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Fatma Zohra Study of the Antifungal Activity of Aqueous Extracts of *Cleome arabica* Against *Fusarium proliferatum* and *Alternaria alternata* Isolated from Potato (*Solanum tuberosum*) in the El Oued Region

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Abstract

This study aims to analyze the chemical composition of aqueous extracts from different parts of *Cleome arabica* and evaluate their antifungal activity against the phytopathogenic fungi *Alternaria alternata* and *Fusarium proliferatum*, isolated from post-harvest potato tubers in the El Oued region. The aqueous extracts were obtained through maceration under agitation for 24 hours. Phytochemical tests conducted on the aqueous extracts revealed the presence of various chemical compounds, including tannins, flavonoids, saponins, alkaloids, and reducing compounds. HPLC analysis revealed that the aqueous extract of *Cleome arabica* leaves contains acetylsalicylic acid, ascorbic acid, gallic acid, and pyrogallol. In contrast, the stem extract is characterized by the presence of vitamin D3 and ascorbic acid, while the root extract contains gallic acid and vitamin B1. The antifungal activity of the aqueous extracts was evaluated using the direct contact method on the mycelial growth of the tested strains.The results highlighted a strong antifungal potential against these strains. The aqueous extract of *Cleome arabica* leaves exhibited 100% antifungal inhibition against *Fusarium proliferatum* and *Alternaria alternata* at concentrations of 1%, 10%, and 15%. The aqueous extract from the stem of *Cleome arabica* showed total antifungal activity (100%) against both tested fungi at concentrations of 10% and 15%. Regarding the root extract, concentrations of 10% and 15% resulted in complete (100%) inhibition of *Alternaria alternata* and *Fusarium proliferatum*. These findings highlight the potential of *Cleome arabica* leaves, stems, and roots as a natural alternative to synthetic chemical fungicides.

Keywords: Potato, phytopathogenic fungi, *Cleome arabica*, aqueous extracts, antifungal activity.

1. Introduction

The potato is one of the most important crops in the world, ranking after wheat, rice, and maize **(Benkeblia, 2020)**. According to **Devaux et al. (2020)**, its global production covers approximately 19 million hectares, with an estimated yield of 378 million tons. Recent data indicate that fungal diseases have primarily affected essential food crops such as rice, wheat, maize, and potatoes, causing economic losses amounting to billions of dollars **(Peng et al., 2020).** According to **Shuping and Eloff (2017)**, around 8,000 fungal species were implicated in nearly 100,000 plant diseases. In recent years, a global increase in the number of phytopathogenic fungi has been observed, exceeding 19,000 species **(Jain et al., 2019)**.

Currently, synthetic fungicides are widely used to protect plants against these pathogens. However, their production entails high costs and can result in the presence of toxic residues on treated surfaces. Moreover, the repeated use of synthetic fungicides from the same category has led to a loss of effectiveness in several phytopharmaceutical products due to resistance developed by fungal pathogens **(Cantrell et al., 2012).**

Additionally, their low selectivity and non-biodegradability pose risks to the environment and human health. Furthermore, they promote the development of resistance in microorganisms, which can compromise food security **(Ahmad et al., 2020).**

It is therefore imperative to develop new approaches to combat fungal infections. Among the promising alternatives, plant-based fungicides have attracted researchers' attention in recent years. Numerous studies have demonstrated that phytochemical compounds derived from plants possess antifungal properties **(Bhandari et al., 2021)**.

According to **Suteu et al. (2020)**, organic substances can be classified into primary metabolites (proteins, carbohydrates, lipids) and secondary metabolites (terpenes, steroids, anthocyanins, anthraquinones, phenols, alkaloids, etc.). Numerous studies have demonstrated that several aromatic plants, as well as their essential oils and aqueous extracts, possess antifungal properties and could potentially be used as antifungal agents.

In this context, we evaluated the antifungal activity of various aqueous extracts from different parts of *Cleome arabica* from southern Algeria against potato phytopathogenic fungi. These natural substances could serve as an alternative to chemical products used in agriculture.

2. Materials and Methods

2.1. Plant Material

The plant used in this study belongs to the Capparidaceae family. In September 2018, during its peak production period, its aerial and underground parts were collected from the Ghardaïa region in southeastern Algeria.

