

Research Article

Chemical Composition and Evaluation of Antifungal and Insecticidal Activities of Essential Oils Extracted from *Jambosa caryophyllus* (Thunb.) Nied: Clove Buds

Otmane Zouirech,¹ Reem Alajmi,² Hiba El jeddab,¹ Aimad Allali ,³ Mohammed Bourhia ,⁴ Abdelfattah El Moussaoui,⁵ Azeddin El Barnossi ,⁵ Ashraf M. Ahmed,² John P. Giesy,^{6,7,8,9} Mourad A. M. Aboul-Soud ,¹⁰ Badiaa Lyoussi,¹ and El houssine Derwich^{1,11}

¹Laboratory of Natural Substances, Pharmacology Environment, Modeling, Health and Quality of Life, Faculty of Sciences Dhar El Mahraz, University Sidi Mohamed Ben Abdellah, Fez, Morocco

²Department of Zoology, College of Science King Saud University, Riyadh 11451, Saudi Arabia

³Laboratory of Animal and Plant Production, and Agro-Industry, Faculty of Sciences, Ibn Tofail University, BP 133, Kenitra 14000, Morocco

⁴Higher Institute of Nursing Professions and Technical Health, Laayoune 70000, Morocco

⁵Laboratory of Biotechnology, Environment, Agrifood and Health, Faculty of Science, University of Sidi Mohamed Ben Abdellah, Fez 30050, Morocco

⁶Toxicology Centre, University of Saskatchewan, SK S7N 5B3, Saskatoon, Canada

⁷Department of Veterinary Biomedical Sciences, University of Saskatchewan, SK S7N 5B4, Saskatoon, Canada

⁸Department of Integrative Biology, Michigan State University, East Lansing 48824, MI, USA

⁹Department of Environmental Sciences, Baylor University, Waco 76706, TX, USA

¹⁰Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, P.O. Box 10219, Riyadh 11433, Saudi Arabia

¹¹Unity of GC/MS and GC-FID, City of Innovation, Sidi Mohamed Ben Abdallah University, Fez, Morocco

Correspondence should be addressed to Mohammed Bourhia; bourhiamohammed@gmail.com

Received 25 August 2022; Revised 12 September 2022; Accepted 5 October 2022; Published 17 October 2022

Academic Editor: Mohamed Z. M. Salem

Copyright © 2022 Otmane Zouirech et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Jambosa caryophyllus has been used in traditional phytotherapy as a treatment against infections. In the present work, essential oils extracted from clove buds (*Jambosa caryophyllus*) (EO-JC) were investigated for their composition, antifungal, and insecticidal properties. Extraction of EO-JC was performed by use of hydrodistillation using a Clevenger-type apparatus, and the EOs were analyzed by gas chromatography coupled with mass spectrometry (GC-MS). Antifungal activity of EO-JC was evaluated by the use of solid-state diffusion (disc method) and microdilution to determine the minimum inhibitory concentration (MIC), against three strains of fungus, *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum*. Insecticidal activity of EO-JC against the cowpea weevil, *Callosobruchus maculatus*, was determined to assess utility of EO-JC to control this pest. Several exposures including inhalation and contact were used to determine lethality, as well as the repulsion test was conducted at concentrations of 4, 8, 16, and 32 μ L EO-JC. Characterization of EO-JC by GC/MS revealed 34 compounds accounting for 99.98% of the mass of the extract. The predominant compound was eugenol (26.80%) followed by β -caryophyllene (16.03%) and eugenyl acetate (5.83%). The antifungal activity of EO-JC on solid media exhibited inhibitions in the range of 49% to 87%, and MIC was between 3.125 and 7.80 μ g EO-JC/mL. Insecticidal activity, as determined by the use of the inhalation test, and expressed as the LD₅₀ and LD₉₅ after 96 hours of exposure was 2.32 and 21.92 μ L/L air, respectively. In the contact test, a 96-hour exposure resulted in LD₅₀ and LD₉₅ of 5.51 and 11.05 μ L/L of air, respectively. EO-JC exhibited insecticidal activity against fungi and pest chickpea weevil.

1. Introduction

Chickpea (*Cicer arietinum* L.) is a very important crop in Morocco's production systems, coming in second place after fava beans with an area of 59,000 ha in 2014–2015 (MAPM's annual report 2016) [1]. It is an important source of carbohydrates and proteins, contains a significant amount of all essential amino acids, and is an important source of important vitamins such as riboflavin, niacin, thiamine, folate, and the precursor of vitamin A, β -carotene [2]. However, it is attacked by several insects of which the most dreaded is *Callosobruchus maculatus*, a beetle, in the leaf cutter family, Chrysomelidae which is among the most important species of insect pests of food legumes and is responsible for significant losses by reducing the quality and/or quantity of stored chickpea seed yield products during storage [3, 4].

The control of insects and other pests in warehouses has been adequately controlled by chemical control methods, including fumigation of stored products with carbon disulfide, phosphine, or spraying with malathion, carbaryl, pirimiphos-methyl, or permethrin. These chemicals have been reported to be effective against *C. maculatus* and other insect pests. However, uses of synthetic insecticides raise problems related to their cost as well as concerns for health of humans and effects on the environmental, as well as development of resistance to insecticides [5, 6]. These dangers have pushed the WHO to ban the use of certain chemical insecticides from more than one chemical family (organochlorine compounds, carbamates and thiocarbamates, organophosphates, and synthetic pyrethroids) [7, 8]. Hence, in order to avoid synthetic insecticides, the importance of obtaining effective natural alternatives that are cost effective, easily applicable, of minimal toxic potency to humans and wildlife, nonpersistent, biodegradable, and environmentally benign [9].

Due to adverse effects on both quality and quantity, exposure of plants and preserve seeds to fungal pathogens can lead to considerable economic losses. Various fungal contaminants can cause considerable losses and attack chickpea seeds and other foodstuffs. In particular, *Aspergillus niger* is one of the fungi most frequently isolated from peanuts, pecans, pistachios, hazelnuts, walnuts, kola nuts, coconuts, and copra [10]. *Aspergillus flavus* is a saprotrophic and pathogenic fungus of cosmopolitan distribution. It is best known for its colonization of cereals, legumes, and the fungus [11], *Fusarium oxysporum* f. sp. *albedinis* is the agent of vascular wilt of date palm (*Phoenix dactylifera*) [12].

Aromatic and medicinal plants are a source of chemical molecules resulting from secondary metabolism, belonging to diverse chemical classes, such as alkaloids, phenols, flavonoids, terpenoids, and steroids [13, 14]. Clove buds (*Jambosa caryophyllus*) are medicinal and aromatic parts of a plant of the Myrtaceae family that contain various constituents, including phenylpropanoids such as carvacrol, thymol, eugenol, and cinnamaldehyde [15].

