^{R} Plant II) was used as recommended by the manufacturer (MACHEREY-NAGEL, www.mn-net.com) to extract DNA.

1.3.3.4. Desoxyribonucleic acid amplification of ITS regions

DNA was amplified by polymerase chain reaction (PCR) with the universal primers ITS1 and ITS4. The ITS1-5.8S rDNA-ITS4 gene region was amplified in a system including 10 µl of Dream Taq Green Master Mix (2%), 2 µl of primers ITS1 (5 µm), 2 µl of primers ITS4 (5 µm), 4.6 µl of deionized distilled water and 2 µl of DNA templates. The PCR conditions were 95°C for 5 minutes (denaturation) followed by 35 cycles of 95°C for 30 seconds, 50°C for 45 seconds and 72°C for 1 minute, after which the reaction was kept at 72°C for 10 minutes (extension).

1.3.3.5. Agarose gel electrophoresis

To check if the PCR was carried out effectively, gel electrophoresis was performed using a 1% agarose gel prepared by dissolving 1 g of agarose in 100 mL of Tris-acetate ethylenediaminetetraacetate (TAE) at 1%. Thus, gel was cover with TAE. Two µl of Gel loading buffer was add to 4 µl of DNA samples and 4 µl of the mixture obtained were transferred on the gel. Four microliters of DNA ladders of 100 and 1000 base pairs were used as markers. The voltage was set at 70 volts and 40 mA for 5 minutes then, 100 volts and 40 mA for 30 minutes. Ten microliters of SYBR SAFE were added to allow visualization of DNA bands. The gel was exposed to ultraviolet light under the transluminator, and the DNA fragments were visualized.

1.3.3.6. Purification and sequencing

PCR amplicons were purified by mixing 10 µL of samples with 4 µL of clean sweep PCR purification enzyme (Applied Biosystems by Thermo Fisher Scientific, Lithuania). The purification conditions were 37°C for 15 minutes then 18°C for 15 minutes.

The BLAST (Basic Local Alignment Search Tool) algorithm was used to screen the NCBI (National Center for Biotechnology Information)/GenBank database of fungal nucleotide sequences to identify ITS sequences homologous to those obtained from pathogenic fungi. A sequence similarity dendrogram was developed with the nucleotide sequences of the pathogens and those of reference strains available in NCBI GenBank.

1.3.4. Inhibition assay of fungal conidia germination

The inhibition test of conidial germination was performed using the microdilution method in 96-well plates as described by Toghueo *et al*. (2016). Briefly, 100 µL of sterilized potato dextrose broth (PDB) medium supplemented with chloramphenicol (500 mg/L) was distributed in all wells, followed by the addition of 100 µL of stock essential oil solution to the first well. After thorough mixing, a twofold serial dilution of the first term (5.67 µL/mL) and the last term (0.09 µL/mL) was performed by successive transfer of 100 µL from the first wells into subsequent wells. Then, 100 µL of fungal conidia prepared in physiologic water was inoculated in the wells except those of the sterility control to achieve a final tested charge of 10^6 conidia/mL. The sterility control consisted of PDB medium without treatment and inoculation. A negative control was prepared with Tween 80, while positive control wells were prepared using Propiconazole Tilt 25 EC. The test was performed in triplicate. Upon incubation at 25±2°C for 20 h, the microdilution plates were read on a microplate reader at 450 nm. The obtained value of optical density (OD) served to determine the conidia germination inhibition percentage [CGI (%)] as follows:

$$CGI\ (\%) = ((OD_c - OD_t)/(OD_c)) \times 100$$

OD_c is the mean OD value from the negative control well, and OD_t is the mean OD value from the test well.

The lowest concentration given 100% of CGI (%) was the MIC.

1.4. Statistical analysis

The doses causing 50% (LD_{50}) and 90% (LD_{90}) mortality as well as those inducing 50% and 90% (KD_{50} and KD_{90}, respectively) knockdown were determined by probit analysis (Finney, 1952) using Online Tool (OPSTAT) (http://14.139.232.166/Probit/probitanalysis.html). The differences between means were tested through analysis of variance (ANOVA) followed by Fisher's least significant difference test at P=0.05 level of significance using the Statgraphics Plus 5.1 statistical package.