2.2. Fungal Material

In this study, two phytopathogenic fungal strains were isolated from potato tubers collected in the El Oued region (N33°07', E7°11'). The identified species were *Fusarium proliferatum* and *Alternaria alternata*. These strains were purified and characterized by PCR, then referenced in the GenBank database of the NCBI (National Center for Biotechnology Information) under accession numbers OQ606246.1 and OQ860003.1 **(Benhaoued et al., 2024)**.

2.3. Preparation of Aqueous Plant Extracts

Aqueous extracts were prepared by macerating 15 g of plant powder in 100 ml of distilled water under agitation at 200 rpm for 24 hours at 25°C **(Razak et al., 2009; Beddou et al., 2015)**. After filtration using Whatman filter paper, the mixture was centrifuged at 600 rpm for 30 minutes. The obtained filtrate was then evaporated to dryness under reduced pressure at 40°C using an evaporator.

2.4. Phytochemical Tests

The phytochemical study aims to identify the different categories of molecules present in a plant **(Bruneton, 1999)**. To this end, the obtained extracts were subjected to various phytochemical tests based on color and precipitation reactions **(Bruneton, 1999; Mojab et al., 2003; Karumi et al., 2004; Oloyede, 2005; Koffi et al., 2009).**

2.5. Characterization of Polyphenols in Aqueous Extracts

The qualitative identification of phenolic compounds present in the aqueous extracts was performed using high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS), following the conditions described in Table 1.

Table 1: Analytical conditions for high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS).

Injection volume	10 µL
Time	65.00 min
Max Pressure	40.0 MPa
Min Pressure	0.0 MPa
Maximum Temperature	90° C
Mobile Phase	A (ultrapure water) B (Ethanol)
Processed by	HPLC (SHUMADZU)
Column Name	Ultra C18
Column ID	250 x 4.6 mm
Particle Size	5 um

2.6. Antifungal Activity

2.6.1. Direct Contact Methods

To evaluate the antifungal efficacy of *Cleome Arabica* aqueous extracts, we used the direct contact method **(Mohammedi et al., 2012)**.

2. 6.1.1. Mycelial Growth

Mycelial growth (in mm) was measured at the end of the experiment, after 10 days of incubation (240 hours), by calculating the average of three perpendicular diameters. The results obtained were systematically compared to those of the control cultures, initiated on the same day under identical conditions.

6.1.2. Determination of the Antifungal Index

The antifungal index of each aqueous extract was calculated according to the method of **Kordali et al. (2003)**, by assessing the reduction in fungal colony diameter compared to the control, using the following formula:

$$I(\%) = [1-(D_{test} / D_{control})] \times 100$$

D_{test}: Diameter of the tested colony (in mm).
$D_{control}$: Diameter of the control colony (in mm).

2.7. Statistical Analysis

Means and analysis of variance (ANOVA) were calculated based on the results obtained for each parameter using the XLSTAT software (2019).

3.Results and Discussion

3.1. Phytochemical Screening

The results of the phytochemical characterization tests of *Cleome arabica* aqueous extracts are presented in Table 2. These analyses provide a qualitative assessment of the presence or absence of bioactive molecules in the three aqueous extracts.

Table 2: Phytochemical tests performed on the aqueous extracts of *Cleome Arabica*.

Test	Leaf	Stem	Root
Alkaloid	+	--	--
Tannins	+	--	--
Flavonoid	+	+	+
Saponosin	+	+	+
Free anthocyanin	--	--	--
Anthocyanin	--	--	--
Terpenoid	+	--	--
Reducing compoun	+	+	+
Sterol and triterpen	+	--	--

-: Absence **+:** Presence

The obtained results indicate that the aqueous extracts of *Cleome arabica* exhibit a relatively similar chemical composition. Chemical analysis revealed the presence of polyphenols in the form of tannins in the leaves, while they are absent in the roots and stem. Additionally, flavonoids are abundant in all extracts, whereas anthocyanins and free anthocyanidins are absent. The analyzed extracts tested positive for alkaloids, steroidal terpenoids, and triterpenes, which are present in the leaves but absent in the stem and roots. Regarding steroids, which belong to the terpene group, they were detected only in the leaves, while being absent in the stem and roots. Conversely, saponins and reducing compounds (sugars) are present in all extracts.

