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ANTIFUNGAL ACTIVITY OF *CITRUS AURANTIUM L.* ESSENTIAL OIL AGAINST CROWN ROT OF WHEAT CAUSED BY *FUSARIUM GRAMINEARUM*

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A B S T R A C T

Fusarium graminearum a causal agent of crown rot of wheat is an important soil-borne pathogen that causes yield reduction on cereal crops. Controlling this disease is crucial to ensure food security. The present study was conducted toward the research of biological method against this pathogen. Our research aimed to evaluate the antifungal activity of *Citrus aurantium* essential oil (EO) at different concentrations against *F. graminearum in vitro*. Also, we assessed the inhibitory effect of EO on disease development in greenhouse conditions. The results showed that EO exhibited a potent antifungal activity with no significant difference compared to chemical fungicide, Difenoconazole. The tested EO effectively inhibited radial growth of the pathogen *in vitro*, and it revealed significant decreasing on disease incidence from 91.7% to 32.8% in the *in vivo* bioassay. In addition, the seed treatment with *C. aurantium* EO improved significantly the growth parameters of the wheat seedlings compared to the untreated control. The chemical characterization of *C. aurantium* essential oil using the gas chromatography analysis allowed the identification of monoterpene hydrocarbon as major group of volatile compounds. These were Limonene (17.6%), Geranyl acetate (11.9%), Geranial (10.8%), Citral (10.3%), Linalool (9.9%) and Linalyl acetate (5.7%). From the current study, we can deduce that essential oils extracted from *C. aurantium L.* leaves can be used as an antifungal bio-product against phytopathogenic *Fusarium* species.

Keywords: *Fusarium graminearum*, bio-fungicide, antioxidant activity, Chromatography analysis.

INTRODUCTION

Fusarium crown rot is a worldwide serious constraint that causes significant reduction on yield and quality of wheat grain (Tunali *et al.,* 2008). Several pathogens are the main responsible of this disease, especially *Fusarium culmorum*, and *F. graminearum (*teleomorph *Gibberella zeae*) (Beccari *et al*., 2011). These fungi can have a negative impact on various plant organs at different stages of their development. Chemical control is the most used method against these fungal diseases. However, the adverse effects on human health (carcinogenic, endocrine disrupting) caused by synthetic products (Kim *et al.,* 2017), the negative influences on the environment, including water, soil and air contamination (Chambers *et al.,* 2014). Moreover, the appearance of pathogen populations resistant to fungicides are serious disadvantages of this method (Hahn, 2014). Therefore, agricultural researches have continually focused on biological control of plant diseases. Finding antifungal compounds in plants is an efficient way to produce new pollution-free pesticides.

Medicinal plants contain some organic compounds and bioactive substances include tannins, alkaloids, terpenoids, steroids and flavonoids (Yadav *et al.,* 2011). They are commonly used in human therapy, veterinary, agriculture and scientific research. A wide range of phytochemicals

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from various chemical classes have showed an inhibitory effect on a large group of microorganisms (Vaou *et al*., 2021).

Essential oils are volatile oily liquids extracted from various plant parts that are widely used as food flavorings, also used for their antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties (Elgayyar *et al.,* 2001). Different essential oils have been found to be more effective and an alternative method against pathogens (Ali *et al.,* 2018).

Citrus aurantium L. popularly known as "bitter orange" is commonly used as a rootstock for his beneficial properties, including resistance to different biotic and abiotic stresses, tolerance to climate change, improvement of fruit quality (Yildiz *et al.,* 2013), also it can be used as an ornamental tree. Furthermore, the plant's leaves, fruit peel and flowers are commonly used for medicinal purposes because of the several bioactive compounds it contains, such as vitamins, tannins, volatile oils, and proteins (Yu *et al.,* 2020).

The aim of the present study is to evaluate the antifungal activity of the essential oil and aqueous extract of *Citrus aurantium* against Fusarium crown rot of wheat caused by *Fusarium graminearum* in both, *in vivo* and *in vitro* experiments. Our research was executed as an alternative approach to chemical control, for this purpose, the GC-MS analysis was used to acquire chromatographic data on chemical components.

MATERIAL AND METHODS

Plant material: *Citrus aurantium* leaves were harvested from their natural habitat in Mascara province the North-West of Algeria in March 2022. The plant material was slightly dried at ambient temperature for 7 days.

