

Clove essential oil against tomato fungal pathogens

Hanane Abed^{1,2*}, Souad Boumaiza², Nouari Sadrati^{2,3}, Soraya Hihat^{2,4} and Ahmed Chouchou²

ABSTRACT

Syzygium aromaticum L. (clove), renowned for its antiseptic properties, holds significant potential in the food industry for controlling various food-borne microorganisms. A bibliometric analysis was performed using the Scopus database to explore the scientific landscape concerning the antifungal activity of clove (*Syzygium aromaticum*) essential oil. Most published studies do not specify the plant's physical state. This research gap directly supports the experimental framework of the current study, which compares the antifungal efficacy of EOGC and EOUGC. The evaluation of the antifungal activity of essential oils (EOs) extracted from both ground and unground clove buds against phytopathogenic fungi isolated from infected tomatoes. Antifungal efficacy was assessed using the agar diffusion method on potato dextrose agar (PDA), while the minimum inhibitory concentrations (MICs) were determined on Sabouraud dextrose agar (SDA). Macroscopic and microscopic examinations enabled the identification of the following fungal species: *Aspergillus* sp., *Geotrichum* sp., *Fusarium* sp., *Arthrographis* sp., and *Penicillium* sp. The results demonstrated that the pure essential oils exhibited notable antifungal activity, as evidenced by distinct inhibition zones against all tested strains. The strongest inhibitory effect was observed for the essential oil derived from ground cloves (EOGC), with an inhibition zone of 50 ± 10 mm against *Penicillium* sp. The MIC values were below 2.5 $\mu\text{L/mL}$ for all fungal isolates, although 10 $\mu\text{L/mL}$ was required in the vapour-phase (micro-atmosphere) assay. Overall, the findings support the efficacy of clove essential oil, whether extracted from ground or unground buds, as a promising natural alternative to synthetic antifungal agents.

Keywords: *Syzygium aromaticum* L., antifungal activity, grinding, essential oils, tomato fungi, volatile compounds

MS History: 11.02.2026(Received)-02.03.2026(Revised)- 01.04.2026 (Accepted)

Citation: Hanane Abed, Souad Boumaiza, Nouari Sadrati, Soraya Hihat and Ahmed Chouchou. 2026. Clove essential oil against tomato fungal pathogens. *Journal of Biopesticides*, 19(1):32-42. DOI: 10.57182/jbiopestic.19.1.32-42

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) are grown worldwide for their nutritional value and use in both fresh and processed foods. However, tomato growth is sometimes affected by plant diseases that affect the leaves, stems, and fruit. Diseases caused by fungi, bacteria, and viruses in tomatoes lead to significant yield losses, low-quality produce, and economic damage (Gunasekaran et al., 2025). Fungi such as *Fusarium*, *Aspergillus*, *Penicillium*, *Geotrichum*, *Alternaria*, and *Botrytis* attack tomatoes causing soft rot, mycelial formation, decay, and yield reduction among others (Panno et al., 2021; Ma et al., 2023; Abuzaitoun et al., 2026).

Traditional chemicals work on the principle of compounds that are toxic to pathogenic

organisms via their mechanism of action. These chemicals must be capable of eliminating pathogenic organisms without harming any other living beings and having negligible effects on the environment (Hazem et al., 2026). Synthetic fungicides are widely used to control fungal infections in agricultural and postharvest systems. However, the extensive and regular application of these fungicides gives rise to serious issues such as the development of resistance, contamination of products, harmful effects on the environment, toxicity to non-target organisms, and restrictions put forward due to food safety guidelines (Mutlu-Ingok et al., 2020; Elshafie et al., 2026). These disadvantages have spurred the search for natural antifungal agents that are efficient, environmentally friendly, and

safe for use in agricultural and postharvest applications. In this regard, plant essential oils have garnered significant attention due to their antimicrobial properties, complex mixture, volatility, and multiple mechanisms of action against fungi (Keyal *et al.*, 2017; Elshafie *et al.*, 2026). Essential oils have been proposed as potential biopesticides due to their antimicrobial potency and negligible toxicity to mammals (Lili *et al.*, 2026).

Myrtle essential oil is one such oil, owing to its antifungal properties and ability to inhibit numerous pathogenic fungi affecting plant life (Ayed *et al.*, 2024). In contrast, clove essential oil has gained popularity due to its high eugenol content and its use in foods, where it acts as an edible coating for fruits, extending their shelf life (Giménez *et al.*, 2025). Moreover, clove essential oil (CEO) is highly potent against pathogenic fungi, specifically *Aspergillus* and *Trichothecium*. This oil is used as coatings for fruits and vegetables, thereby increasing their shelf life (Peralta *et al.*, 2020).

The present study was designed with two primary objectives. First, a keyword-focused bibliometric analysis was conducted using the Scopus database (2000–2025) to situate the study within the existing literature. This review aimed to identify research trends and knowledge gaps regarding the antifungal properties of clove essential oil. Following this, an experimental investigation was conducted to evaluate the antifungal efficacy of clove essential oil against five major fungal pathogens of tomato: *Geotrichum* sp., *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and *Arthrographis* sp. This involved comparing two distinct extraction methodologies (ground versus unground clove buds) and testing multiple essential oil concentrations. The antifungal effectiveness was determined *in vitro* by establishing minimum inhibitory concentrations (MICs) and assessing the activity of the oil's volatile components. This holistic methodology endeavours to enhance our understanding of how preparation variables impact the biological potency of clove essential oil and to investigate its applicability in sustainable plant disease management.