Essential oils of clove buds (EO-JC) are of particular interest because of their biological activities [15, 16]. EO-JC

has broad medicinal properties and applications among other uses, as anticancer, antibacterial, antifungal, analgesic, antiseptic, antidiabetic, antiobesity, anti-inflammatory, antioxidant, antiviral, and aphrodisiac [17–23].

In this study, in order to find a less expensive, effective, and accessible alternative to synthetic insecticides and fungicides, essential oils extracted from clove buds, were characterized by GC-MS, and their insecticidal activity on *C. maculatus*, and antifungal activity, were investigated.

2. Materials and Methods

2.1. Plant Material. *Jambosa caryophyllus* was collected in Fez City-Morocco in 2021 before being identified by a botanist and given a voucher number JC22/Fez-03. Next, the flower buds of the clove were dried by the use of an oven set to 40°C for 3 days before being ground into a fine powder prior to extraction.

2.2. Extraction of EO-JC. Essential oils (EO) of clove buds were obtained by hydrodistillation, using a Clevenger-type extractor [24]. This technique is based on the power of water vapor to transport essential oils. The essential oil yield was determined through the ratio of the mass of flower buds of clove (g) by the mass of essential oil (g) (%).

$$Rd (\%) = \frac{\text{Quantity of oil in g}}{\text{Quantity of plant material in g}} * 100. \quad (1)$$

2.3. Composition of EO-JC. Gas chromatography (Ultra GC Trace), linked to a mass spectrometer (Polaris Q), ion trap in electron impact (EI) mode with ionization energy of 70 eV, was used to separate and identify chemical components of EO-JC. This chromatographic apparatus was equipped with a silica capillary column type (CP-SIL5CB) (WCOT fused silica), 60 m in length, 0.32 mm diameter, and 1.25 μ m thickness. The column temperature is designed to rise at a rate of 3°C/min from 45 to 290°C. The injector is set to 270°C, while the detector (ionization source) was 200°C. The carrier gas (helium) flow rate was 1 mL/min. The injected sample contains 1.0 μ L of diluted oil in hexane. The constituents of EO-JC were identified by matching their mass spectra to those of the library NIST-MS and by calculating the retention index and comparing it with the retention index of literature Adams (2007) [25].

2.4. Insecticidal Activity of EO-JC

2.4.1. Conditions of Collection and Rearing of Insects. Chickpea weevils (*C. maculatus*) were maintained on chickpea seeds (*Cicer arietinum*) in transparent boxes of capacity (1 L) covered inside with a transparent fabric to raise the bruchids, deposited in a temperature of about 25 \pm 2°C keeping the relative humidity of about 65% \pm 5% and a photoperiodic cycle of 14 h (light)/10 h (dark).

2.4.2. Toxicity of EO-JC by the Contact Test. Toxic potency of EO-JC was determined by contact by previously described methods [3, 26, 27], with some modifications. 0.1 kg of chickpeas were infected with five pairs of 0–48 hold insects, placed in plastic containers (0.25 L). For each concentration of EO-JC, we use a perforated top and a thin transparent cloth. After that, using an automated pipette, EO-JC was added to the beans and mixed for 2 minutes. A control group of 0.1 kg chickpeas infected with five pairs of oil-free insects was utilized at the same time. After 48 h of confinement, the mortality of adults was evaluated as described elsewhere [28]. Based on the results obtained in the preliminary tests, a range of concentrations was determined (4, 8, 16, and 32 $\mu\text{L}/100\text{ g}$). The tests were repeated three times for each concentration. Insecticidal potency of EOs was evaluated by daily counting the number of dead *C. maculatus* adults under a stereoscopic microscope (Equation).

$$P_c (\%) = \frac{P_o - P_t}{100 - P_t} * 100, \quad (2)$$

where P_c is the corrected % mortality; P_o is the observed mortality in the trial, and P_t is the mortality in the negative control.

Eggs were counted 12 days after the experiment began, and emerging individuals were counted 30 days later. The decrease in fecundity of females and emerging adults for each concentration of EO-JC was calculated.

$$PR (\%) = \left(1 - \frac{N_t}{N_c}\right) * 100, \quad (3)$$

where PR is the reduction rate compared to the control, N_c is the number of eggs or insects in the control, and N_t is the number of eggs in the trial.

2.4.3. Toxicity of Essential Oils Tested by Inhalation. Effects of EO-JC on adult *C. maculatus* were determined after exposure via inhalation. Purpose, these adults were placed in 1-liter glass vials, and small cotton masses were suspended with a thread attached to the inside of the lid. Concentrations of 4, 8, 16, or 32 μL EO-JC/L were applied to the cotton with a ProPette™. 10 bruchids (male and female) of *C. maculatus* aged 0–48 hours were placed in each jar with a seal. Three replicates were performed for each dose. In comparison with an untreated control sample, calculation of the mortality rate according to the formula of Abbott is given as follows (Equation) [29]:

$$P_c (\%) = \frac{P_o - P_t}{100 - P_t} * 100, \quad (4)$$

where P_c is the corrected % mortality, P_o is the mortality in the trial, and P_t is the mortality in the negative control.

2.4.4. Repellent Effect of EO-JC. The preferable area technique on the filter paper was used to investigate the repellent effect of EOs against adult *C. maculatus* [29]. Discs were divided into two halves, each having a surface area of 31.80 cm^2 . A volume of 500 μL of each concentration of EO-JC previously prepared in acetone (4, 8, 16, and 32 $\mu\text{L}/\text{mL}$) was evenly distributed for

one of the two halves, resulting in doses of 0.016, 0.079, 0.157, and 0.315 $\mu\text{L}/\text{cm}^2$ per disc, while the other half received only 500 μL of each concentration of EO-JC previously prepared in acetone. The other half received only 500 μL of solvent (negative control). After that, the Petri dishes were covered with Parafilm® for 30 minutes. The number of bruchids on the EO-treated half of the disc was compared to the number of bruchids on the untreated half of the disc. For each experiment, three replicates were placed in the same setting as the insect rearing. Repulsion was measured according to the formula of Lehman [29].

$$Pr (\%) = \frac{N_c - N_t}{N_c + N_t} * 100. \quad (5)$$

Pr (%) is the percent repulsion, N_c is the number of insects in the negative control area, and N_t is the number of insects in the treatment area with EO-JC.