Fungal material: The *Fusarium graminearum* FG10 isolate (GenBank accession No. ON685926) was obtained from diseased durum wheat seedling presenting typical symptoms of crown rot, collected from the North-West of Algeria in 2019. It was identified based on morphological and molecular characteristics. The strain was selected based on their high pathogenicity against sensible durum wheat variety (cv. Vitron) (Unpublished data). For the experiment, the target fungus was sub-cultured on potato dextrose agar medium (PDA) at 25 °C for 7 days.

Essential Oil and Aqueous Extract Extraction: Essential oil was extracted from dried leaves by the hydro-distillation process for 3 h using a Clevenger-type device. The EO obtained was stored in glass dark vials at 4°C. The Aqueous Extract (AE) was prepared using the protocol described by Chen *et al.* (2007). After grinding Bitter Orange dried leaves, 20 g of plant powder were mixed with 200 ml of boiling distilled water for 30 min. After stirring and cooling down the mixture at ambient temperature, the extract was filtered through a filter paper Whatman N° 1, and the filtrate was freeze-dried.

Qualitative phytochemical analysis: To assess the presence of organic compounds, such as proteins, fixed oils, tannins, saponins, flavonoids and steroids. The medicinal plant screening was carried out using standard methods described by Yadav *et al.* (2011).

Antioxidant Activity: The antioxidant activity was determined using the DPPH radical scavenging method as described by Brand-Williams *et al.* (1995). DPPH solution was freshly prepared by mixing 4mg of DPPH with 100ml of methanol 95%. Five concentrations of essential oil were tested by adding 1.5 ml of methanolic DPPH solution to 250 µl of each concentration, then incubated in the dark at room temperature for 16 min. The absorbance was measured in triplicate at 517 nm using a JENWAY 6400 spectrophotometer. The methanolic DPPH solution and the ascorbic acid were used as negative and positive control respectively. The DPPH free radical scavenging activity was calculated using the following formula:

Radical scavenging activity (I%) = [(Abs control – Abs test) / Abs control] x 100

Abs control: absorbance of the control (DPPH solution)

Abs test: absorbance after addition of the essential oil

GC-MS analysis of essential Oil: The Shimadzu GCMS TQ8030 was used for the Gas Chromatography–Mass Spectrometry analysis. Capillary gas chromatograph directly coupled to the mass spectrometer system type GC-2010 plus, GCMS-TQ8030 from Shimadzu. A capillary column (30 mx 0,25 mm, 0,25 µm film thickness) was used under the following conditions: Column Oven Temperature: 50,0°C: Injection Temperature: 220 °C: Injection Mode: Split; Pressure: 100 kPa; Total Flow: 50.0 ml/min; Column Flow: 4.55 mL/min; Linear Velocity: 77.4 cm/sec; Purge Flow: 3,0 ml/min and Split Ratio: 50.0. The column temperature was programmed from 50.0°C to 220.0°C with a rate of 5.00°C/min. The

mass spectrometer (MS) conditions were as follows: Ion Source Temperature: 200.00 °C; Interface Temperature: 220.0 °C; Solvent Cut Time: 3,00 min and Detector Gain: 0.98 kV. The total running time was 31 minutes (Scan Start: 45.00 m/z and Scan End: 500.00 m/z) and Scan Speed: 1666. Authentic chemicals are identified by the database PubChem.

In vitro antifungal assays: The inhibitory effect of the essential oil and the aqueous extract was evaluated measuring the radial growth of *F. graminearum in vitro* using the poisoned food method as described by Soylu *et al.* (2006). For each treatment six concentrations were tested. Different volumes of the testing extracts were added separately to conical flasks containing 100 ml sterile PDA medium before its solidification to obtain the concentrations 50, 100, 200, 250, 500 and 1000 µl L^{-1}. A few drops of the emulsifier Tween 80 (3% of water volume) were added to obtain an aqueous emulsion feature. In addition, the systemic fungicide Score (250 g/L Difenoconazole) of Syngenta crop protection society obtained from Mascara market was used as comparing treatment. The controls consisted only of PDA medium with added Tween 80. Around 18 ml of the prepared medium was poured into each Petri dish.

Mycelial discs (6 mm Ø) taken from the periphery of an actively growing PDA culture of the tested fungi *(F. graminearum*) were placed at the center of the prepared Petri dishes. All plates were incubated at 25°C until the tested fungi reached full growth in the check treatment (approximately 7 days). Three replicates were used for each treatment. The percentage of fungal growth inhibition was calculated according to the following formula:

$$IC(\%) = \frac{DT - DT}{DT} \times 100$$

Where, IC = mycelial growth inhibition (%); Dt = the average diameters of fungal colony of control, DT = the average diameters of fungal colony of treatment.