MATERIALS AND METHODS

Bibliometric study

A bibliometric analysis was performed using the Scopus database to explore the scientific landscape concerning the antifungal activity of clove (*Syzygium aromaticum*) essential oil. The search strategy was formulated using the following string: TITLE-ABS-KEY ("clove extract" OR "clove essential oil") OR "*Syzygium aromaticum* extract" OR "*Syzygium aromaticum* essential oil") and (antifungal OR "antifungal activity"). This search retrieved a total of 183 original research articles published between 2000 and July 2025. The selection was limited to English-language publications in the disciplines of agriculture, medicine, biochemistry, pharmacology, immunology, chemistry, environmental science, and chemical engineering. Author keyword co-occurrence analysis was conducted using VOS viewer (version 1.6.20), setting a minimum threshold of three occurrences per keyword. This process generated a network map comprising 40 significant keywords. This visualization provided insights into the major research themes and potential gaps related to the antifungal applications of clove-derived essential oils.

Plant materials

Healthy and mature clove buds (*Syzygium aromaticum*, synonym: *Eugenia caryophyllata*) were purchased from the local market of Bordj Bou Arreridj (northwest Algeria). The samples were washed thoroughly to remove dust and impurities, air-dried, and then dried at 30°C in a ventilated oven. After drying, they were stored in plastic bags at room temperature. The cloves were subsequently divided into two lots: the first was left whole (unground), while the second was finely ground using a blender. To preserve the integrity of the active compounds, all samples were protected from direct sunlight throughout the preparation process.

Essential oil extraction

The ground and unground clove buds were hydrodistilled separately for 3–4 hrs using a Clevenger-type apparatus. After distillation, the essential oils were collected in glass flasks and dried with anhydrous sodium. The essential oils,

namely EOGC (Essential Oil of Ground Clove) and EOUGC (Essential Oil of Unground Clove), were stored in sealed glass vials at 4°C until further use. The percentage oil yield was calculated according to the following equation:

$$\text{Oil yield (\%)} = \frac{w(EO)}{w(P)} \times 100$$

where w (EO) is the weight of the essential oil and w (P) is the weight of the plant material (Kalagatur *et al.*, 2018).

Isolation and identification of the fungus

Tomato fruits showing signs of infection were selected for fungal isolation. Tissue sections (1 × 1 cm), composed equally of healthy and infected tissue, were surface-sterilised by immersion in 2% (v/v) sodium hypochlorite (NaClO), rinsed with distilled water, and dried on sterile filter paper. These sections were then placed on potato dextrose agar (PDA) in Petri dishes and incubated at 26 ± 2°C for 24 to 72 hrs. For purification, mycelium was taken from the outermost edge of the growing colony and transferred to fresh PDA plates until pure cultures were obtained (Salvador *et al.*, 1999). Fungal identification at the genus level was performed based on microscopic morphology by mounting mycelium on a drop of methylene blue on a glass slide, followed by the placement of a coverslip. Identification was guided using dichotomous taxonomic keys.

Screening of antifungal activity

Preliminary screening of antifungal activity of clove essential oils (EOs) was performed using the agar well diffusion assay. A spore suspension of each isolate was prepared by excising a single fungal disc aseptically with a 5 mm cork borer from the sporulating culture and mixing it in 20 mL of sterile distilled water to which 50 µL of Tween-80 was added. The total number of spores was determined by counting under a microscope using a hemocytometer. Fungal spore count was adjusted to 1 × 10⁶ spores/mL by making appropriate dilutions (Yalemtesfa *et al.*, 2010). Petri plates containing PDA medium were inoculated aseptically with 500 µL of spore suspension using the spread plate technique and then kept in the laminar flow for 30 min. Wells of 5 mm diameter were punched aseptically in the centre of the Petri plates using a sterile cork borer. Into each well, 10 µL of each clove EO

was dropped, followed by 40 µL of dimethyl sulfoxide (DMSO) so as to make a total volume of 50 µL in the well. Plates were kept in the refrigerator at 4°C for 3 hrs to allow EOs and DMSO to diffuse into the agar medium. Plates were finally incubated at 28±1°C for 5 days. DMSO without clove oil was set as a negative control. The diameter of the zone of inhibition around each well was measured in mm.

In vitro determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined for all fungal isolates through the agar dilution method. PDA plates were prepared with increasing concentrations of clove EOs (2.5, 5, 10, 7.5, and 15 µL/mL; v/v). Tween-20 (0.05%) was used to dissolve EOs in the medium. Plates were inoculated in the center with a 4 mm mycelial disc and incubated for 5 days, followed by measurement of mean radial colony diameters. Three replicates were made for each treatment, and the medium with Tween-20 alone was set as the control. The plate with the lowest concentration of clove EO, showing no visible growth, was regarded as the MIC (Shin, 1998). The percentage inhibition in mycelia growth was calculated using the following formula:

$$\text{Per cent mycelial growth inhibition} = \frac{\text{mycelial growth in control} - \text{mycelial growth in treatment}}{\text{mycelial growth in control}} \times 100$$

100

Antifungal Activity of Volatile Compounds of Clove Essential Oil

The method for testing the antifungal activity of volatile compounds in essential oils was the micro-atmosphere method of Neri *et al.* (2006). It was carried out in 90 mm Petri dishes containing 25 mL of PDA medium. The PDA medium was inoculated with a 10 mm diameter piece excised from the edge of the seven-day-old fungal colony on PDA. Next, a 1 cm² disc of Whatman paper is positioned in the center of the Petri dish lid and impregnated with varying amounts of essential oil. Concentrations ranging from 2.5 to 15 µL/mL were tested. An untreated control was also prepared under identical conditions, that is, a piece of paper coated with distilled water. To avoid the evaporation of volatile

compounds from the culture, Petri dishes were hermetically sealed using Parafilm. The cultures were incubated inverted (lid down) at 25°C for 7 days. Each test was performed in triplicate.