2. Results

2.1. Insecticidal activity assay of *Cymbopogon citratus* essential oil through ingestion toxicity

2.1.1. Larvicidal effect of *Cymbopogon citratus* essential oil on *Tuta absoluta*

The results showed that *C. citratus* (lemongrass) essential oil exerted a larvicidal effect on *T. absoluta* in a dose-dependent manner, while positive and negative controls induced no mortality at the concentration tested after 4 hours of exposure (Figure 1). However, 2.5% mortality was observed with the reference insecticide treatment after 24

hours of exposure, showing the rapid action of essential oil compared to the positive control. The LD_{50} and LD_{90} values were 29.762 µL/mL and 64.931 µL/mL, respectively, after 4 hours of treatment (Table 1).

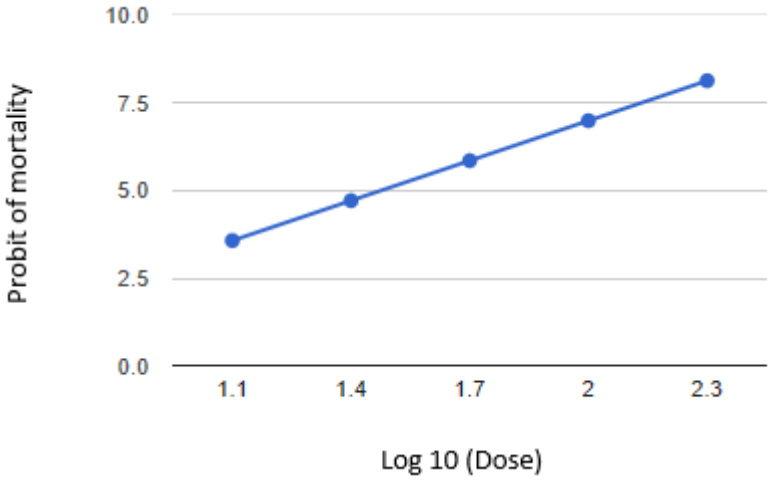

Figure 1: Larval mortality of *Tuta absoluta* as a function of *Cymbopogon citratus* essential oil doses by ingestion route as per the probit transformation model

Table 1: LD_{50} and LD_{90} values of *Cymbopogon citratus* oil on *Tuta absoluta* larvae by ingestion toxicity after 4 hours of exposure

Essential oil	LD_{50}[a] (FL 95%)[b] (µL/mL)	LD_{90}[c] (FL 95%) (µL/mL)	Slope ±SE	Intercept ±SE	$(\chi 2)$[d]
Lemongrass oil	29.762 (25.463-34.787)	64.931 (55.552-75.895)	3.783±0.297	-5.574±0.451	97.130
Lynx®	ND	ND	ND	ND	ND

SE Standard Error, ND Not Determined, positive control plates were tested at a single concentration and there was no dead up to 4 hours
[a]Dose causing 50% mortality
[b]95% fiducial limits
[c]Dose causing 90% mortality
[d]Chi square

2.1.2. Knockdown activity of *Cymbopogon citratus* essential oil on *Tuta absoluta* larvae

The results revealed that only three doses (12.5 µL/mL, 25 µL/mL, and 50 µL/mL) effectively knocked down (immobilized but not killed) the larvae from the five doses tested (12.5 µL/mL, 25 µL/mL, 50 µL/mL, 100 µL/mL and 200 µL/mL) (Figure 2). At the higher doses (100 µL/mL and 200 µL/mL), all the larvae exposed to the essential oil died. The probit model showed that the percentage of knockdown larvae increased with the doses of *C. citratus* essential oil. No knockdown was observed on positive and negative controls after 4 hours. However, 2.5% of larvae knocked down were observed with Lynx® treatment after 24 hours exposition. The KD_{50} and KD_{90} values were 27.499 µL/mL and 49.044 µL/mL, respectively, after 4 hours of exposure (Table 2).

