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Research Article

Preparation and Characterization of a Biopesticide Based on *Artemisia herba-alba* Essential Oil Encapsulated with Succinic Acid-Modified Beta-Cyclodextrin

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Numerous essential oils have been researched as biopesticides because of their effectiveness and environmental safety. Nevertheless, encapsulation is necessary to improve its physical, chemical, and biological properties. Therefore, this paper aims to investigate the physicochemical characteristics and antifungal activity of the *Artemisia herba-alba* essential oil (HAEO) encapsulated in succinic acid-modified β-cyclodextrin (SACD). Hydrodistillation was used to extract a yellowish oil from the plant *A. herba-alba*, and gas chromatography coupled to mass spectrometry was performed to determine the chemical composition. The scanning electron microscope, Fourier transform infrared, thermogravimetric analysis, and docking studies were combined to validate the molecular inclusion of HAEO with SACD. The antifungal activity was examined against *Botrytis cinerea* by direct contact with a potato dextrose agar. The results showed that the main compound in HAEO was α-thujone (65.0%). Furthermore, HAEO has been successfully incorporated into SACD with a binding energy value of $-5.3\ \text{kJ}\cdot\text{mol}^{-1}$, and its thermal stability improved. At a 0.1% concentration, HAEO in encapsulated form showed a higher ability to inhibit *B. cinerea* (38.34%), compared to EO in free form (12.00%). Thus, these findings produced evidence that the strategy of encapsulating EOs by SACD can develop novel *B. cinerea* inhibitors and a promising alternative biopesticide.

1. Introduction

Botrytis cinerea (*B. cinerea*) is known as a phytopathogenic fungus affecting over 200 plant species, with the potential for significant crop losses of $10 billion to $100 billion annually [1, 2]. The postharvest rot has long been controlled with chemical fungicides; however, overuse of traditional chemical fungicides has resulted in a slew of issues, including fungicide residues, contamination of the environment, and increased pathogen resistance to fungicides [3]. Therefore, natural pesticide products, the essential oils extracted from aromatic plants, are promising substitutes for chemical fungicides. Current studies based on plant essential oils were investigated as potential alternatives for *B. cinerea* control [4, 5].

Artemisia herba-alba is known as the wormwood of the desert and its Arabic name is Sheeh [6]. Recently, a large number of studies have been published on *A. herba-alba* [7] because of its economic value, therapeutic uses, and medicinal uses. Several studies were reported that the yield recorded for *Artemisia herba-alba*'s essential oil is 0.5 and 1.23% [8, 9]. This difference observed between EO yields can

be attributed to many factors such as vegetative stage, climatic condition soil, and extraction technique [10]. Additionally, *A. herba-alba* essential oil (HAEO) was distinguished by its abundance of oxygenated monoterpenes, including camphor, α-thujone, β-thujone, eucalyptol, and chrysanthenone [11]. Thus, HAEO has a wide range of biological properties, mainly insecticidal, antioxidant, anti-inflammatory, antibacterial, and antifungal [12]. The impact of *A. herba-alba* essential oil on *B. cinerea* was also examined, and at a concentration of 2000 μL/L, total inhibition of mycelium growth was verified [13]. Indeed, the efficacy of EOs altered under light, air, and temperature. Therefore, an effective stabilization process is required, and the inclusion complex currently appears a suitable approach for encapsulating EOs.

β-cyclodextrin (βCD) is a series of cyclic oligosaccharides, composed of seven glucopyranose units connected by α-(1,4) bonds [14]. It is recognized by a slightly conical hollow cylindrical ring structure, with a hydrophobic cavity and a hydrophilic surface (Figure 1). Among the CDs, βCD is the most common and commercially available, which can form a suitable inclusion complex for mono and sesquiterpenes [15]. In some cases, chemical modification of βCD was performed to raise its low water solubility (1.85 g/100 mL) and boost biological activity by adding hydroxyalkyl [16] and sulfobutylether groups [17]. Thus and more recently, the succinic acid has been attached to hydroxyl groups on βCD molecules to produce succinic acid-modified β-CD (SACD), as shown in Figure 1, which is 50 times more water-soluble than βCD [18] and exhibited antimicrobial properties [19].