Statistical analysis

Statistical analyses and graphical representations were performed using IBM SPSS Statistics 26 and JASP 0.19.3 software. The non-parametric Wilcoxon test was used to compare the effect of each extract on individual fungal species. The Mann-Whitney test was applied to evaluate the effect of the two extracts across all species. Finally, the Kruskal-Wallis test, followed by Dunn's post hoc test with multiple-comparisons corrections (Bonferroni and Holm), was used to detect significant differences between species for each extract type.

RESULTS AND DISCUSSION

Trends and Gaps in the Bibliometric Landscape

The keyword co-occurrence analysis revealed seven thematic clusters, reflecting the multidimensional nature of research on *Syzygium aromaticum* Eos (Figure 1A). The central cluster (red cluster) focused on fundamental concepts such as clove (*Syzygium aromaticum*), essential oil, and antifungal activity. The other peripheral clusters highlight emerging topics such as nanoemulsion, biocontrol, encapsulation, shelf life, synergism, and green synthesis.

The research identified on the map is divided into two main directions: on the one hand, investigations into clove essential oils, and on the other hand, studies of their effects on phytopathogenic fungi such as *Penicillium digitatum* and *Botrytis cinerea*. The temporal evolution of research topics related to the antifungal activity of clove EO (Figure 1B) highlights those earlier studies (shown in dark blue/purple) focused primarily on evaluating antifungal activity against fungi responsible for human infections, particularly *Candida albicans*. Since 2018, research has increasingly shifted toward more recent topics (in green/yellow), reflecting a growing interest in extraction methods, formulation strategies, and technological optimization of EOs. This temporal shift indicates a transition from fundamental studies to more applied approaches,

with greater emphasis on the stability, bioavailability, and effectiveness of clove-derived bioactive compounds.

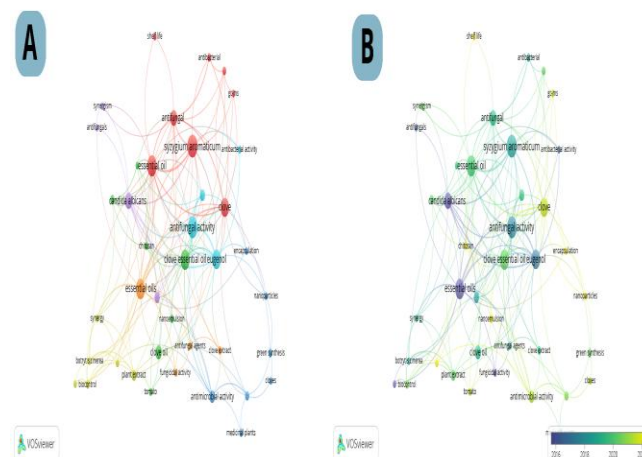


Figure 1. Keyword co-occurrence network of research on clove EO's antifungal activity (2000–2025). (A) Cluster Visualisation: Nodes represent keywords; colours indicate distinct thematic clusters. (B) Temporal Overlay: Node colours reflect the average publication year, with purple indicating earlier studies and yellow representing the most recent research trends.

However, several research gaps persist: The mechanisms of antifungal action are still insufficiently understood, particularly at the molecular level. The limited linkage of the terms "*synergy*" and "*synergism*" suggests that most studies focus on parallel comparisons rather than exploring potential combinatory or potentiating effects. Finally, most published studies do not specify the physical state of plant material before extraction, and no systematic comparative analysis between ground and unground clove has been explored. This lack of methodological precision may impact the reproducibility and comparability of findings. This research gap directly supports the experimental framework of the current study, which compares the antifungal efficacy of EOGC and EOUGC. The aim is to investigate how grinding as a pre-distillation step may influence oil yield and biological activity, an aspect rarely differentiated in existing research.

Extraction Yield

The results indicate that ground clove samples yielded a higher EO extraction rate than unground samples. Specifically, the EO yield from unground clove buds was 4.76%, whereas

ground buds reached a slightly higher yield of 5.06% (Figure 2). These findings differ from those reported in previous studies. For instance, Ben Hassine *et al.* (2021) reported an EO yield of $7.1 \pm 0.8\%$ for unground clove buds and a significantly higher yield of $14.3 \pm 0.6\%$ for ground buds. Similarly, Kapadiya and Desai (2017) and Ben Hassine *et al.* (2010) observed yields around 11.35%. Selles *et al.* (2020), working with ground *Syzygium aromaticum* buds from Algeria, reported a yield of 11.6%. Derguin *et al.* (2024) obtained a clove essential oil yield of 9%. These variations underscore the influence of the grinding process on EO extraction. Grinding increases the surface area in contact with boiling water, thereby enhancing the efficiency of hydrodistillation (Johner *et al.*, 2018).

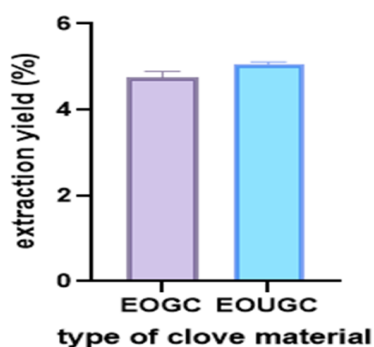


Figure 2. Extraction yield of essential oils from ground clove (EOGC) and unground clove (EOUGC). Additionally, grinding accelerates the extraction process and improves overall yield. However, excessive grinding may impair steam circulation, potentially reducing the extraction rate (Ben Hassine *et al.*, 2010). Alfikri *et al.* (2020) reported that buds from young trees had higher essential oil yields than those from mature trees. Other factors, such as the environment, geographical location, and the method of essential oil extraction, also affect the extraction yield (Mutlu-Ingok *et al.*, 2020).