3.2. HPLC Chromatography

The aqueous extract of *Cleome arabica* leaves contains the following compounds: ascorbic acid, gallic acid, pyrogallol, and acetylsalicylic acid. The aqueous extract of the stem contains three major polyphenols, including ascorbic acid, vitamin D3, and quercetin. As for the root extract, it is composed of vitamin B1, vitamin D3, gallic acid, vanillin, and acetylsalicylic acid.

Our study on the aqueous extracts of *Cleome arabica* leaves, stems, and roots revealed partially consistent results with other research, highlighting the presence of certain chemical families while noting the absence of others.

This variation can be explained by several factors, including geographical, physicochemical, and biological parameters. Among these factors are the collection site and its environment, exposure to light, precipitation levels, topography, season, soil composition, harvest period, the plant's genetic background, the extraction method used, and the specific plant part analyzed **(Malik et al., 2012; Sujana et al., 2013; Akhtar et al., 2015).**

3.3. Antifungal Activity

3.3.1. Mycelial Growth

Antifungal activity is observed through the inhibition or presence of mycelial growth in fungal strains.

3.3.1.1. *Cleome arabica* Leaf

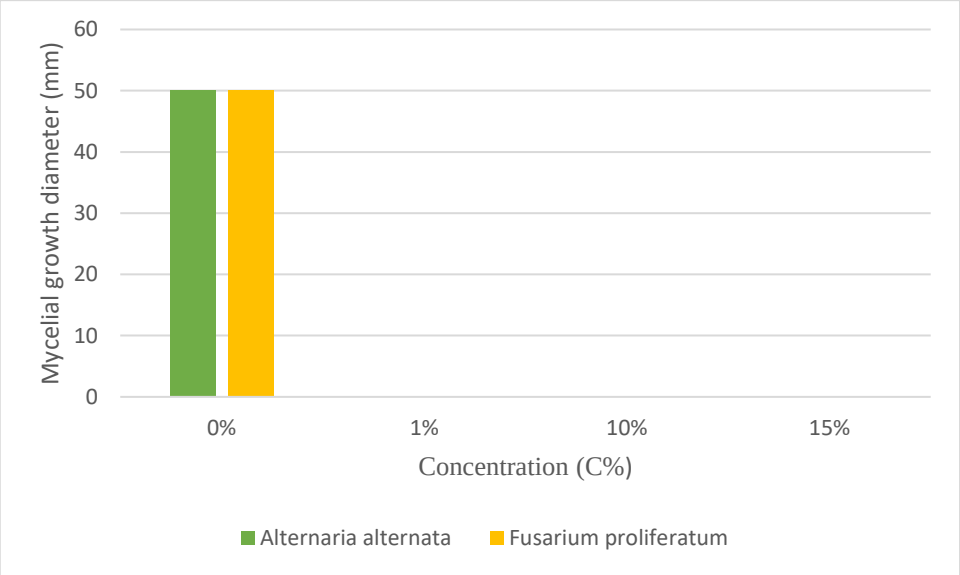

Fig 1: Effect of different concentrations of *Cleome arabica* aqueous leaf extract on fungal strains.

Fig 1 illustrates the effects of the aqueous extract of *Cleome arabica* on the growth of fungal species. The results show that the aqueous extract from *Cleome arabica* leaves completely inhibits the growth of *Fusarium proliferatum* and *Alternaria alternata*. No mycelial development was observed at any tested concentration (1%, 10%, and 15%). In contrast, in the control samples, both strains grew normally in the absence of the aqueous extract.