Effect of EO on emergence, coleoptile and coleorhizae growth: To determine the efficacy of EO against *F. graminearum* pathogenicity, on emergence percentage, coleoptile and coleorhiza growth. The method mentioned by Abdallah-Nekache *et al.* (2019) was adopted. Conical flaks containing 50 ml of Potato Dextrose Broth were inoculated by four mycelial discs (6 mm Ø) taken from the periphery of 7 days' old growing PDA culture of the tested fungi. Flaks were incubated for 7 days at 25 °C in incubator shaker. Mycelium was extracted by centrifugation (5000 g for 10 min), diluted to 13 mg/mL and homogenized by adding drops of the emulsifier Tween 80 (3%). Sterile filter paper was deposited on sterile Petri dishes, then, 8 ml of the previously prepared inoculum were added to the filter paper and a second filter paper was placed on top of the inoculum.

The essential oil formulation was prepared by fusing 500µl of EO with 1 L of sterile distilled water then adding a few drops of the emulsifier Tween 80 (3% of water volume) (Moutassem *et al*., 2019).

For seed inoculation, susceptible durum wheat variety (cv. Vitron) was used in this experiment, which was obtained from the Cereals and Pulses Cooperative (CCLS) in Mascara, Algeria.

Durum wheat seeds (cv. Vitron), were disinfected with 2% sodium hypochlorite, then rinsed thrice with sterile distilled water, and dried between two sterile layers of filter paper.

The disinfected seeds were soaked in the homogenized essential oil formulation and were air-dried at ambient temperature for 30 min. After, the seeds were placed on each Petri plates, at the rate of 10 seeds per plate, and four replicates for each test.

The test included:

Seeds soaked in the homogenized essential oil formulation with pathogen inoculation.

Seeds soaked in distilled water with pathogen inoculation

Seeds soaked in distilled water only.

The plates were incubated at 22 °C in the dark for 6 days. The emergence percentage (%), coleoptile growth (mm) and coleorhiza length (mm) were measured at the end of the experiment.

Effect of essential oil on Fusarium crown rot of wheat in pot experiment: A pot experiment was carried out to examine the *in vivo* efficacy of the tested essential oil against crown rot of wheat.

Pathogenicity test was performed by soaking disinfected seeds (cv. Vitron) in the homogenized essential oil formulation (prepared as the previous experiment) and were air-dried at ambient temperature for 30 min. After, the seeds were sown into a 7cm diameter plastic pot, filled with a sterilized soil mixture and peat (V/V), and inoculated with explants of 6 mm diameter taken

from a 7-day FG10 culture (Demirci and Dane, 2003).

The experiment included two control treatments, seeds treated with sterile distilled water, and inoculated with FG10were used as positive control, whereas, seeds treated with sterile distilled water and uninoculated with FG10 (healthy seedlings without fungus) were used as negative controls.

Pots were placed in greenhouse and kept under observation for 30 days after sowing. All tests were performed in triplicate.

At the end of the experiment, plants were carefully removed and washed to evaluate plant height, root length, fresh and dried root and stem.

Disease assessment

Disease development of each treatment was evaluated in terms of disease incidence (DI), and disease severity (DS), 30 days after sowing using the following formula:

(DI) (%) = (Number of infected plants/ Total number of inoculated plants) x 100

Disease severity (DS) of Fusarium crown rot was estimated using the disease scale (0-3) described by Grey and Mathre (1984), [0 = absence of crown browning, 1 = 1-25% of crown browning, 2 = 26-50% of crown browning, 3 = > 50% of crown browning].

STATISTICAL ANALYSIS

The normality test of the data obtained was done using Shapiro-Wilk normality. The results were statistically evaluated using two-way analysis of variance (ANOVA) using R functions (R Core Team 2021). Mean separation was tested using Honesty Significance Difference of Tukey, HSDT. Significance was evaluated at $p < 0.05$ for all tests. Each test was done in triplicate.

RESULTS

Qualitative phytochemical analysis: Preliminary phytochemical screening of leaves extract showed the presence of various metabolites such as flavonoids, tannin, saponins, terpenoids, volatile oil, proteins and amino acids, carbohydrates, reducing sugars, and absence of alkaloids and anthraquinones.

Antioxidant Activity: The DPPH scavenging activity increased with increasing essential oil concentration. The oil exhibited a potent antioxidant activity (80.8%) but lower than the synthetic Ascorbic Acid (98.1%) on 1000 µg/ml (Figure 1).