Identification of Fungal Strains

A total of five distinct fungal genera were identified in the present study: *Geotrichum*, *Aspergillus*, *Fusarium*, *Penicillium*, and *Arthrographis*. The first isolate exhibited hyaline, septate hyphae and produced hyaline, cylindrical-to-rectangular arthroconidia (Figure 3A) arranged in chains and easily detached. Based on both macroscopic and microscopic

characteristics, this isolate was identified as a member of the genus *Geotrichum*. The second isolate showed hyaline, septate hyphae, along with both macroconidia and microconidia (Figure 3B), morphological traits characteristic of the genus *Fusarium*. The third isolate also displayed hyaline (non-pigmented) mycelium and was characterized by a long, smooth conidiophore originating from a foot cell and terminating in a vesicle (Figure 3C). These features are consistent with the diagnostic morphology of the genus *Aspergillus*. The fourth isolate was distinguished by brush-like conidiophores (Figure 3D), a hallmark feature of the genus *Penicillium*. The fifth isolate presented hyaline, septate hyphae, generally narrow and smooth-walled. The branched conidiophores exhibited a dendritic or tree-like structure, arising from vegetative hyphae. Additionally, this isolate produced arthroconidia ranging from cylindrical to doliiform. These combined characteristics were consistent with those of the genus *Arthrographis* (Figure 3E).

The results on the association of fungi with tomato post-harvest fruit rot indicated significant variation among fungal isolates. A total of five different fungal genera, *Geotrichum* sp., *Aspergillus*, *Fusarium*, *Penicillium*, and *Arthrographis*, were identified as pathogens that cause tomato fruit damage. Similar to the current study, Sajad *et al.* (2017) observed that tomato fruits were frequently affected by fruit rot disease caused by *Alternaria alternata*, *Aspergillus niger*, *Geotrichum candidum*, *Alternaria solani*, *Mucor racemosus*, *Aspergillus flavus*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Penicillium digitatum*, *Rhizopus stolonifer*, *Alternaria alternata*, *Colletotrichum lycopersici*, *Sclerotium rolfsii*, *Myrothecium roridum*, *Phomadestructiva*, and *Trichothecium roseum*. Nizamani *et al.* (2021) reported six different fungi, viz., *A. solani*, *A. niger*, *A. alternata*, *Geotrichum candidum*, *F. oxysporum*, and *Rhizopus stolonifer*, as associated with tomato postharvest rot.

Screening of antifungal activity

The results of the preliminary screening shown in Figure 4 clearly indicate that both clove essential oils (CEO) are highly antifungal

towards all tested fungal species, with mean inhibition zone diameters of (EOGC) and (EOUGC).

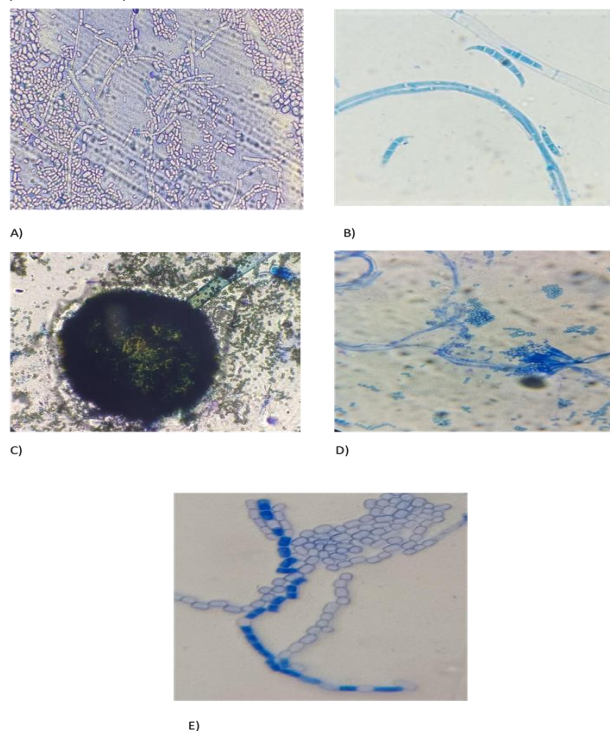


Figure 3. Microscopic identification of fungal isolates: A) *Geotrichum*, B) *Fusarium*, C) *Aspergillus*, D) *Penicillium*, and E) *Arthrographis*.

Species-wise, *Penicillium* sp. appeared to be the most sensitive, with inhibition diameters of 50 ± 10 mm and 46.66 ± 5.77 mm for EOGC and EOUGC, respectively (Table 1). In contrast, *Arthrographis* sp. was the least sensitive, with inhibition diameters of 27.33 ± 2.30 mm (EOGC) and (EOUGC). Both essential oils produced comparable inhibition diameters, with no statistically significant difference between them. However, for the EOGC extract, a statistically significant difference was observed only between *Penicillium* sp. and *Arthrographis* sp. ($p = 0.017$) (Figure 4b). The findings of the current study are superior to those reported by Rana et al. (2011) in a preliminary screening study, clearly indicating that clove essential oil possesses strong antifungal activity against all the studied fungal species. There was a remarkable variation in inhibition zone diameters among the fungal isolates, ranging from 12 to 22 mm, with sensitivity to clove essential oil. Razzouk et al. (2022) reported that *Aspergillus niger* was the most sensitive towards

the CEO, with an inhibition diameter of 44.00 mm, followed by the yeast species *C. albicans* at 14.00 mm and *C. glabrata* at 24.67 mm.