3.3.1.2. Stem of *Cleome Arabica*

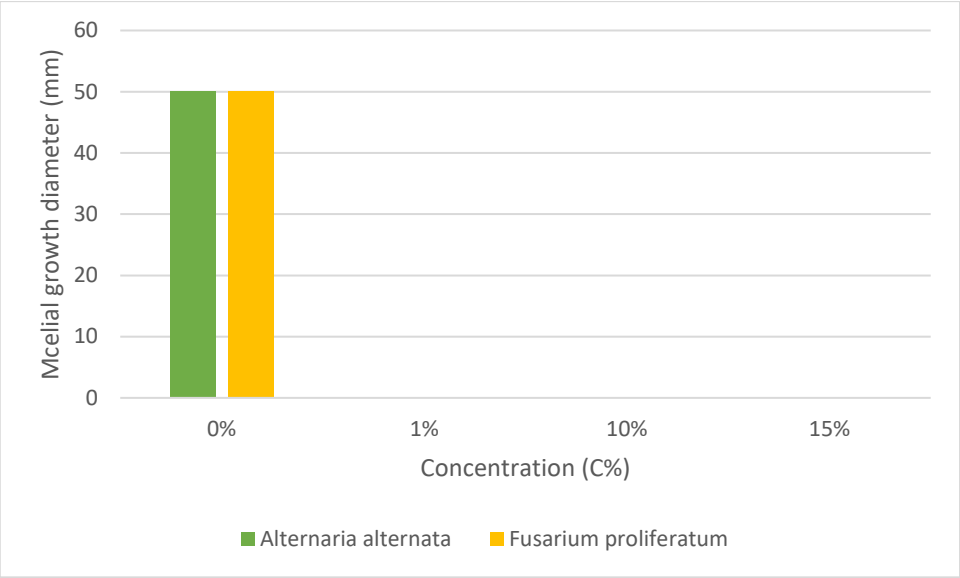

Fig 2: Effect of different concentrations of *Cleome arabica* aqueous stem extract on fungal strains.

Fig 2 shows that the largest mycelial growth diameter (50 mm) was observed in the control for both fungi. *Alternaria alternata* exhibited a mycelial growth of 50 mm in the presence of a 1% concentration of aqueous stem extract, while no growth was observed at concentrations of 10% and 15%. As for *Fusarium proliferatum*, no mycelial growth was recorded at any concentration of the aqueous stem extract of *Cleome arabica*.

3.3.1.3. Root of *Cleome Arabica*

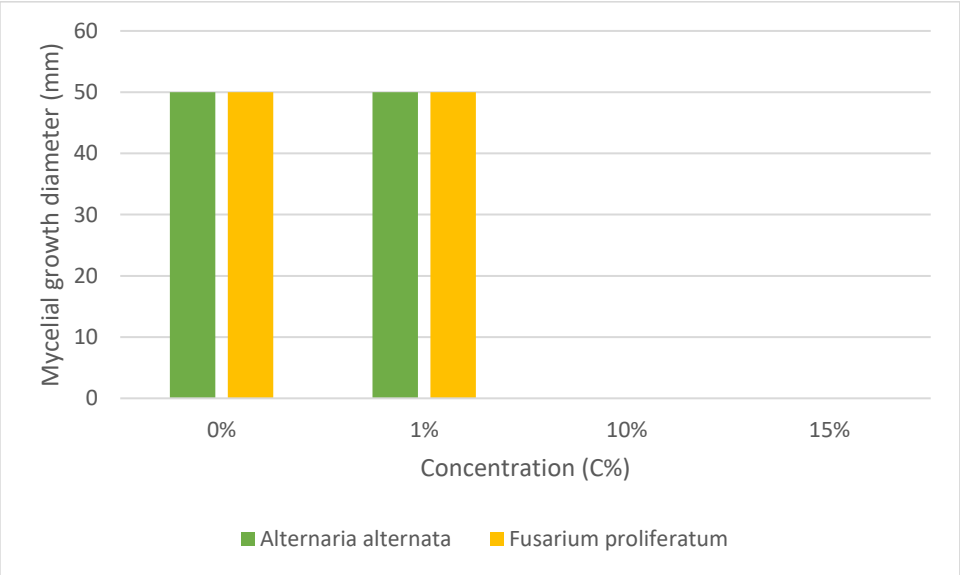

Fig 3: Effect of different concentrations of *Cleome arabica* aqueous root extract on fungal strains.