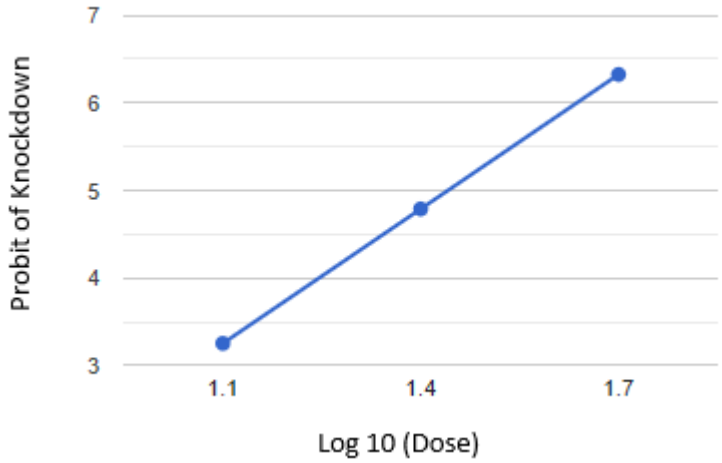

Figure 2: Larval knockdown of *Tuta absoluta* as a function of *Cymbopogon citratus* essential oil doses by ingestion route as per the probit transformation model

Table 2: KD_{50} and KD_{90} values of *Cymbopogon citratus* oil on *Tuta absoluta* larvae by ingestion toxicity after 4 hours of exposure

Essential oil	KD_{50}[a] (FL 95%)[b] (µL/mL)	KD_{90}[c] (FL 95%) (µL/mL)	Slope± SE	Intercept ± SE	$(\chi 2)^2$[d]
Lemongrass oil	27.499 (16.101-46.964)	49.044 (28.716-83.762)	5.100± 0.467	-7.341±0.675	26.023
Lynx®	ND	ND	ND	ND	ND

SE Standard Error, ND Not Determined, positive control plates were tested at a single concentration and there was no knockdown up to 4 hours
[a]Dose causing 50% knockdown
[b]95% fiducial limits
[c]Dose causing 90% knockdown
[d]Chi square

2.1.3. Effect of treatments on larval and pupal durations of surviving larva

At 12.5 µL/mL, the larval duration results revealed that no significant difference was observed upon exposure to lemongrass oil (2 days 12 hours) and the negative control (Tween 80) (2 days 12 hours), while the oil significantly decreased ($p < 0.001$) the larval period compared to the positive control (λ-cyhalothrin 15 g/L + acetamiprid 20 g/L) (2 days 22 hours) (Figure 3). At the same concentration, *C. citratus* oil (7 days 9 hours) significantly prolonged ($p < 0.001$) pupal duration compared to negative (6 days 21 hours) and positive (7 days 5 hours) controls.

At a dose of 25 µL/mL, lemongrass oil (2 days 16 hours) significantly lengthened ($p < 0.001$) the larval duration relative to the negative control (2 days 12 hours); however, the larval duration of the oil decreased significantly ($p < 0.001$) relative to the positive control (2 days 22 hours). The pupal duration results showed that lemongrass oil (7 days

12 hours) significantly prolonged ($p < 0.001$) the pupal period compared to the negative (6 days 21 hours) and positive (7 days 5 hours) controls.

At 50 µL/mL, *C. citratus* oil significantly lengthened ($p < 0.001$) larval and pupal durations compared to controls. For larval duration, periods were 3 days for the oil, 2 days 12 hours and 2 days 22 hours for negative and positive controls, respectively. For the pupal duration, the recorded durations were 8 days for the oil, 6 days 21 hours and 7 days 5 hours for the negative and positive controls, respectively. At concentrations above 50 µL/mL, there was no transition from the larval to the pupal stage or from the pupal to the adult stage.