Table1. Inhibition zones of mycelial growth of different isolated fungi (mm) mean $\pm$ SD, ground clove (EOGC), unground clove (EOUGC), * $p < 0.05$.

Ext ract	<i>Aspergillus</i>	<i>Geotrichum</i>	<i>Fusarium</i> sp	<i>Arthrographis</i>	<i>Penicillium</i> sp	Total
EOGC	33.33 ± 5.77	41 ± 8 . 54	29.33 ± 1.15	27.33 $\pm$ 2.31	50 ± 1 0	36.20 $\pm$ 10.24
EOUGC	37.66 ± 4.04	38.33 ± 2.88	38.33 ± 2.88	27.66 $\pm$ 4.72*	46.66 $\pm$ 5.77*	37.73 ± 7.18

Ahmad et al. (2005) reported that clove oil possesses very high antifungal activity against *C. albicans*, *C. neoformans*, and *A. fumigatus*. Xu et al. (2016) reported that eugenol, eugenol acetate, β -cariofilen, α -humulene, β -pinene, limonene, farnesol, benzaldehyde, 2-heptanone, and ethyl acetate were the most common compounds. These phenolic compounds are responsible for the antibacterial and antifungal properties of clove essential oil.

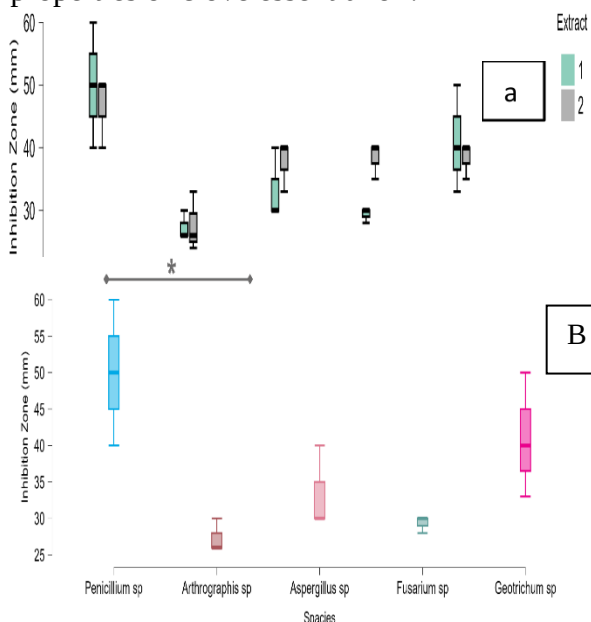


Figure 4. Effect of clove essential oils on mycelial growth of different isolated fungi. a) Inhibition zones of mycelial growth, b) the susceptibility of strains to clove essential oils. 1 EOGC, 2 EOUGC, * $p < 0.05$.

Minimum inhibitory concentration (MIC)

The MIC results showed that clove oil had an MIC of 2.5 μ L/mL, which completely inhibited the growth of all studied strains (Figure 5). Statistical analysis showed a significant difference between EO from ground and

unground clove buds. These findings were in perfect agreement with Ben Hassine *et al.* (2021), who demonstrated that clove bud EO was highly active against Gram+ and Gram-bacteria, with inhibition zone diameters ranging from 17.2 to 35.5 mm. Pinto *et al.* (2009) determined an MIC of clove oil in contact with *Aspergillus* sp. to be 1.25 µl/ml, while Uwineza *et al.* (2018) reported an MIC of 0.312 µl/ml against *Fusarium culmorum*. However, our MIC value was lower than that reported by Yage *et al.* (2011), whose results indicated that clove oil had antifungal activity against *A. flavus* and *P. citrinum* with an MIC of 25 µl/mL. These observations indicate that clove oil is effective against phytopathogenic fungi across a broad range. It was more active, and MIC values obtained for all the tomato strains were comparable. Therefore, due to its enormous potential, it may be used as a natural antifungal agent for use in the management of phytopathogens on tomato fruits. In the study by Thabet and Khalifa (2018), the MIC of clove oil was 4% against *F. oxysporum* and *Rhizoctonia solani*. This concentration, however, led to reductions of 88.5% and 91.8% in the growth of *F. semitectum* and *F. solani*, respectively.

Antifungal volatile compounds

At the lowest concentration of 2.5 µl/mL, the two oils examined (EOGC and EOUGC) showed extremely weak antifungal activity against all strains, with inhibition percentages ranging from 0 to 30.76%. On the other hand, the highest concentration of 15 µl/mL of EOUGC was found to be highly effective against the various fungal isolates, having inhibition rates between 21.42% and 56.92%, with *Fusarium* sp. being the most susceptible (Figure 6b). The 15 µl/mL concentration of EOGC also revealed high inhibition activities, with inhibition rates ranging from 28.57% to 61.53%, with *Arthrographis* sp. being the most susceptible strain. In this case, the two oils showed a statistically significant difference in their effects ($p < 0.0001$; Figure 6a). Our results showed that the volatile compounds in clove essential oil were highly active against all fungi