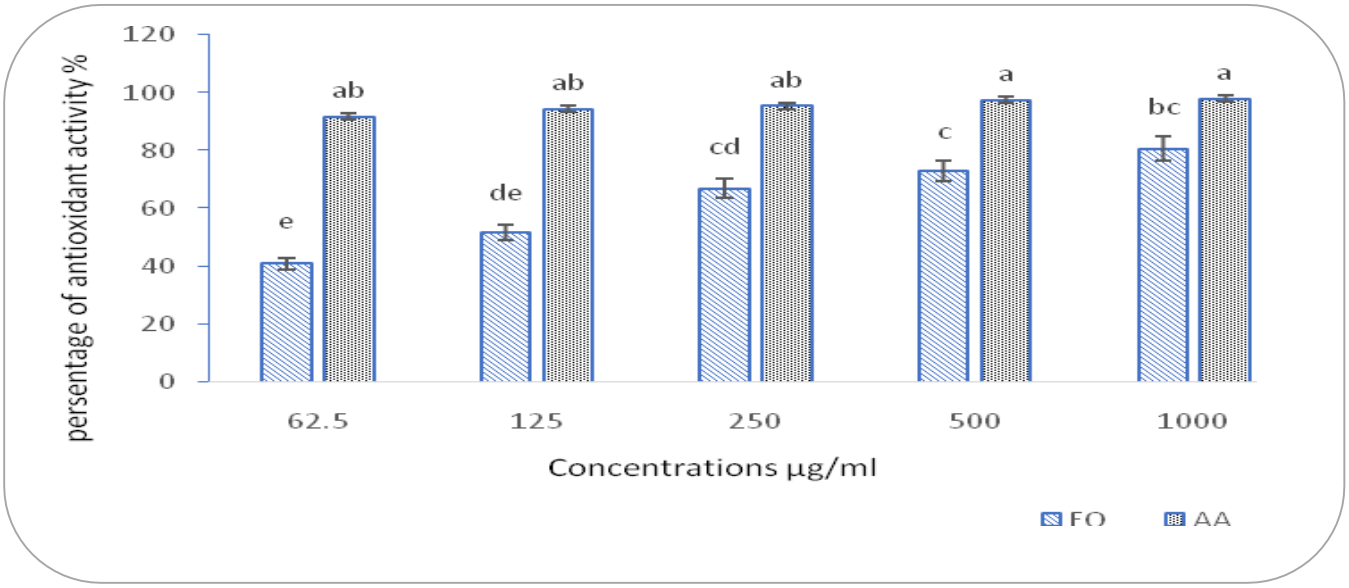

Figure 1. Antioxidant activity of *Citrus aurantium EO* at different concentrations, measured by DPPH radical. Ascorbic acid (AA) was used as standard.

GC-MS analysis of Essential Oil: The yield of essential oil from leaves was 0.5% through hydro distillation. The oil was pale yellow with aromatic odor. The GC-MS analysis showed the existence of monoterpene hydrocarbon as major group of volatile compounds. These were Limonene (17.6%), Geranyl acetate (11.9%), Geranial (10.8%), Citral (10.3%), Linalool (9.9%), Linalyl acetate (5.7%), Geraniol (4.9%), Nerol (4.6%), α- Terpineol acetate (4.1%), Neryl acetate (3.9%), β-Ocimene (2.6%), Citronellal (1.9%), Eucalyptol (1.65%), Citronellol (1.1%) and other minor compounds (Table1).

Table 1. Chemical composition of essential oil from *Citrus aurantium* leaves.

Compounds	Height %	Area %	Retention time
Limonen	17.6	14.7	3.07
Geranyl acetate	11.8	14.5	14.00
Geraniol	10.8	6.7	14.04
Citral	10.3	14.6	12.85
Linalool	9.9	12.2	10.06
Linalyl acetate	5.7	5.7	10.15
Geraniol	4.9	4.5	16.68
Nerol	4.6	4.3	15.61
α- Terpineol acetate	4.1	4.0	13.23
Neryl acetate	4.0	4.0	14.64
β-Ocimene	2.6	1.6	3.85
Citronellal	1.9	1.9	8.30
Eucalyptol	1.6	0.9	3.13
Citronellol	1.1	1.1	14.92
α- Ocimene	0.8	0.5	3.59
γ-Caryophyllene	0.7	0.9	10.59
α- Terpineolene	0.7	0.4	4.27
Sulcatone	0.6	0.4	5.36
Other compounds	6.5		