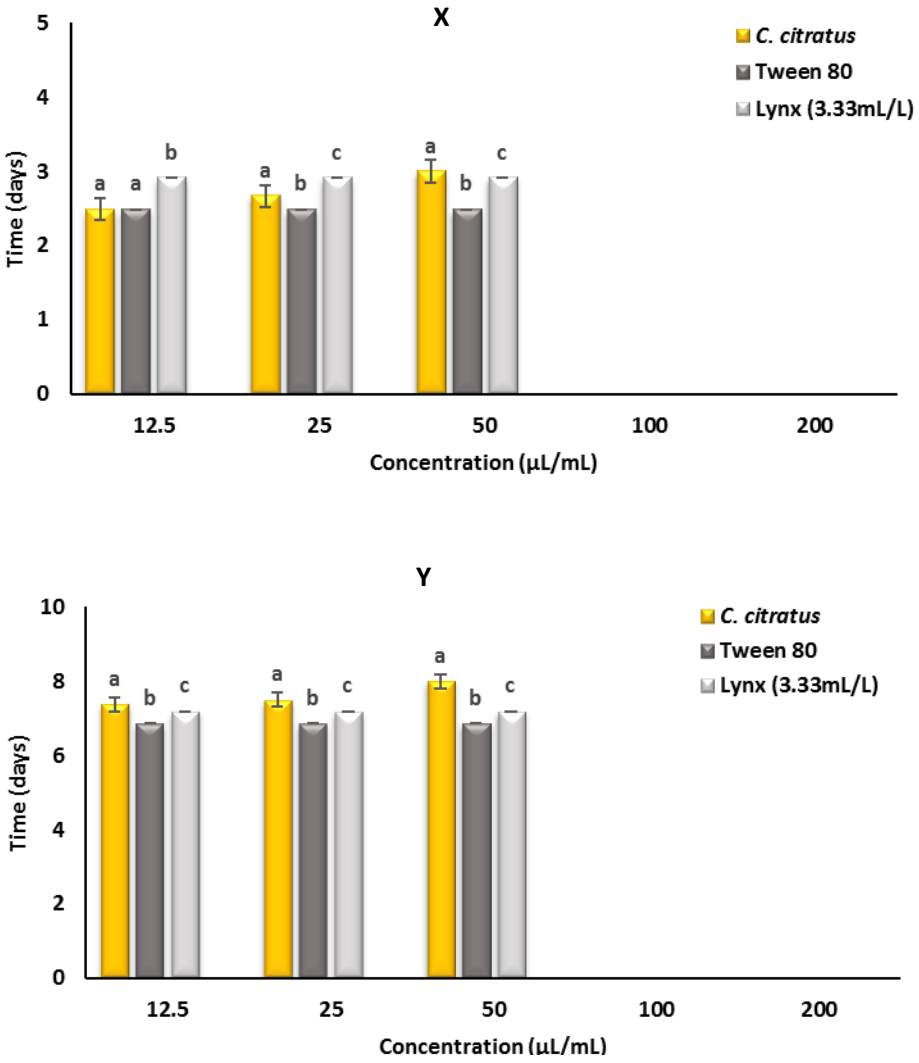

Figure 3: Larval (x) and pupal (y) durations of surviving *T. absoluta* through exposure to *C. citratus* oil by ingestion

Bars bearing different letters depict significant difference as referred to the Least Significant Difference (LSD) test. At doses above 50 µL/mL, all the larva and pupa died

2.2. Antifungal activity of *Cymbopogon citratus* essential oil against *Geotrichum candidum*

2.2.1. Isolation of *Geotrichum candidum* and pathogenicity test

From fruits infested by *T. absoluta* and presenting sour rot symptoms, 25 strains were isolated. The pathogenicity test revealed that only 3 isolates showed sour rot characteristics with water-soak lesions that contained white mycelium. As decay progressed, fruits became soft with a distinctive sour odor. The three pathogenic isolates were named G1, G2 and G3. The disease incidence was 100% for each pathogen, meaning that all fruits inoculated with G1, G2 and G3 developed sour rot characteristics (Figure 4). In addition, fungal colonization measurements showed that there was a significant difference between the width fungal growth of each pathogen. Thus, in a decreasing manner, the growth diameter of G2 isolate (58.33 mm) on infected fruits was significantly higher than those of G3 isolate (35.83 mm) and G1 isolate (14.38 mm) ($p<0.05$) (Table 3). The evaluation of disease virulence revealed that all the pathogens were highly virulent, as the calculated rot areas were more than 21 mm^2 (**Chehri, 2015**).