tested except *Penicillium* sp. Kacániová *et al.* (2021), in situ antimicrobial study using bread, proved that the microscopic filamentous fungi from the *Penicillium* genus were inhibited by CEO vapours at every concentration tested, a finding that differs from our results. Hmiri *et al.* (2011) applied the micro-atmosphere method described by Neri *et al.* (2006) to measure the antifungal effect of essential oils. *B. cinerea* was inhibited starting at 156 µl/l, while *A. alternata* and *P. expansum* were resistant to concentrations of up to 300 µl/l. Neri *et al.* (2006) and Amiri *et al.* (2008) also established that eugenol can be, depending on its concentration, either fungistatic or fungicidal against *B. cinerea* and *P. expansum*. The assessment of the grinding effect on clove buds EO was performed initially. It was demonstrated that ground buds exhibited higher antifungal activity via volatile compounds than intact buds. Grinding can indirectly lead to the decomposition of plant material and thus reduce the amount of volatile chemical compounds. These results are in accordance with those stipulated by Herzi (2013). In the current study, the volatile compounds of clove essential oil showed fungistatic activity, whereas direct application of CEO showed fungicidal activity. These results are in accordance with those reported by Chee and Lee (2007), who found that the volatile vapour of clove essential oil showed fungistatic activity on a solid medium, whereas the oil demonstrated fungicidal activity in broth medium. Jain and Agrawal (2002) demonstrated the fungistatic activity of the volatile vapour of several essential oils against fungi. Dan Wang *et al.* (2019) indicated that essential oil would be faster and more efficient in preventing fungal infection when used as a vapour rather than by direct contact. However, direct application of essential oils to fruit surfaces via fumigation was prone to fruit injury and quality deterioration due to rapid or uncontrolled evaporation. Although the exact mechanism of fungal growth inhibition by the volatile vapours of essential oils remains unclear, further research is needed to investigate

the antifungal mechanism of clove essential oil. The volatile compounds of the different essential oils (ground and unground) from clove buds were analyzed by GC-MS. A comparison between the EO chemical composition obtained from ground and unground buds was elaborated by Ben Hassin *et al.* (2021). We indicated that eugenol was the major component for both ungrounded and grounded buds (68.7%). However, β -caryophyllene was also reported as one of the major components (Xu *et al.* 2016).

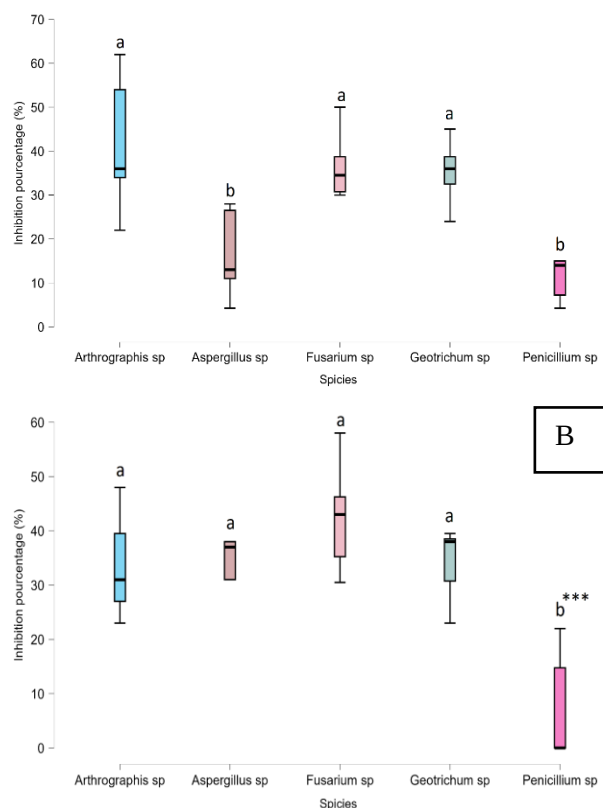


Figure 6. Determination of antifungal volatile compounds of clove essential oil. A) EOGC and B) EOUGC. Superscript concentrations with the same letter were not significantly different from each other by the Mann-Whitney test (***) $p < 0.001$.

Dan Wang *et al.* (2019) found that the absorbance at 260 nm of the groups treated with 200, 300, and 400 $\mu\text{L/L}$ clove oil was higher than that of the control group and 100 $\mu\text{L/L}$ treatment, suggesting that a high concentration of essential oil increased membrane permeability and caused the release of nucleic acid from *C. gloeosporioides*. Dan Wang *et al.* (2019) also revealed that the protein content of fungal cells

was markedly decreased with increasing

concentrations of essential oil (100–400 $\mu\text{L/L}$), indicating a disruption of the cell membrane permeability barrier. The efficacy of clove oil is due to its major components, including phenylpropanoids such as carvacrol, thymol, eugenol, and cinnamaldehyde. The authors indicated that clove EO may be developed as a potential natural fumigant or repellent for controlling the pulse beetle.

AUTHOR CONTRIBUTIONS

Project conceptualization, A.H., B.S., S.N. and C.A.; experimentation, A.H., B.S., S.N. and C.A.; data analysis, A.H., B.S., S.N. and C.A.; preparation of the draft manuscript, A.H., B.S. and C.A.; review and editing of the manuscript, A.H., B.S., S.N. and C.A. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGEMENT

We thank two anonymous reviewers for their helpful comments on earlier drafts of this manuscript. We thank the Algerian Government and the Algerian Ministry of Higher Education and Scientific Research for providing resources.

Conflicts of interest: The authors declared no conflicts of interest.