Efficacy of plant extracts and synthetic fungicide against *F. graminearum* in vitro: Effect of essential oil, aqueous extract and difenoconazole on *F. graminearum* was evaluated measuring the radial growth inhibition *in vitro*. The results are presented in (Table 2). Essential oil treatment at 100, 200 and 250 µl L^{-1} reduced the mycelial growth by 56.7, 69.6 and 100.0%, respectively. However Aqueous extract treatment showed lower inhibition activity on mycelial growth by 40.4, 47.5 and 66.5%, respectively. This treatment exhibited a phenotypic modification represented in yellow pigmentation (Figure 2). Fungicide difenoconazole used as positive control showed a higher inhibitory effect on mycelial growth by 70.42, 82.92 and 100% respectively. Statistically analyzed result clearly indicated that the fungitoxicity of *C. aurantium* essential oil was significantly higher than aqueous extract. Compared to difenoconazole, essential oil treatment was slightly less inhibitory and aqueous extract treatment was significantly less inhibitory.

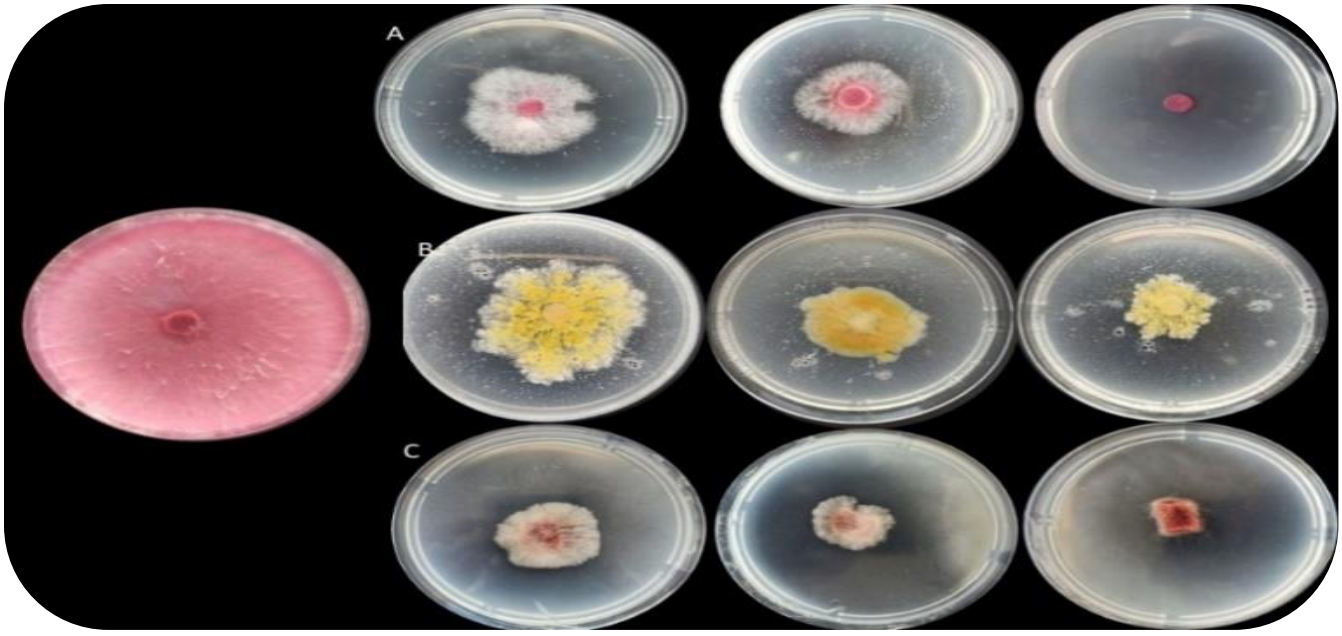

Figure 2. *F. graminearum* (FG10) colony development on PDA medium amended with, A (Essential Oil), B (Aqueous Extract) and C (difenoconazole) from left to right 100, 200 and 250 µl L^{-1} compared to the control.

Table2. Effect of essential oil, aqueous extract of *citrus aurantium*, and difenoconazole on mycelia growth inhibition (%) of *Fusarium graminearum* (FG10).

Concentration (µl L^{-1})	Difenoconazole	Essential oil	Aqueous extract
50	61.67±0.83c	41.25±3.81d	17.92±2.53e
100	70.42±1.81bc	56.66±1.81c	40.42±1.10d
200	82.92±2.20b	69.58±2.31bc	47.5±0.72d
250	100±0.00a	100±0.00a	66.46±1.78c
500	100±0.00a	100±0.00a	70±0.72bc
1000	100±0.00a	100±0.00a	74.17±1.10bc

The values represent the mean of the 3 replicates ± standard error; data marked by the different letters in the columns indicate a significant difference at $P \leq 0.05$.