2.5. Antifungal Activity of EO-JC

2.5.1. Evaluation of Fungal Activity. Evaluation of antifungal activity of EO-JC was performed by the use of the disk diffusion method [30]. Petri dishes containing MH (Muel-ler–Hinton) medium were grown with the three fungal strains and inoculated with *A. niger*, *A. flavus*, and *F. oxysporum* by the agar plot method. Sterile Whatman paper discs (6 mm diameter) were placed in the center of the Petri dish and then impregnated with 20 μL of EO-JC (1 mg/mL) and fluconazole (positive controls) at a dose of 5 mg/mL, whereas the fungal strains were incubated at 37°C. 7 days for *A. niger*, *A. flavus*, and *F. oxysporum*, percent inhibition was evaluated [31, 32]. The percentage of inhibition was calculated according to the mathematical formula:

$$I (\%) = \frac{D_c - D_s}{D_c} * 100, \quad (6)$$

where I (%) is the percentage of inhibition; D_c is the diameter of the control; D_s is the diameter in the presence of essential oils.

2.5.2. Determination of the Minimum Inhibitory Concentration. The minimum inhibition concentration (MIC) of EO-JC was determined by using the microdilution technique [30]. Microplates were prepared under aseptic conditions, each sterile 96-well microplate was labeled, 100 μL of EO used for the test in 10% (v/v) DMSO was pipetted into the first column of the plate, and 50 μL of sterile ME (malt extract) for the fungal strains was added to all other wells; serial dilutions were made using a multichannel pipette, and finally 30 L after 7 days at 37°C for *A. niger*, *A. flavus*, and *F. oxysporum* [28, 30]. The MIC endpoint is established by direct observation of good development or colorimetric technique 0.2 percent (w/v) [32, 33].

3. Statistical Analysis

The results were reported as the mean of three replicates with standard deviations (standard deviation). Shapiro–Wilk

tests were used to validate the normality of the variables, and Levene's test was used to examine the homogeneity of variances, and both were performed using GraphPad Prism (version.8.0.1). Analysis of variance (one way-ANOVA) and Tukey's multiple comparison test were used to examine the differences between the means. At a probability level (p) 0.05, differences were considered statistically significant.

4. Results

4.1. EO-JC Extraction Yield. The yield of OE-SA obtained by the four-hour hydro-distillation technique in a Clevenger apparatus was $1.344 \pm 0.030\%$, with a characteristic yellow to clear brown color and aromatic odor.

4.2. Chemical Composition of the EO-JC. Instrumental analysis by GC-MS of the EO-JC showed that the oil consists of 34 compounds representing identified components (Figure 1). These compounds represent more than 99.98% of the total essential oil (Table 1).

4.3. Effect of EO-JC on Mortality by Direct Contact with Adult *C. maculatus*. There was a significant, direct relationship between the concentrations of EO-JC and the mortality of adult *C. maculatus*, and there was also a significant relationship between durations of exposure (Figure 2). During the four observation periods (24, 48, 72, and 96 hours), at the least dose of $4 \mu\text{L}$ EO-JC/L of air, mortalities at each duration of exposure were 6.68 ± 5.76 , 13.33 ± 5.77 , 23.33 ± 5.76 , and $46.66\% \pm 5.775$, respectively; mortalities at $8 \mu\text{L}$ EO-JC/L were 20, 36.667 ± 5.7 , and 53.33 ± 5.76 , 70%, respectively; at exposure to $16 \mu\text{L}$ EO-JC/L air, mortalities were $33.33\% \pm 5.777$, $56.66\% \pm 5.76$, 80 ± 10 , and 100%, respectively. The maximum concentration of $32 \mu\text{L}$ EO-JC/L air caused complete mortality of approximately 100% during the four durations of exposure.

4.4. Effect of Inhalation of EO-JC on Mortality of Adult *C. maculatus*. Mortality of adult *C. maculatus* was significantly and directly proportional to the concentration of EO-JC to which they were exposed. Exposure for 24 hours to doses of 0, 4, 8, 16, or $32 \mu\text{L}$ EO-JC/L of air resulted in mortalities of less than 50%; mortalities were 0.0, 0.0, 13.33 ± 5.77 , and $46.66 \pm 11.54\%$, respectively. Exposure for 48 hours to the same concentrations caused mortalities of 0.00, 16.67 ± 5.78 , 23.33 ± 5.77 , $30 \pm 1.0\%$, and $83.33 \pm 15\%$, respectively. Exposure for 96 hours to 0, 4, 8, 16, and $32 \mu\text{L}$ EO-JC/L air resulted in mortalities greater 50% with values of 66.66 ± 5.77 , 83.33 ± 5.76 , 86.67 ± 5.77 , and 100%, respectively. Furthermore, the minimum concentration of the essential oil tested to achieve 100% mortality of *C. maculatus* adults was $32 \mu\text{L}/\text{L}$ of air volume after 72 hours of exposure. Relatively, great mortality was observed in adult chickpea bruchids exposed for 96 hours, which showed the potent insecticidal effect of EO-JC. Direct contact to EO-JC for 96 hours resulted in LD_{50} and LD_{95} of 5.51 and $11.05 \mu\text{L}$ EO-JC/L, air. LD_{50} and LD_{95} values obtained in the inhalation

test were greater than those obtained with the contact test, based on statistical analysis. Lethal doses to 50% (LD_{50}) and 95% (LD_{95}) of individual adult *C. maculatus* were determined for EO-JC (Figure 3). For the inhalation test, the DL_{50} and DL_{95} after 96 hours of exposure were 2.32 and $21.92 \mu\text{L}$ EO-JC/L, air, respectively.

4.5. Effect of EO-JC on Mortality of *C. maculatus* Larvae. Exposure of female *C. maculatus* to EO-JC reduced oviposition in a dose-dependent manner (Figure 4(a)), but none of the concentrations of EO-JC completely prevented oviposition. The number of eggs laid per unexposed female *C. maculatus* was 111.0 ± 9.0 . At the least concentration of $4 \mu\text{L}$ EO-JC/100 g, the mean number of eggs per female was 52 ± 12 , which represents a decrease of 64 ± 10.8 eggs compared to the negative control (Figure 4(b)). The greatest dose of $32 \mu\text{L}$ EO-JC/100 g resulted in a 100% reduction in oviposition compared to the unexposed control females. Exposure to $32 \mu\text{L}$ EO-JC/100 g also eliminated the emergency of juveniles.

4.6. Repellent Activity of the EO-JC. EO-JC was moderately repellent of *C. maculatus* in a dose-dependent manner with a maximum repellence of $93.33 \pm 11.55\%$ after exposure to $0.503 \mu\text{L}$ EO-JC/ cm^2 for 60.