REFERENCES

- Abuzaitoun, S. Y., Salman, M. N., Hamdan, Y. Y., Jamous, R. M., and Ali-Shtayeh, M. S. 2026. Antifungal efficacy of essential oils and nanoformulations against *Fusarium* wilt of tomato: Systematic review (2000–2025). *Plants*, **15**(8): 1268. <https://doi.org/10.3390/plants15081268>
- Ahmad, N., Alam, M. K., Shehbaz, A., Khan, A., Mannan, A., Rashid, H. S., Bisht, B., and Owais, M. 2005. Antimicrobial activity of clove oil and its potential in the treatment of vaginal candidiasis. *Journal of Drug Targeting*, **13**(10): 555–561. <https://doi.org/10.1080/10611860500422958>
- Alfikri, F. N., Pujiarti, R., Wibisono, M. G., and Hardiyanto, E. B. 2020. Yield, quality, and antioxidant activity of clove (*Syzygium aromaticum* L.) bud oil at the different phenological stages in young and mature trees. *Scientifica*, **4**: 1–8.
- Ayed, A., Caputo, L., De Feo, V., Elshafie, H. S.,

- Fратиanni, F., Nazzaro, F., Hamrouni, L., Amri, I., Mabrouk, Y., and Camele, I. 2024. Antimicrobial, Anti-Enzymatic, and Antioxidant Activities of Essential Oils from Some Tunisian Eucalyptus Species. *Heliyon*, **10**: e34518.
- Ben Hassine, D., Ben Ismail, H., Jribi, C., Khouja, M. L., and Abderrabba, M. 2010. Chemical composition of some *Tunisian Eucalyptus* essential oils as obtained by hydrodistillation using a Clevenger-type apparatus. *Biosciences, Biotechnology Research Asia*, **7**(2): 647–656.
- Ben Hassine, D., Kammoun El Euch, S., Rahmani, R., Ghazouani, N., Kane, R., Abderrabba, M., and Bouajila, J. 2021. Clove bud essential oil: The impact of grinding on the chemical composition and its biological activities involved in consumers. *BioMed Research International*, Article ID 9940591, 11 pages. <https://doi.org/10.1155/2021/9940591>
- Derguini, A., Chikhoune, A., Benberkane, A., Belazougui, K., Djinni, I., Idres, T., Boudjellaba, S., and Iguer-Ouada, M. 2024. Evaluation of antifungal activity of free and encapsulated clove oil in β -cyclodextrin against an Algerian isolate of *Fusarium oxysporum* f. sp. *radicislycopersici*. *The Annals of the University Dunarea de Jos of Galati, Fascicle VI – Food Technology*, **48**(1): 178–191.
- Elshafie, H. S., Crescenzi, A., and Camele, I. 2026. Investigating the biological effects of plant essential oils on plant-decaying pathogens. *Plants*, **15**(4): 542. <https://doi.org/10.3390/plants15040542>
- Gunasekaran, H., Rajkumar, S., and Kirubhadharsini, B. L. 2026. Lightweight deep learning for tomato disease detection: trends, challenges, and edge AI perspectives. *Front. Plant Sci.* **16**:173720 8. doi: 10.3389/fpls.2025.1737208
- Giménez-Santamarina, S., S., Torres-Pagan, N., Larran, S., Roselló, J., and Santamarina, M.P. 2025. Clove essential oil and eugenol as natural Antifungal Agents to Reduce Postharvest Losses in Melon (*Cucumis melo*). *International Journal of Sciences*, **26**: 9603.
- Hazem, S., Elshafie, Aniello, C., and Ippolito, C. 2026. Investigating the Biological Effects of Plant Essential Oils on Plant-Decaying Pathogens. *Plants*, **15**(4): 542; <https://doi.org/10.3390/plants15040542>
- Herzi, N. 2013. Extraction et purification de substances naturelles: comparaison de l'extraction au CO₂ supercritique et des techniques conventionnelles [Thèse de doctorat, Institut National Polytechnique de Toulouse].
- Jain, S. K., and Agrawal, S. C. 2002. Fungistatic activity of some perfumes against otomycotic pathogens. *Mycoses*, **45**(3): 88–90.
- Johner, J. C. F., Hatami, T., Carvalho, P. I. N., and Meireles, M. A. A. 2018. Impact of the grinding procedure on the yield and quality of the extract from clove buds using supercritical fluid extraction. *Open Food Science Journal*, **10**(1): 1–7.
- Kalagatur, N., Ghosh, O. N., Sundararaj, N., and Mudili, V. 2018. Antifungal activity of chitosan nanoparticles encapsulated with *Cymbopogon martinii* essential oil on the plant pathogenic fungus *Fusarium graminearum*. *Frontiers in Pharmacology*, **9**, 1–13. <https://doi.org/10.3389/fphar.2018.00610>
- Kapadiya, S., and Desai, M. A. 2017. Isolation of essential oil from buds of *Syzygium aromaticum* using hydrodistillation: Multiresponse optimization and predictive modeling. *International Journal of Advanced Science and Technology*, **6**(1): 405–418.
- Lili, H., Liming, D., Yifan, L., Tianwei, Y., Yun, Z., Liming, F., Fawu, S., Zhiying, C., and Min, Y., 2026. Multi-Target antifungal mechanism of vapor-phase *Cymbopogon citratus* essential oil: Effective control of Postharvest *Botrytis cinerea* and Powdery Mildew. *Foods*, **15**: 583 <https://doi.org/10.3390/foods15030583>
- Ma, M., Taylor, P. W. J., Chen, D., Vaghefi, N., and He, J.-Z. 2023. Major soilborne pathogens of field-processed tomatoes and management strategies. *Microorganisms*, **11**(2): 263.