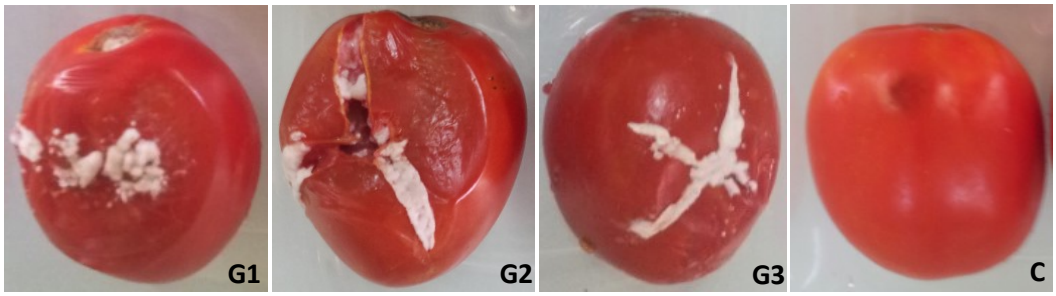

Figure 4: Rot symptoms caused by G1, G2 and G3 isolates on wounded tomato fruits (cv. Rio grande) compared to the control (C)

Table 3: Rot diameter and virulence of pathogens after five days of incubation

Pathogens	Rot diameter (mm)	Rotted area (mm^2)	Virulence (a-d scale)	Virulence status
G1	14.38 ±1.08[a]	162.91 ±24.98[a]	D	High virulent
G2	58.33 ±2.95[b]	2677.10±267.48[b]	D	High virulent
G3	27.29± 0.36[c]	585.06±15.4[c]	D	High virulent

Means followed by different letters in the same column are significantly different ($p<0.05$) according to the least significant difference (LSD) test.

2.2.2. Identification of *Geotrichum candidum*

2.2.2.1. Morphological identification

The three pathogens morphologically belong to *Geotrichum* sp. based on colony morphology on PDA. Indeed, fungi showed milky white colony, cream reverse color, conidiogenesis belonging to arthric mode, hyphae were hyaline, septate and differentiated into disarticulated hyphae, then into rectangular cells called arthric conidia.

Specifically, after 10 days of culture (25±2°C) on PDA, G1 pathogen was characterized by a soft and farinose aspect and hairless, and the diameter of fungal growth was 90 mm with a predominance of vegetative hyphae and few arthroconidia. G2 showed a hairy aspect, and the diameter of fungal growth was 52 mm. Microscopic observation revealed more arthroconidia than hyphae. G3 was characterized by hairless, flat and smooth aspects, the colony growth size was 90 mm, and there was a proportionality between vegetative hyphae and arthroconidia. The width of the main branches of hyphae varied from 8 to 11 µm for all pathogens. On the basis of these characteristics, all three pathogens G1, G2 and G3 were the isolates of *Geotrichum candidum.*

2.2.2.2. Molecular identification

Sequence analysis

Sequencing of the ITS1-5.8S rDNA-ITS4 gene region revealed that the identity of the pathogens previously presumed to be *Geotrichum candidum* upon morphological identification was confirmed. Similarity with sequences submitted to the GenBank database was established. Indeed, G1 showed 98% homology with *Geotrichum candidum* isolate GC333-ITS1F. G2 presented 99% homology with *Geotrichum candidum* strain JYC546. G3 showed 98% homology with *Geotrichum candidum* culture CBS:11616 (Figure 5). The isolates of *Geotrichum* sp. (G1, G2 and G3) were therefore named *Geotrichum candidum*.