4.7. Antifungal Activity of EO-JC. EO-JC exhibited antifungal activity against a variety of fungi pathogenic to plants (Table 2). The greatest activity was observed against *A. flavus* (MTCC 9606), with inhibition between 50 ± 2.5 and $87 \pm 2.4\%$. MIC values ranged from 2.9 ± 0.0 to $5.9 \pm 0.02 \mu\text{g}$ EO_JC/mL, which was significantly ($p \leq 0.05$) greater than that of fluconazole (Table 2).

5. Discussion

The main compound in EO-JC is eugenol [17], which has been reported to represent 49.0 to 56% of EO-JC [34, 35]; however, it has been reported to comprise as much as 87.1% of the mass of extracted EOs [33]. However, in the present study, EO-JC constituted only 26.8% (Table 2). Variability in the proportion of EO-JC contributed by eugenol and total yield of EO-JC might be due to various factors such as geographical variations, environmental conditions, physiological variations, genetic factors as well as differences in methods used for extraction of EOs [36, 37]. The insecticidal activity of EO-JC observed against adults of *C. maculatus*, observed in this study, is consistent with the results of previous studies [15].

In the present work, the insecticidal and fungicidal power of EO-JC can be attributed to eugenol and β -caryophyllene as they are known for their insecticidal effects, particularly when they act on synapses of herbivorous insects and block the production of neurotransmitters, recorded literature [38–40]. Results of this study indicate that EO-JC can be used for the control of *C. maculatus* populations which is possible. Essential oils of *J. caryophyllus*

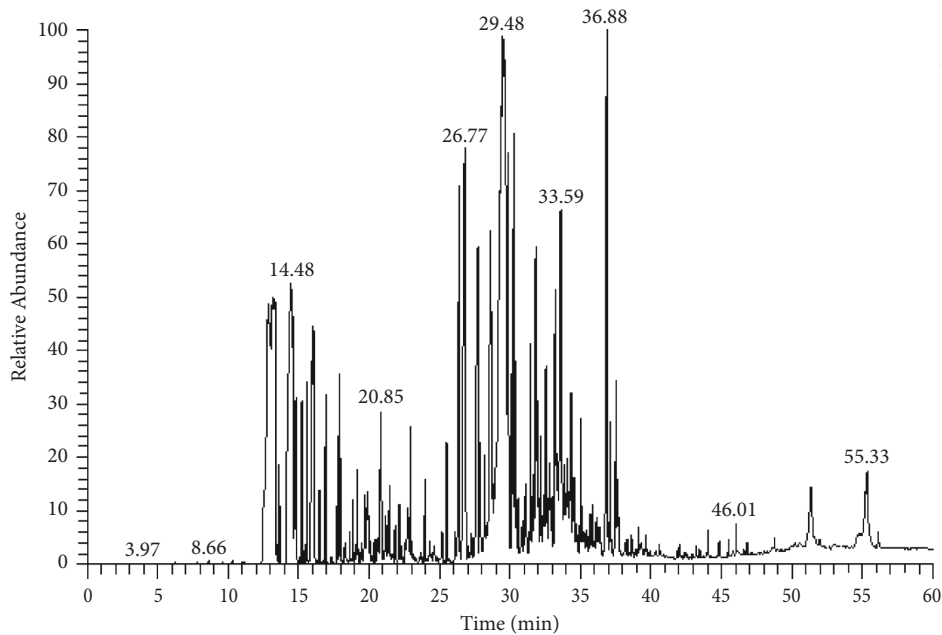


FIGURE 1: GC-MS chromatogram of essential oils from *J. caryophyllus* clove buds.

act by gaseous diffusion [41]. This allows them to reach all interstices in the stored seeds [41]. Thus, they can be used as a fumigant [42, 43]. Like EOs, *J. caryophyllus* powder has been shown to be effective in eliminating individuals of (*C. maculatus*) within 3 days [42, 43]. Furthermore, our results show that treated EO-JC inhibits reproduction of *C. maculatus* F., which is consistent with the results of previous studies [43].

The fact that exposure of adult female *C. maculatus* to EO-JC reduced fecundity might be due to its main component, eugenol, which is a volatile substance contained in the EOs of *J. caryophyllus* [44, 45], and this monoterpene has been evaluated for its toxicity towards *C. maculatus*, which can eventually reach its larvae hidden inside the treated seeds [44–46].

Based on the LD₅₀ and LD₉₅ of EO-JC against adults of *C. maculatus*, it is likely that it can be used to protect grains from this pest. Insecticidal activity of EOs of *J. caryophyllus* against pests of stored products in particular (*Cicer arietinum*) has been the subject of only a few works such as those of Allali and coauthors [47], but EO-JC has been shown to be effective at controlling several species of insect pests in stored grain [48].

The mechanism of action of these oils is not yet well known. It is therefore important to carry out toxicological studies in order to determine the mode of action of the essential oils and to prevent possible resistance phenomena that might occur during treatments. The fact that the tested EOs were effective on both adults, eggs, and larvae suggests that they act via the respiratory pathway. In *C. maculatus* eggs, the oil vapors act through the egg respiratory tract [49, 50]. This is consistent with reports that the components of essential oils act on the respiratory chain by inhibiting the activity of monoxygenases in the treated insect [51]. It has

been reported that the respiratory activity of *C. maculatus* eggs is six times less than that of neofomed larvae. This reflects a low activity of monoxygenases in eggs and therefore a higher tolerance to products that inhibit these enzymes [52]. Eggs, which are classified as a quiescent stage, are more tolerant to synthetic insecticides [53]. Since EO-JC is composed of volatile matter, contact treatment is not applicable. Indeed, contact treatment does not take into account limitations related to evaporation of the active ingredients. Hence, there are difficulties encountered in determining the effective concentration. Fumigation allows large masses of seeds to be treated without the need to mix or move them. Thus, due to its volatility and biodegradation are advantages of using EOs as pesticides [54].

The results of this study that demonstrated broad antifungal activity of EO-JC are consistent with the results of previous studies [55, 56]. It was observed in similar studies that this oil possessed inhibitory activity against *Aspergillus niger* [57, 58]. Similarly, it has been shown that EO-JC has inhibitory effects on strains of *Aspergillus flavus* [59, 60]. The fungicidal activity of *J. caryophyllus* essential oil has also been reported on strains of *Fusarium oxysporum* [56]. The mode of action of the antifungal activity of volatile oils is not well known. However, some compounds in EOs appear to be strong inhibitors of malt amylase and catalase in some fungi [61]. Recently, studies have reported that some volatile oils affect the disruption of the endomembrane system of the fungal cell, including the plasma membrane and mitochondria, as well as inhibition of synthesis of ergosterol, malate dehydrogenase, mitochondrial ATPase, and succinate dehydrogenase [62, 63], all of which result in damage to the cytoplasmic membrane and subsequent leakage of intracellular components such as DNA [64].