Fig 3 reveals that the aqueous root extract of *Cleome arabica* inhibits the growth of *Fusarium proliferatum* and *Alternaria alternata*. Indeed, no mycelial growth was observed at concentrations of 10% and 15%. However, in the presence of a 1% concentration, a growth diameter of 50 mm was recorded. In contrast, in the controls, *Fusarium proliferatum* and *Alternaria alternata* grow normally in the absence of the aqueous extract.

3.3.2. Antifungal Index

The effect of the aqueous extract from the leaves, stems, and roots of *Cleome arabica* on the growth of different fungal strains varies depending on the concentrations and species tested.

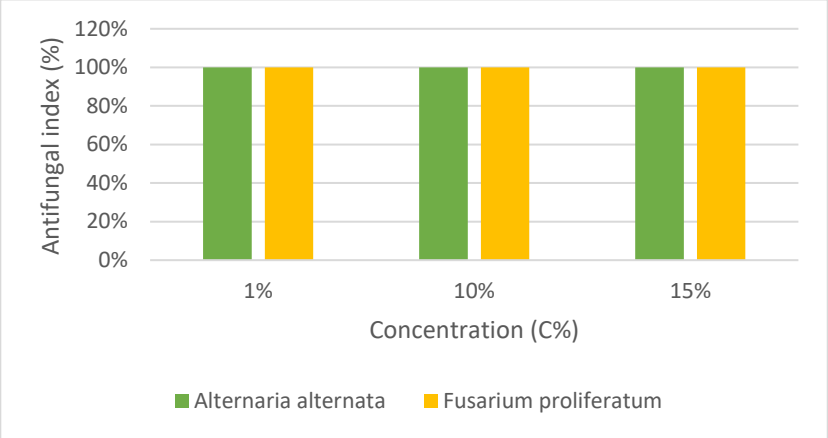

Fig 4: Antifungal index of mycelial growth inhibition of *Fusarium proliferatum* and *Alternaria alternata*. based on the concentration of *Cleome arabica* aqueous leaf extract.

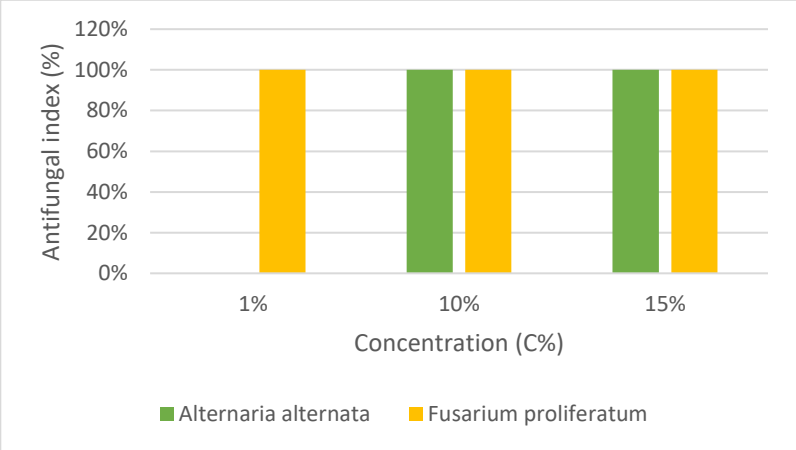

Fig 5: Antifungal index of mycelial growth inhibition of *Fusarium proliferatum* and *Alternaria alternata* based on the concentration of *Cleome arabica* aqueous stem extract.