- <https://doi.org/10.3390/microorganisms11020263>
- Mutlu-Ingok, A., Devecioglu, D., Dikmetas, D. N., Karbancioglu-Guler, F., and Capanoglu, E. 2020. Antibacterial, antifungal, antimycotoxigenic, and antioxidant activities of essential oils: An updated review. *Molecules*, **25**(20): 1–49. <https://doi.org/10.3390/molecules25204711>
- Neri F., M. Mari, and S. Brigati. 2006. Control of *Penicillium expansum* by volatile compounds. *Plant Pathol.*, **55**:100-105.
- Nizamani, S., Khaskheli, A. A., Jiskani, A. M., Khaskheli, S. A., Khaskheli, A. J., Poussio, G. B., Jamro, H., and Khaskheli, M. I. 2021. Isolation and identification of the fungi causing tomato fruit rot disease in the vicinity of Tandojam, Sindh. *Agricultural Science Digest*, **41**(Special Issue): 186–190.
- Panno, S., Davino, S., Caruso, A. G., Bertacca, S., Crnogorac, A., Mandić, A., Noris, E., and Matic, S. 2021. A review of the most common and economically important diseases that undermine the cultivation of tomato crops in the Mediterranean Basin. *Agronomy*, **11**(11): 2188. <https://doi.org/10.3390/agronomy11112188>
- Peralta-Ruiz, Y., Grande Tovar, C., Sinning-Mangonez, A., Bermont, D., Pérez Cordero, A., Paparella, A., and Chaves-López, C. 2020. Colletotrichum Gloesporioides Inhibition Using Chitosan-Ruta Graveolens L Essential Oil Coatings: Studies In Vitro and In Situ on Carica Papaya Fruit. *Int. J. Food Microbiol.* **326**: 10864.
- Sajad, A. M., and Abid, H. Q. J. 2017. Fungi associated with the spoilage of post-harvest tomato fruits in different markets of Jabalpur, Madhya Pradesh, India. *International Journal of Current Research Review*, **9**:12–16.
- Salvador, L., Miranda, S. P., Aragón, N., and Lara, V. 1999. Recubrimiento de quitosán en aguacate. *Journal of the Mexican Chemical Society*, **43**(1): 18–23. <https://www.redalyc.org/articulo.oa?id=47543204>
- Sebei, K., Sakouhi, F., Herchi, W., Khouja, M. L., Boukhchina, S. 2015. Chemical Composition and Antibacterial Activities of Seven Eucalyptus Species Essential Oils Leaves. *Biology Research* **48**: 7.
- Selles, S. M. A., Kouidri, M., Belhamiti, B. T., and Ait Amrane, A. 2020. Chemical composition and in vitro antibacterial and antioxidant activities of *Syzygium aromaticum* essential oil. *Journal of Food Measurement and Characterization*, **14**(4): 2352–2358.
- Shin, T. Y. 1998. Effect of *Syzygium aromaticum* extract on immediate hypersensitivity in rats. *Journal of Ethnopharmacology*, **60**: 125–131.
- Thabet, M., and Khalifa, W. 2018. Antifungal activities of clove oil against root rot and wilt pathogens of tomato plants. *American-Eurasian Journal of Agricultural & Environmental Sciences*, **18**(3): 105–114. <https://doi.org/10.5829/idosi.aejaes.2018.105.114>
- Uwineza M. S., El Yousfi B., and Lamiri A. 2018. Antifungal activities *in vitro* of essential oils of *Mentha pulegium*, *Eugenia aromatica*, and *Cedrus atlantica* on *Fusarium culmorum* and *Bipolaris sorokiniana*. *Revue Marocaine de Protection des Plantes*, **12**: 19-32.
- Wang, D., Zhang, J., Jia, X., Xin, L., and Zhai, H. 2019. Antifungal effects and potential mechanism of essential oils on *Colletotrichum gloeosporioides* in vitro and in vivo. *Molecules*, **24**(18): 3386. <https://doi.org/10.3390/molecules24183386>
- Xu, J. G., Liu, T., Hu, Q. P., and Cao, X. M. 2016. Chemical composition, antibacterial properties, and mechanism of action of essential oil from clove buds against *Staphylococcus aureus*. *Molecules*, **21**(9): 1194, 1–13. <https://doi.org/10.3390/molecules21091194>
- Yage, X., Qinglian, X., Xihong, L., Zhenmin, C., and Juan, Y. 2011. Activités antifongiques de l'huile de girofle contre *Rhizopus nigricans*, *Aspergillus flavus* et *Penicillium citrinum*, in vitro et sur des fruits blessés. doi.org/10.1111/j.1745-4565.2011.00347.x
- Yalemtesfa, B., Alemu, T., and Santhanam, A. 2010. Solid substrate fermentation and conversion of orange waste into fungal biomass using *Aspergillus niger* KA-06 and

Chaetomium spp. KC-06. *African Journal of Microbiology Research*, **4**: 1275–1281.

Yang, C.-J., Gao, Y., Du, K.-Y., and Luo, X.-Y. 2020. Screening of 17 Chinese medicine plants against phytopathogenic fungi and active components in *Syzygium aromaticum*. *Journal of Plant Diseases and Protection*, **127**: 237–244.
<https://doi.org/10.1007/s41348-019-00281-6>

Hanane Abed^{1,2*}, **Souad Boumaiza**², **Nouari Sadrati**^{2,3}, **Soraya Hihat**^{2,4} and **Ahmed Chouchou**²

¹Laboratory of Health and Environment, University of Mohamed El Bachir El Ibrahimi, Bordj Bou Arreridj, Algeria.

² Faculty of Nature and Life Sciences, University of Mohamed El Bachir El Ibrahimi, Bordj Bou Arreridj, Algeria.

³Laboratory of Characterization and Valorization of Natural Resources, University Mohamed El Bachir El Ibrahimi of Bordj Bou Arreridj, Bordj Bou Arreridj, Algeria.

⁴Laboratory of Biomathematics, Biophysics, Biochemical and Scientometry, University of Bejaia, 06000 Bejaia, Algeria

***Corresponding author**

E-mail:hanane.abed@univ-bba.dz

Tel: +213 659 858 464