TABLE 1: Chemical composition of EO extracted from *J. caryophyllus* clove buds.

Peak	RT	Compounds	Molecular formula	Chemical class	RI		Relative Area (%)
					Cal	Lit	
1	13.15	β -Caryophyllene	C15H25	ST.H	1417	1419	16.03
2	15.24	α -Thujone	C10H16O	MO.O	1102	1102	0.79
3	15.58	3-Carene	C10H16	MO.H	1007	1011	0.76
4	16.0	Thymol	C10H14O	MO.O	1285	1290	4.37
5	16.49	α -Terpinene	C10H18O	MO.O	1015	1017	0.26
6	16.93	α -Pinene	C10H16	MO.H	936	939	0.8
7	17.88	Camphene	C10H16	MO.H	950	954	1.75
8	18.84	β -Pinene	C10H16	MO.H	974	979	0.24
9	19.17	1.8-Cineole	C10H18O	MO.O	1030	1031	0.48
10	19.93	Limonene	C10H16	MO.H	1020	1029	1.20
11	20.85	Verbenol	C10H16O	MO.O	1139	1141	1.45
12	21.43	Terpinene-4-ol	C10H18O	MO.O	1172	1177	0.87
13	22.15	Seychellene	C15H24	ST.H	1446	1446	0.37
14	22.97	α -Copaene	C15H24	ST.H	1377	1376	1.71
15	23.95	α -Ylangene	C15H24	ST.H	1418	1420	0.33
16	25.50	B-Patchoulene	C15H24	ST.H	1381	1381	0.46
17	26.40	Sativene	C15H24	ST.H	1390	1391	3.74
18	26.77	Eugenyl acetate	C12H14O3	O	1520	1524	5.83
19	27.71	Aristolone	C15H22O	ST.O	1760	1763	3.33
20	29.48	Eugenol	C10H12O2	MO.O	1359	1356	26.8
21	30.31	Borneol	C10H18O	MO.O	1149	1169	5.0
22	31.47	Solanone	C13H22O	O	1360	1361	0.71
23	31.85	β -Phellandrene	C10H16	MO.H	1029	1029	3.13
24	32.54	Myrcene	C10H16	MO.H	991	990	1.04
25	33.59	α -Humulene	C15H24	ST.H	1452	1454	5.55
26	34.33	Cymene	C10H14	MO.H	1425	1426	1.81
27	36.88	β -Caryophyllene epoxide	C15H24O	ST.O	1665	1667	5.03
28	37.12	Bornyl acetate	C12H20O2	O	1285	1288	0.67
29	37.51	Terpinyl acetate	C12H20O2	O	1310	1317	1.59
30	51.34	Terpinolene	C10H16	MO.H	1080	1088	1.50
31	51.44	Eudesmol	C15H26O	ST.O	1660	1663	2.34
32	51.55	Trans-verbenol	C10H16O	MO.O	1140	1144	0.01
33	54.01	Caranone	C10H16O	MO.O	1200	1200	0.02
34	55.33	Linalool	C10H18O	MO.O	1090	1090	0.01
Chemical class							
Monoterpene oxygenated (MO.O)							40.06
Monoterpene hydrocarbons (MO.H)							12.23
Other (O)							8.80
Sesquiterpene oxygenated (ST.O)							10.70
Sesquiterpene hydrocarbons (ST.H)							28.19
Total							99.98

RT: retention time; RI: retention index; Cal: calculated; Lit: literature.

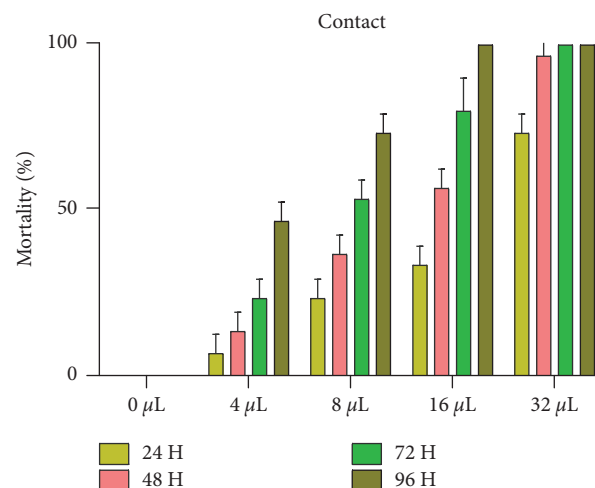


FIGURE 2: Mortality (%) of adult *C. maculatus* exposed to various concentrations of EO-JC in air.

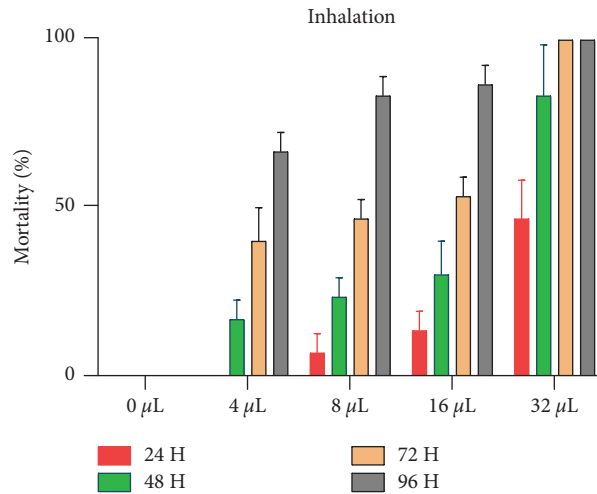


FIGURE 3: Mortality (%) of adult *C. maculatus* exposed via inhalation to 5 concentrations of EO-JC for 4 durations.