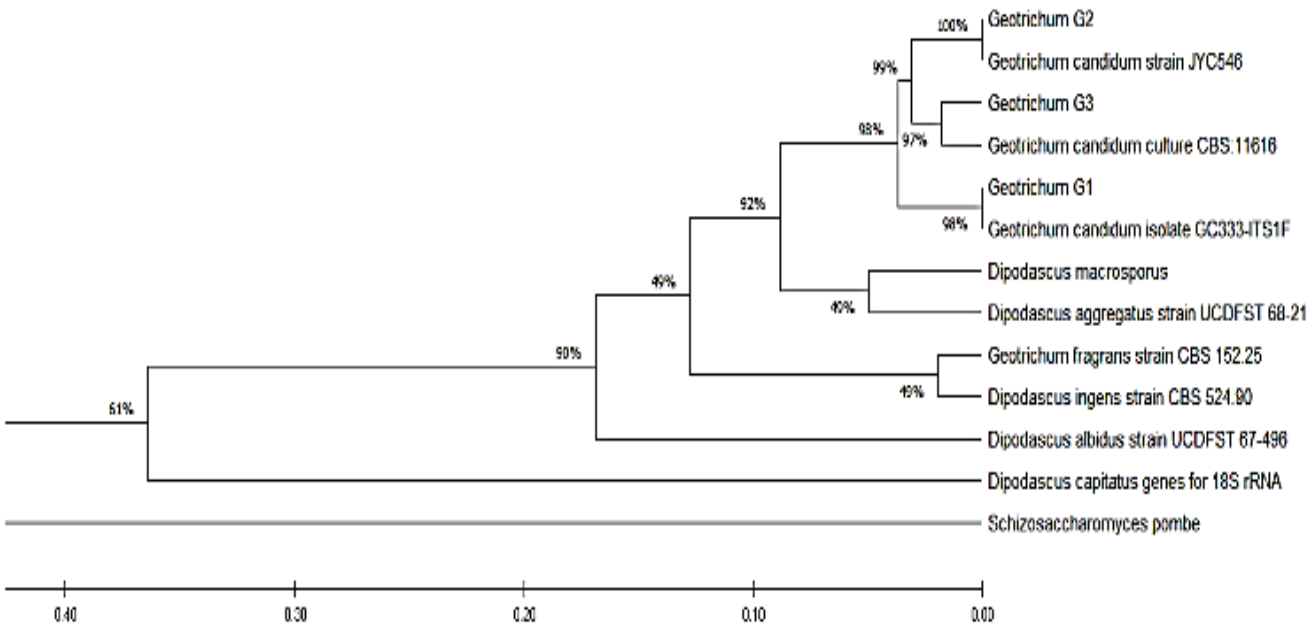

Figure 5: Phylogenetic tree based on comparison of ITS1-5.8 s-ITS4 sequences of pathogenic *Geotrichum* sp. to the reference strains available in NCBI GenBank

2.2.3. Inhibition assay of fungal conidia germination

The percentage of inhibition significantly ($p < 0.05$) increased with the oil concentration (Table 4). At the lowest concentration (0.09 µL/mL), inhibition percentages were 65.09, 62.69 and 80% on *G. candidum* G1, *G. candidum* G2 and *G. candidum* G3, respectively. A 100% inhibition percentage was obtained at 5.67, 1.42 and 0.71 µL/mL for *G. candidum* G1, *G. candidum* G2 and *G. candidum* G3, respectively, corresponding to the MIC values ($P < 0.05$). This result revealed that *G. candidum* G3 was the most sensitive to *C. citratus* oil. The positive control, Propiconazole Tilt 25 EC, totally suppressed the conidia germination of pathogens at all the concentrations tested, and the MIC was undetermined.

Table 4: Conidial germination inhibition percentage of *Cymbopogon citratus* essential oil against *Geotrichum candidum* G1, *Geotrichum candidum* G2 and *Geotrichum candidum* G3

	Inhibition percentage (%)		
Concentrations (µL/mL)	*G. candidum* G1	*G. candidum* G2	*G. candidum* G3
0.09	65.09±0.00 [a]	62.69±1.75[a]	80±0[a]
0.18	67.76±7.56[b]	85.63±5.35[b]	92.35±0.00[b]
0.35	80.51±1.46[c]	88.50±1.74[c]	99.33±1.74[c]
0.71	92.02±0.00[d]	99.81±0.00[d]	100±0[c]
1.42	91.91±0.00[d]	100±0[d]	100±0[c]
2.83	93.85±0.00[e]	100±0[d]	100±0[c]
5.67	100±0[f]	100±0[d]	100±0[c]

Means followed by different letters in the same column are significantly different ($p<0.05$) according to the least significant difference (LSD) test.