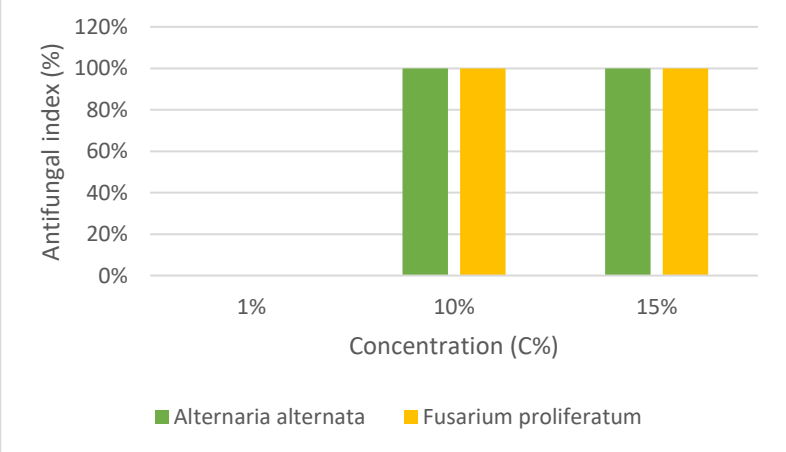

Fig 6: Antifungal index of mycelial growth inhibition of *Fusarium proliferatum* and *Alternaria alternata*. based on the concentration of *Cleome arabica* aqueous root extract.

Analysis of **Fig 4** highlights a total inhibition (100%) of mycelial growth in both fungal strains, *Fusarium proliferatum* and *Alternaria alternata*, at all tested concentrations (1%, 10%, and 15%). **Fig 5** also confirms a complete (100%) inhibition of *Fusarium proliferatum* at all three concentrations, while *Alternaria alternata* shows no inhibition at 1% but total inhibition (100%) at concentrations of 10% and 15%.

Furthermore, **Fig 6** shows that the aqueous root extract of *Cleome arabica* strongly inhibits the growth of both fungal strains. A fungicidal activity of 100% was observed at concentrations of 10% and 15% for all tested strains. However, at a concentration of 1%, no inhibition (0%) was recorded for *Fusarium proliferatum* and *Alternaria alternata*.

The use of new natural substances as an alternative to chemical agents represents a promising approach for crop protection. This study highlights, for the first time, the antifungal activity of *Cleome arabica* extracts against two fungal strains responsible for post-harvest diseases in potatoes.

The analysis of the effect of varying concentrations of *Cleome arabica* aqueous extracts on the mycelial growth of *Fusarium proliferatum* and *Alternaria alternata* revealed that their antifungal activity is significant at high concentrations.

The increase in the concentration of aqueous extracts from the leaves, stems, and roots of *Cleome arabica* resulted in strong antifungal activity, accompanied by a rise in the antifungal index on the mycelial growth of the tested strains. These results are consistent with the findings of **Probavathy et al. (2006)**, who demonstrated a similar activity of aqueous extracts against *Aspergillus* and *Fusarium.*

It is likely that this activity stems from the nature and molecular structure of the active compounds present in the aqueous extracts. These substances cross the cell membrane, enter the cell, and interact with essential intracellular sites, such as enzymes and proteins, ultimately leading to cell death **(Omideygi et al., 2007)**.

In general, antimicrobial activity is linked to the chemical composition of phenolic substances. Their structure, characterized by an aromatic ring associated with hydroxyl groups in various positions, allows them to establish hydrogen bonds with the -SH groups of the active sites of target enzymes, thereby leading to their deactivation in fungi **(Ultee et al., 2002; Zongo et al., 2011)**. According to **Sepúlveda et al. (2011)**, identified tannins and flavonoids are known for their ability to inhibit the growth of various microorganisms, including bacteria and fungi. Additionally, phenolic compounds, terpenes, and steroids detected in the extracts are considered key constituents that play a protective role for the plant against fungal and bacterial infections **(Raven et al., 2000)**.

4.Conclusion

This study focused on evaluating the antifungal effect of aqueous extracts from different parts (leaves, stems and roots) of *Cleome arabica*. The direct contact method demonstrated the antifungal potential of these extracts against *Fusarium proliferatum* and *Alternaria alternata* strains. The results revealed significant antifungal activity, particularly for leaf extracts, which exhibited a total inhibitory effect (100%) on *Fusarium proliferatum* and *Alternaria alternata* at all tested concentrations (1%, 10%, and 15%).

The antifungal activity of stem and root extracts was particularly evident at concentrations of 10% and 15%, with the antifungal index reaching 100%. However, at a concentration of 1%, the antifungal index was 100% for *Alternaria alternata* but null for *Fusarium proliferatum*. Furthermore, the root extract showed no antifungal activity against the tested strains. These results suggest that the aqueous

extract of *Cleome arabica* could serve as a natural alternative to synthetic antifungal agents, offering a promising solution for agro-industries.

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