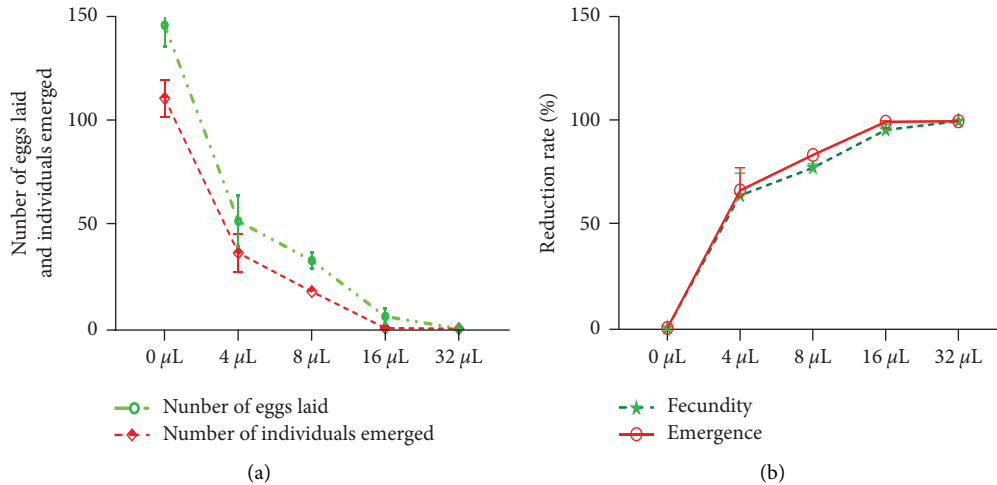


FIGURE 4: (a) Fecundity and emergence of new individuals after direct contact with 5 doses of EO-JC. (b) Percent reductions of fecundity and emergence after direct contact with five doses of EO-JC.

TABLE 2: Antifungal activity and minimum inhibition concentrations (MIC) of essential oils extracted from seeds of *J. caryophyllus* (EO-JC) and the synthetic fungicide, fluconazole.

		<i>Aspergillus niger</i> (MTCC 282)	<i>Aspergillus flavus</i> (MTCC 9606)	<i>Fusarium oxysporum</i> (MTCC 9913)
EO-JC	Antifungal activity	50 ± 2.5% ^a	89 ± 2.4% ^b	56 ± 2.5% ^c
	MIC (μg/mL)	2.9 ± 0.02 ^a	5.9 ± 0.02 ^b	2.9 ± 0.0 ^a
Flu	Antifungal activity	8.2 ± 2.0% ^a	0.0 ± 0.0% ^b	31 ± 0.58% ^c
	MIC (μg/mL)	7.1 ± 0.0 ^a	—	3.1 ± 0.0 ^b

Means (±SD, $n=3$) followed by different letters in the same row are significantly different (one-way ANOVA; followed by Tukey's multiple range test, $p \leq 0.05$). EO-JC: essential oils of *J. caryophyllus* clove buds; Flu: fluconazole.

6. Conclusions

In the present work, essential oils extracted from *Jambosa caryophyllus* clove buds (EO-JC) were investigated for their composition, antifungal, and insecticidal properties. Results showed that EO-JC possessed promising activities, which

may be attributed to its richness in eugenol, β -caryophyllene and eugenyl acetate. It can be concluded that the essential oils of *Jambosa caryophyllus* clove buds exhibited an interesting insecticidal effect, which is promising as a natural bioinsecticide that can be applied to grain crops to protect the seeds of various pests in crops and stored

grains that might be able to be used instead of more expensive and hazardous pesticides.

Data Availability

Data used to support the findings are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

J. P. Giesy was supported by distinguished visiting professorship from the Department of Environmental Sciences of Baylor University, Waco, Texas, USA. This research was funded by the Researchers Supporting Project number (RSP-2021/99), King Saud University, Saudi Arabia.

References

- [1] M. Ministry of Agriculture, Maritime Fisheries, Rural Development and Water and Forests, *Agriculture in Figures*, <https://www.devex.com/organizations/ministry-of-agriculture-maritime-fisheries-rural-development-and-water-and-forests-map/mdref-morocco-137577>, 2018.
- [2] A. K. Jukanti, P. M. Gaur, C. L. L. Gowda, and R. N. Chibbar, "Nutritional Quality and health benefits of Chickpea (*Cicer Arietinum* L.): a review," *The British Journal of Nutrition*, vol. 108, 2012.
- [3] L. Ferreira, D. Rafael, and C. Lima, "Industrial crops & products chemical composition and insecticidal effect of essential oils from *Illicium verum* and *Eugenia caryophyllus* on *Callosobruchus maculatus* in cowpea," *Industrial Crops and Products*, vol. 145, Article ID 112088, 2020.
- [4] A. Boughdad, R. Elkasimi, and A. Boughdad, *Essentielles de mentha sur Related Papers*, 2011.
- [5] G. Keneni, E. Bekele, and E. Getu, "Breeding food legumes for resistance to storage insect pests: potential and limitations," *Sustainability*, vol. 3, pp. 1399–1415, 2011.
- [6] I. Pest and M. Decisions, "Influences of stored product insect movements on integrated pest management decisions," *Insects*, vol. 10, pp. 1–20, 2019.
- [7] J. K. Willcox, S. L. Ash, and G. L. Catignani, "Antioxidants and prevention of chronic disease," *Critical Reviews in Food Science and Nutrition*, vol. 44, no. 4, pp. 275–295, 2004.
- [8] P. Cherin, E. Voronska, N. Fraoucene, and C. De Jaeger, "Toxicité aiguë des pesticides chez l'homme Acute toxicity of pesticides in human," *Médecine & Longévité*, vol. 4, no. 2, pp. 68–74, 2012.
- [9] M. Scholler, S. Prozell, A. G. Al-Kirshi, and C. Reichmuth, "Towards biological control as a major component of integrated pest management in stored product protection," *Journal of Stored Products Research*, vol. 33, no. 1, pp. 81–97, 1997.
- [10] S. E. Tjamos, P. P. Antoniou, A. Kazantzidou, D. F. Antonopoulos, I. Papageorgiou, and E. C. Tjamos, "Aspergillus Niger and Aspergillus carbonarius in corinth raisin and wine-producing vineyards in Greece: population composition," *Journal of Phytopathology*, vol. 152, no. 4, pp. 250–255, 2004.
- [11] I. Methods, Contents Introduction *Aspergillus flavus Aspergillus oryzae*, <https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/aspergillus-oryzae>, 2014.
- [12] D. A. Komeil, M. Y. Abdalla, A. F. El-Bebany, and A. B. A. Basyony, "Resistance marker detection in ten date palm cultivars to the wilt pathogen, *fusarium oxysporum*," *Asian Journal of Plant Sciences*, vol. 20, no. 2, pp. 363–369, 2021.
- [13] F. Bongomin, "Global and multi-national prevalence of fungal diseases—estimate precision," *Journal of Fungi*, vol. 3, 2017.
- [14] R. Tiwari, "Plant secondary metabolites: a review," *International Journal of Engineering Research and General Science*, vol. 3, 2015.
- [15] K. Chaieb, H. Hajlaoui, T. Zmantar et al., "The chemical composition and biological activity of clove essential oil, eugenia caryophyllata (*Syzygium aromaticum* L. Myrtaceae)," *Phytotherapy Research*, vol. 21, no. 6, pp. 501–506, 2006.
- [16] K. Hostettmann and J. Wolfender, *The Search for Biologically Active Secondary*, vol. 471, 1997.
- [17] K. Chaieb, T. Zmantar, R. Ksouri, H. Hajlaoui, and K. Mahdouani, "Antioxidant properties of the essential oil of *Eugenia Caryophyllata* and its antifungal activity against a large number of clinical *Candida Species*," *Mycoses*, vol. 50, pp. 403–406, 2007.
- [18] H. P. Devkota and A. Adhikari-devkota, "Cold pressed clove (*Syzygium Aromaticum*) oil," *Cold Pressed Oil*, pp. 273–276, 2020.
- [19] S. Hussain, R. Rahman, A. Mushtaq, and A. El Zerey-belaskri, "Clove a review of a precious species with multiple uses clove," *International Journal of Chemical and Biochemical Sciences*, vol. 11, 2017.
- [20] S. M. El-Darier, A. M. D. El-Ahwany, E. T. Elkenany, A. A. Abdeldaim, and A. A. Abdeldaim, "An in vitro study on antimicrobial and anticancer potentiality of thyme and clove oils," *Rendiconti Lincei. Scienze Fisiche e Naturali*, vol. 29, no. 1, pp. 131–139, 2018.
- [21] M. Saeed, M. S. Khan, and M. Alagawany, "Clove (*Syzygium aromaticum*) and its phytochemicals in ruminant feed: an updated review," *Rendiconti Lincei. Scienze Fisiche e Naturali*, vol. 32, 2021.
- [22] M. Aghrouch and H. Techniques, "Antibacterial Activity of essential oils extracts from cinnamon, thyme, clove and geranium against a gram negative and gram positive pathogenic bacteria," *Journal of Disease and Medicinal Plants*, vol. 3, 2017.
- [23] K. Kaur and S. Kaushal, "Phytochemistry and pharmacological aspects of *Syzygium Aromaticum*: a review," *Journal of Pharmacognosy and Phytochemistry*, vol. 8, 2019.
- [24] J. B. Harborne, "Methods of plant analysis," *Phytochemistry Methods*, Chapman & Hall, London, UK, 1973.
- [25] R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, Allured Publishing Corporation, Carol Stream, IL, USA, 4th edition, 2007.
- [26] N. Pajaro-castro, K. Caballero-gallardo, R. Hernandez-lambra, E. Stashenko, and J. Olivero-verbel, "Essential oils from plants of the genus *Cymbopogon* as natural insecticides to control stored product pests," *Journal of Stored Products Research*, vol. 62, pp. 81–83, 2015.
- [27] E. M. Abdelfattah, A. Aimad, M. Bourhia et al., "Insecticidal and antifungal activities of chemically-characterized essential oils from the leaves of *withania frutescens* L.," *Life*, vol. 12, no. 1, p. 88, 2022.