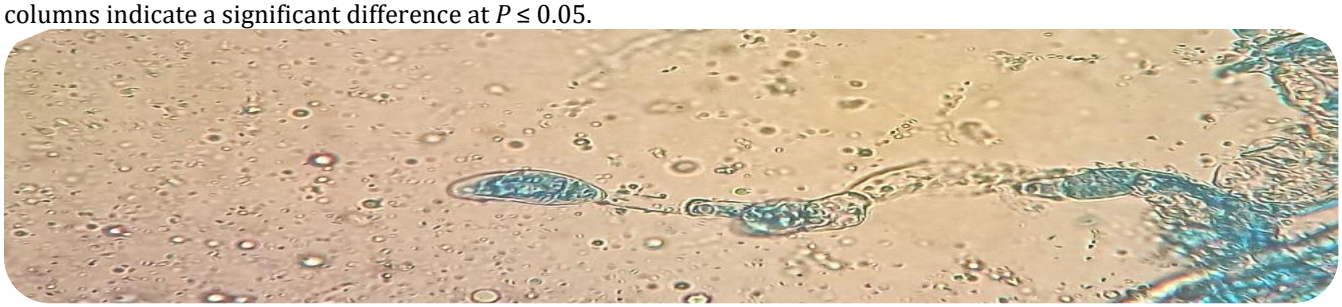

Figure 3. mycelial membrane degradation and vacuolization due to EO treatment.

Effect of EO on emergence, coleoptile and coleorhizae growth: The efficacy of EO, on emergence percentage, coleoptile and coleorhiza length was assessed six days after sowing. The data of our experiment indicate that *F. graminearum* ON685926 was responsible for 33.33% of seed emergence inhibition, in addition, it caused 62% and 68% on coleoptile and coleorhiza length reduction respectively, compared to the control. Whereas, *C. aurantium* essential oil application on infected seeds increased emergence by 13%, and 30% of coleoptile length.

Efficacy of essential oil on Fusarium crown rot of wheat: The EO effect on disease development 4 weeks after treatment is reported in (Fig 5**).** Wheat seedlings of positive control (inoculated with pathogen only) showed symptoms of crown rot after 11 days of sowing (Figure 4). The symptoms of the disease were evident from day 20. Whereas the plants treated with *C. aurantium* EO showed significant effectiveness on the disease reduction. Between the positive control and the treated, the results revealed significant decreasing on disease incidence from 91.7% to 32.8%. Also, the severity was significantly decreased from 57% to 12%.

Figure 4. Essential oil effect on disease development *in vivo*, A (seedling treated with EO solution and inoculated with pathogen) and B (seedling inoculated with pathogen only, positive control), A1-A2 (absence of crown and root rot symptoms), B1-B2 (crown rot and dry rot of root), A3-B3 (crown transversal cross-sections).