3. Discussion

The obtained results revealed that lemongrass oil exhibited larvicidal activity against *T. absoluta* by ingestion toxicity. The mortality rate of insect pest increased with concentration in a dose-dependent manner. Previous works reported the insecticidal activity of *C. citratus* oil against *Agrotis ipsilon* by the ingestion route (Moustafa *et al*., 2021). The results showed that at 96 h post treatment, the LC_{50} value was 2623.06 mg/L on second-instar larvae of the insect pest. Moreover, Sammour *et al*. (2018) determined the insecticidal activity at 0.078% of formulated thyme oil on *T. absoluta* larvae through ingestion toxicity. Ghanim and Ghani (2014) reported the ingestion action of aqueous plant extracts on *T. absoluta* larvae. The elicited insecticidal activity could be associated with the multitude of compounds of the essential oil and their interactions (Nana *et al*., 2015; Scalerandi *et al*., 2018). The oil components acted by inhibiting acetylcholinesterase causing insect paralysis and death (López and Pascual-Villalobos, 2010).

The study of the impact of lemongrass essential oil treatment on the life cycle duration of surviving larvae revealed that *C. citratus* oil significantly lengthened the pupal period compared to the positive and negative controls. This observation showed that the tested oil could delay the development cycle of *T. absoluta.* In fact, some essential

oils exhibited anti-feeding and starvation effects against insect pests, with consequent growth retardation (Tak and Isman, 2016; Moustafa *et al*., 2021). Thus, Sosa *et al*. (2019) reported significant prolongation of the life cycle development of *Spodoptera frugiperda* in relation to feeding deterrence. Similar results were exhibited by Ngongang *et al*. (2021), who found that lemongrass and thyme oils increased the life cycle duration of *T. absoluta* relative to the reference insecticide [Lynx® (λ-cyhalothrin 15 g/L + acetamiprid 20 g/L)] through fumigant toxicity. Authors found that at 0.8 µL/mL, the pupal period of thyme oil (7 days 18 hours) and lemongrass oil (8 days 23 hours) increased significantly compared to the positive control (7 days 9 hours). Larval duration elicited 3 days 7 hours and 3 days for thyme oil and lemongrass oil, respectively, while the reference insecticide exhibited 2 days 9 hours at 1.6 µL/mL.

From rotted tomato fruits previously infested by *T. absoluta*, three isolates of *Geotrichum candidum* were isolated. Previous studies revealed that sour rot of tomatoes caused by *G. candidum* occurred regularly in commercial fields and postharvest settings (Fiedler, 2014). Moreover, Moline (1984) reported *G. candidum* as a wound pathogen requiring injury to enter and cause sour rot of vegetables and fruits. In addition, Bourret *et al*. (2013) identified *G. candidum* as an opportunistic fungus causing rot on tomato fruits collected in a home garden in Washingston state. Natural products such as essential oils are recorded as sustainable alternatives for phytopathogens control. The results from this study showed the inhibitory effect of *C. citratus* essential oil on the conidia germination of the pathogens through the microdilution method. Accordingly, Plotto *et al*. (2003) highlighted the inhibitory activity of lemongrass oil on the conidial germination of *G. candidum* through fumigation and agar dilution assays. The authors reported that by fumigation, *C. citratus* oil inhibited the conidia germination of *G. candidum* by 100% after 4 days of incubation. The same result (100% inhibition percentage) was obtained through the agar dilution method after 10 days of incubation at 1000 mg/L. The activity of lemongrass oil on the pathogens could be justified by the fact that the essential might create lesions on the wall and cell membrane of arthroconidia. This reduces the elasticity of the membrane and thus increases its rigidity. Moreover, once they pass through the cytoplasm, oil components can induce interactions with protein-like macromolecules, resulting in losses of their biological structure and functions (Luo *et al*., 2004).

Conclusion

This work studied the insecticidal and antifungal activities of lemongrass essential oil against *T. absoluta* fourth instar larvae and *G. candidum* associated with tomato rot. The obtained results highlighted the larvicidal effect of *C. citratus* oil on the insect pest through the ingestion route. Moreover, the oil impaired the life cycle development of surviving larvae. The evaluation of the activity of oil on *G. candidum* associated with *T. absoluta* infestation showed the inhibitory action on conidia germination of the pathogen. Thus, *C. citratus* oil could be used in the development of biopesticides to reduce the population density of insect and fungal colonization in tomato fields.

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Conflict of interest

The authors declare no conflict of interest.

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