- [28] M. Carpena, B. Nuñez-estevez, A. Soria-lopez, P. Garcia-oliveira, and M. A. Prieto, "Essential oils and their application on active packaging systems: a review," *Resources*, vol. 10, pp. 1–20, 2021.
- [29] L. L. McDonald, R. H. Guy, and R. D. Speirs, *Preliminary Evaluation of New Candidate Materials as Toxicants, Repellents, and Attractants against Stored-Product Insects-1*, Agricultural Research Service, Washington, D.C, USA, 1970.
- [30] A. El Barnossi, F. Moussaid, and A. Iraqi Housseini, "Antifungal activity of Bacillus sp. Gn-A11-18 isolated from decomposing solid green household waste in water and soil against *Candida albicans* and *Aspergillus Niger*," *E3S Web of Conferences*, vol. 150, 2020.
- [31] K. Chebbac, H. K. Ghneim, A. El Moussaoui et al., "Antioxidant and antimicrobial activities of chemically-characterized essential oil from artemisia aragonensis lam. against drug-resistant microbes," *Molecules*, vol. 27, no. 3, Article ID 1136, 2022.
- [32] O. Zouirech, A. A. Alyousef, A. El Barnossi et al., "Phytochemical analysis and antioxidant, antibacterial, and antifungal effects of essential oil of black caraway (*Nigella sativa* L.) seeds against drug-resistant clinically pathogenic microorganisms," *BioMed Research International*, vol. 2022, Article ID 5218950, 11 pages, 2022.
- [33] A. El Moussaoui, M. Bourhia, F. Z. Jawhari et al., "Chemical profiling, antioxidant, and antimicrobial activity against drug-resistant microbes of essential oil from *Withania frutescens* L.," *Applied Sciences*, vol. 11, no. 11, Article ID 5168, 2021.
- [34] S. Lee, M. Najiah, W. Wendy, and M. Nadirah, "Chemical composition and antimicrobial activity of the essential oil of *Syzygium aromaticum* flower bud (Clove) against fish systemic bacteria isolated from aquaculture sites," *Frontiers of Agriculture in China*, vol. 3, no. 3, pp. 332–336, 2009.
- [35] B. Amelia, E. Saepudin, A. H. Cahyana, and D. U. Rahayu, "GC-MS analysis of clove (*Syzygium aromaticum*) bud essential oil from java and Manado," *AIP Conference Proceedings*, vol. 1862, 2017.
- [36] J. Wiley, A. C. Figueiredo, J. G. Barroso, L. G. Pedro, and J. J. C. Scheffer, "Factors affecting secondary metabolite production in plants: volatile components and essential oils," *Flavour and Fragrance Journal*, vol. 23, pp. 213–226, 2008.
- [37] L. Harkat-madouri, B. Asma, K. Madani et al., "Chemical composition, antibacterial and antioxidant activities of essential oil of *Eucalyptus globulus* from Algeria," *Industrial Crops and Products*, vol. 78, pp. 148–153, 2015.
- [38] A. Stokłosa, R. Matraszek, M. B. Isman, and M. K. Upadhyaya, "Phytotoxic activity of clove oil, its constituents, and its modification by light intensity in broccoli and common lambsquarters (*Chenopodium album*)," *Weed Science*, vol. 60, no. 4, pp. 607–611, 2012.
- [39] D. N. Moss, "Chemical composition and bio-insecticidal activity of eugenia caryophyllata essential oil against *Spodoptera littoralis*," *Microbiol Hyg Alim*, vol. 25, no. 73, pp. 94–98, 2013.
- [40] F. Achimón, L. E. Leal, and R. P. Pizzolitto, "Insecticidal and antifungal effects of lemon, orange, and grapefruit peel essential oils from Argentina," *AgriScientia*, vol. 39, no. 1, pp. 71–82, 2022.
- [41] D. P. Papachristos and D. C. Stamopoulos, "Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* (Say) (Coleoptera: bruchidae)," *Journal of Stored Products Research*, vol. 38, no. 2, pp. 117–128, 2002.
- [42] S. S. E. Al, "Insecticidal activity of essential oils of four medicinal plants against different stored grain insect pests," *Pakistan Journal of Zoology*, vol. 46, no. 5, pp. 1407–1414, 2014.
- [43] A. Kellouche and D. Biologie, "Activite plantes et de l'huile essentielle d'une d'entre elles sur *Callosobruchus maculatus* (F.)," *International Journal of Tropical Insect Science*, vol. 24, no. 2, pp. 184–191, 2004.
- [44] I. S. Rana, "Evaluation of antifungal activity in essential oil of the *syzygium aromaticum* (L.) by extraction, purification and analysis of its main component eugenol," *Brazilian Journal of Microbiology*, vol. 42, pp. 1269–1277, 2011.
- [45] M. Machado, A. M. Dinis, L. Salgueiro, J. B. Custodio, C. Cavaleiro, and M. Sousa, "Experimental parasitology anti-giardia activity of *Syzygium aromaticum* essential oil and eugenol: effects on growth, viability, adherence and ultra-structure," *Experimental Parasitology*, vol. 127, no. 4, pp. 732–739, 2011.
- [46] T. F. Wahba, M. I. Mackled, S. Selim, and S. R. El-zemity, "Toxicity and reproduction inhibitory effects of some Monoterpenes against the Cowpea Weevil *Callosobruchus Maculatus* F. (Coleoptera: Chrysomelidae: Bruchinae)," *Middle East Journal of Applied Science*, vol. 8, pp. 1061–1070, 2018.
- [47] A. Allali, S. Rezouki, B. Louasté, and T. Najat, "Screening of vegetable powders used as a bio-insecticide against plant archives," *Plant Arch*, vol. 21, pp. 55–65, 2021.
- [48] M. T. Guèye, D. Seck, J. Wathelet, and G. Lognay, "Lutte contre les ravageurs des stocks de céréales et de légumineuses au Sénégal et en Afrique occidentale," *Synthèse Bibliographique*, vol. 15, no. 1, pp. 183–194, 2011.
- [49] J. A. Wightman and B. J. Southgate, "Egg morphology, host, and probable regions of origin of the bruchids (Coleoptera: bruchidae) that infest stored pulses—an identification aid," *New Zealand Journal of Experimental Agriculture*, vol. 10, 2012.
- [50] P. F. Credland and E. Hill, "The structure of bruchid eggs may explain the ovicidal effect of oils," *Journal of Stored Products Research*, vol. 28, no. 1, pp. 1–9, 1992.
- [51] C. B. Bernard, J. Arnason, B. Philogène, J. Lam, and T. Waddell, "Effect of lignans and other secondary metabolites of the asteraceae on the mono-oxygenase activity of the european corn borer," *Phytochemistry*, vol. 28, no. 5, pp. 1373–1377, 1989.
- [52] K. N. Don-pedro, "Mode of action of fixed oils against eggs of *Callosobruchus Maculatus*," *Pesticide Science*, vol. 26, pp. 107–115, 1986.
- [53] G. K. Ketoh, I. Glitho, and Y. Nuto, "Effets de six huiles essentielles sur les oeufs et les larves de *callosobruchus maculatus* F. (Coleoptera: bruchidae)," *International Journal of Tropical Insect Science*, vol. 25, 2005.
- [54] R. C. French, "The bioregulatory action of flavor compounds on fungal spores and other propagules," *Annual Review of Phytopathology*, vol. 23, no. 1, pp. 173–199, 1985.
- [55] R. Essama, F. Menut, and L. De Phytobiochimie, "Chemical composition of essential oils of *eugenia caryophylla* and *mentha* sp cf *Piperita*," *African Journal of Traditional, Complementary and Alternative Medicines*, vol. 11, pp. 40–46, 2014.
- [56] I. Ben and H. Jilani, "Effects of cold-storage facility characteristics on the virulence and sporulation of *Penicillium expansum* and the efficacy of essential oils against blue moldrot of apples," *Folia Horticulturae*, vol. 31, no. 2, pp. 301–317, 2019.