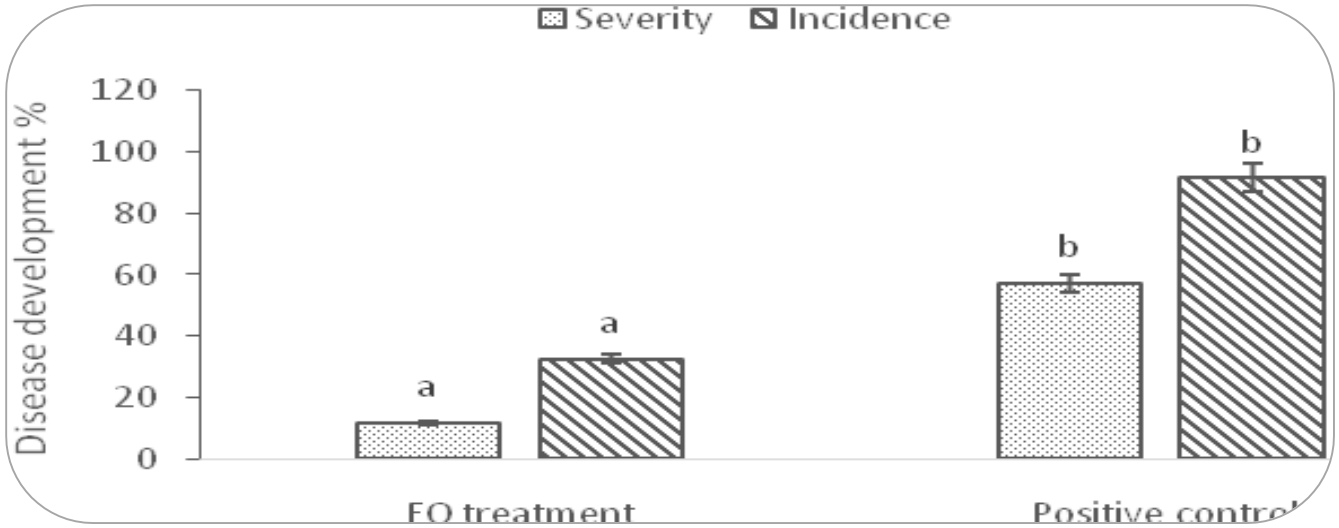

Figure 5. The inhibitory effect of EO on disease development (disease severity and incidence) 30days after inoculation.

Furthermore, our results showed that the crown longitudinally cutting reveals a rot of sap conducting vessels in the untreated plant, compared to the treated plant the sup conduction vessels remain intact (Figure 4, A3-B3). This result may due to the mechanism applied of EO on the pathogens.

The application of EO controlled Fusarium crown rot of wheat. This disease reduction was reflected to growth parameters (table 3). When compared to untreated control, the results showed significant decreases in plant stem height (31 cm and 23 cm), root length (27 cm and 17 cm), fresh stem weight (2.42 g and 0.7 g), dry stem weight (0.58 g and 0.14 g), fresh root weight (1.03 g and 0.34 g) and dry root weight (0.19 g and 0.047 g).

Table 3. Influence of *Citrus aurantium EO* on plant growth parameters in greenhouse experiment.

Treatment	Growth parameters					
	Plant height	Root length	Fresh weight(g)		Dry weight(g)	
	(cm)*	(cm)*	Shoot	Root	Shoot	Root
Control	41.03±1.01^{a}	30.50±0.37^{a}	4.07±0.13^{a}	1.72±0.14^{a}	0.97±0.03^{a}	0.32±0.01^{a}
F.graminearum.	23.7±0.80^{c}	16.97±1.18^{c}	0.70±0.08^{c}	0.34±0.06^{c}	0.14±0.02^{c}	0.047±0.002^{c}
F.graminearum + EO	31.46±1.27^{b}	27.43±0.40^{a}	2.43±0.09^{b}	1.30±0.07^{b}	0.58±0.04^{b}	0.195±0.02^{b}

The values represent the mean of the 3 replicates ± standard error; data marked by the different letters in the columns indicate a significant difference at $P \leq 0.05$.

DISCUSSION

Rutaceae family is known by mixture of bioactive components which act as antimicrobial substances and limited the negative impact of synthetic agents (Jing *et al.,* 2014; Trabelsi *et al.,* 2016). Citrus essential oils are widely used not only in pharmaceutical products for their therapeutic and antiseptics properties, but also as food preserves and additives in several industries, in cosmetics and perfumes, and other industrial fields (Moraes *et al.,* 2009; Yu *et al*., 2020). In recent years, several researchers have focused on the antimicrobial activity of *C. aurantium* EO as a novel approach in controlling human pathogenic and spoilage microorganisms in foods (Badawy *et al*., 2019; Hajlaoui *et al*., 2021), However, their applications in plant protection against phytopathogenic fungi have not yet been studied extensively. Therefore, this study was conducted to evaluate *C. aurantium* EO as alternative to the synthetic pesticides in the control of Fusarium crown rot of wheat.

In our investigation, the essential oil of *C. aurantium* leaves was obtained by the hydrodistillation method. The obtained EO yield (0.5%) is lower than that found by Ouedrhiria *et al*. (2015) from *C. aurantium* leaves and zest with 0.8 and 1.9% respectively. However, this value is greater than that obtained from *C. aurantium* growing in Greece and in Tunisia, which are 0.27% and 0.40% respectively (Sarrou et *al*., 2013; Bnina et *al*., 2019). Ellouze *et al*. (2012) reported a season's variation impact on EO yields from

Tunisian *C. aurantium* leaves, which were ranged from 0.31% to 0.56% according to the harvest season. Generally, the yield of an EO depends on many factors; extraction technique, growth stage, genetic differences, environmental conditions and geographic variations (Ellouze *et al*., 2012; Gaff *et al*., 2020)

The DPPH radical scavenging of our essential oil indicated a potent antioxidant potential. According to Ruberto *et al.* (2000), Badawy *et al.* (2019), the strong antioxidant activity may due to oxygenated monoterpenes (Citronellyl acetate, geranyl acetate and (Z)-limonen oxide, and monoterpenes hydrocarbons (α-terpinene, β-Myrcene, limonene and β-ocimene)). Similar observation has been mentioned by Dosoky and Setzer (2018) and Değirmenci and Erkurt (2020). However, Sarrou *et al*. (2013) and Mejri *et al*. (2022) indicated that EO of old leaves has higher antioxidant activity then young leaves. The chemical profile of the essential oil was determined by gas chromatography analysis. The main compounds detected were Limonene (17.6%), Geranyl acetate (11.9%), Geranial (10.8%), Citral (10.3%), Linalool (9.9%) and Linalyl acetate (5.7%).