- [57] P. Sessou, S. Farougou, and D. Sohounhloùé, "Major component and potential applications of plant essential oils as natural food preservatives: a short review research results," *Food Quality Control and Improvement*, vol. 2, no. 8, pp. 45–57, 2012.
- [58] Z. Benmakhlouf, O. Benserradj, and R. Kellab, "Short communication: identification of phytochemical constituents of *Syzygium aromaticum* L. using gas chromatography coupled with mass spectrometry and evaluation of antimicrobial activity," *Biodiversitas Journal of Biological Diversity*, vol. 23, no. 5, pp. 2586–2593, 2022.
- [59] S. N. Andrianjafinandrasana, M. Chillet, and I. R. Ramonta, "Biological activity of *Syzygium Aromaticum* and *Ravensara Aromatica* essential oils from Madagascar and their possible use against Postharvest mango Anthracnose," *American Journal of Plant Science*, vol. 11, pp. 1682–1697, 2020.
- [60] S. T. Alphonse, "In Vitro activity of *Syzygium Aromaticum* against food spoilage fungi and its potential use as an anti-radical agent," *Journal of Microbiology Research*, vol. 6, 2016.
- [61] B. J. C. Maruzzella, L. Liguori, and A. Root, "The in VZtro antifungal activity of essential oils," *Journal of American Pharmaceutical Association*, vol. 47, pp. 250–254, 1957.
- [62] S. Chouhan, K. Sharma, and S. Guleria, "Antimicrobial activity of some essential oils—present status and future perspectives," *Medicines*, vol. 4, pp. 1–21, 2017.
- [63] F. Silva, S. Ferreira, A. Duarte, D. I. Mendonça, and F. C. Domingues, "Antifungal activity of *Coriandrum sativum* essential oil, its mode of action against *Candida* species and potential synergism with amphotericin B," *Phytomedicine*, vol. 19, no. 1, pp. 42–47, 2011.
- [64] Y. Hu, J. Zhang, W. Kong, G. Zhao, and M. Yang, "Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (*Curcuma longa* L.) on *Aspergillus flavus*," *Food Chemistry*, vol. 220, pp. 1–8, 2017.