The knowledge about the EO composition could help to recognize its mechanism. Previous reports on essential oils extracted from *C. aurantium* leaves have mentioned that they contain almost the same components with a significant variation in concentration depending on leaves age (young or old), variety and location. Chinese samples contained as major components terpinen-4-ol (21.0%), dipentene (limonene) (11.7%), terpinene (9.2%) (Jiang *et al.,* 2011). Another samples from Tunisia contained as major components linalool (43.2% to 66.0%), linalyl acetate (0.8 to 24.8) and α-terpineol (9.3 to 12.1) (Ellouze *et al.,* 2012).

To control Fusarium crown rot, the antifungal effect of essential oil and aqueous extract obtained from *C. aurantium* leaves were assessed in comparison with that of a commercial fungicide. Our results demonstrated that all the treatments used at different concentrations have a differential activity against *F. graminearum* growth in a concentration-dependent manner. The EO applied by incorporation into PDA medium had strongly reduced *F. graminearum* growth, However, the aqueous extract showed the lowest inhibition rates. These findings are in agreement with numerous studies; Elgat *et al.* (2020) found that *C. aurantium* EO can control *Aspergillus* spp, Ortuño *et al.* (2011) reported the antifungal activity of this oil against toward *Penicillium digatum.* Hajlaoui *et al.* (2021) demonstrated that *Citrus* essential oils can be used also as antibacterial product. According to Zabka and Pavela (2018), antifungal effect of essential oils depends on a high content of terpenoids such as limonene. Jing *et al*. (2014) have mentioned some molecules (limonene, octanal, citral, α-terpineol, neral, geranial and β-myrcene) which act against a large group of pathogens. Because of the properties of these bioactive components (low solubility in water and high hydrophobicity), several studies have suggested that volatile compounds may target the cell membrane (Figure 3) (Lang and Buchbauer, 2012; Reyes *et al.,* 2020). As an example, terpenes and phenolic molecules have been shown in previous studies to damage the membranes of both fungi and bacteria, causing proteins denaturation (Wan *et al.,* 2019; Konuk *et al.,* 2020).

Aqueous extract which evoked a yellowish color on mycelia compared to essential oil treatment demonstrated a difference among organic and volatile compounds. O'Mara *et al.* (2020) declared PH as the major factor of *Fusarium* colors. The color change is reversible, after re-cultivating the yellow hypha on new PDA medium, the fungi return to its original red color. This changing may also due to excitation of the medium by organic yellow compounds. According to Qin *et al.* (2020), an oxidative stress test using (H_2O_2) on *Fusarium graminearum* can induce a yellowish coloration.

Under greenhouse trials, *F. graminearum* effect on plant growth parameters revealed a lower percentage of seedling emergence and significant regression in development of root and stem. In addition, a brown necrosis crown was observed on the infected plants associated with leaf yellowing. However, wheat seeds treatment with *C. aurantium* EO reduced the incidence and severity of crown rot disease significantly and improved the growth parameters of wheat seedlings as compared to untreated control. The results of our experiment are consistent with those of Moutassem *et al*. (2019) who reported that thyme and lemongrass EOs were very effective in reducing the Fusarium wilt severity and inducing systemic resistance in chickpea seedlings.

CONCLUSION

Our investigation highlights the valorization of *Citrus aurantium L.* plant in terms of the chemical composition of their essential oil, the potent antioxidant activity and the antifungal effect. The results have clearly indicated that the tested EO can reduce the pathogen growth in both *in vitro* and *in vivo* experiments. From the current study, we can deduce that essential oil extracted from *Citrus aurantium L.* leaves can be used as an antifungal bioproduct against phytopathogenic fungi. Further researches are needed to evaluate the toxicity of this essential oil *in vivo* in order to establish their efficiency as biopesticides and their safety aspects.

Data availability statements: The datasets collected and analyzed during the current study are available from the corresponding author on request. The corresponding author had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure statement: No potential conflict of interest was reported by the authors.

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