In this context, the purpose of this paper was to prepare the inclusion complex of *A. herba-alba* essential oil (HAEO) in SACD by the coprecipitation method. The other aim of this work was to prove the molecular encapsulation of HAEO and study the antifungal effect against *B. cinerea* using the direct contact method before and after the encapsulation procedure.

2. Experimental Section

2.1. Chemicals Used. β-cyclodextrin (βCD, min. 98%) was purchased from APPLICHEM, sodium Hypophosphite (SHP, min. 99%) was supplied by Look Chem, and succinic acid (SA, min. 99%) was provided by Sigma-Aldrich. The remaining chemical reagents were of an analytical grade.

2.2. Plant Material and Essential Oil Isolation. *Artemesia herba-alba* (*A. herba-alba*) was collected at the Moroccan National Institute of Agricultural Research (INRA, Rabat). This plant was identified by Professor Badr Satrani, a botanist at the Forestry Research Center (Rabat, Morocco). The harvested samples were air-dried in the shaded area at room temperature (25–28°C) for 4 days to achieve a constant weight. The *A. herba-alba* essential oil (HAEO) was extracted in triplicate (3×100 g) using the Clevenger-type apparatus for 3 h and dried with anhydrous sodium sulfate to obtain the yellowish essential oil.

2.3. Chemical Analysis of A. herba-alba Essential Oil. The analyses of gas chromatography-mass spectrometry were performed at the Department of Food Technology (INRA). The GC-MS units consisted of a Perkin Elmer, equipped with a capillary column Rxi-5ms (Crossband 5% diphenyl/95% dimethyl polysiloxane, $30\,\text{m} \times 0.25\,\text{mm}$, and film thickness of 0.25 μm). The gas used is helium with a flow rate of 1.00 mL/min. The injection mode was split (1/50), and the volume injected was 0.5 μL by a program of the oven used for the separation of volatile compounds as follows: the initial temperature was 60°C maintained for 3 min, then 1°C/min to 80°C, subsequently at 5°C/min up to 280°C. The ionization was performed with an electron energy of 70 eV, and the source temperature was 230°C with a scan of 50 to 550 Da. The apparatus was controlled by a computer system " Perkin Elmer Version 6.1.0.1963," which manages the operation and follows the evolution of the analyses NIST 2011 mass spectrum library and by comparing their retention indices with those referred to the literature [20].

2.4. Synthesis of SACD. The preparation of succinic acid-modified β-CD (SACD) was detailed by [18]; a mixture of 2.00 g of βCD, 2.00 g of SHP, and 1.48 g of SA was solubilized in 20 mL of deionized water. The solution was transferred onto a plate and dried at 100°C for 5 h. Then, the esterification procedure was performed over 20 min at 140°C. The raw product was dissolved in deionized water and precipitated by absolute ethanol, followed by dehydration under 50°C to afford SACD.

2.5. Encapsulation Process. The inclusion complex (HAEO/SACD) was prepared according to the following procedure: first, the SACD was dissolved in 20 mL of aqueous solution maintained at 40°C for 30 min. Then, 100 μL of HAEO was diluted in ethanol and slowly added over 5 min under continuous agitation for 24 h. In the second step, the obtained suspension solution was refrigerated at 4°C overnight, subsequently, the precipitation of the inclusion complex occurred, and then the IC was washed with ethanol solution (10%) and dried at 50°C. Later, the complex was sealed and stored at room temperature until subsequent experiments.

2.6. Characterization of Encapsulated A. herba-alba Essential Oil. The methods used to characterize the molecular inclusion were the same, as mentioned in our earlier study [21], with a few minor changes. A scanning electron microscope (SEM, JSM-IT500HR) was used to examine the particle morphology of the encapsulated HAEO. After being vacuum coated with a thin layer of gold, the samples (SACD and HAEO/SACD) were evaluated at a 16 kV accelerating voltage. The formation of the HAEO/SACD inclusion complex was also proven by Fourier transform infrared (FTIR, VERTEX 70-BRUKER) spectroscopy with a framework area of 400–4000 cm^{-1}. SACD and HAEO/SACD (solid samples) were ground and combined using the KBr-pellet method, and then the HAEO was made by the KBr window approach [22]. Thermal analysis of HAEO, SACD,

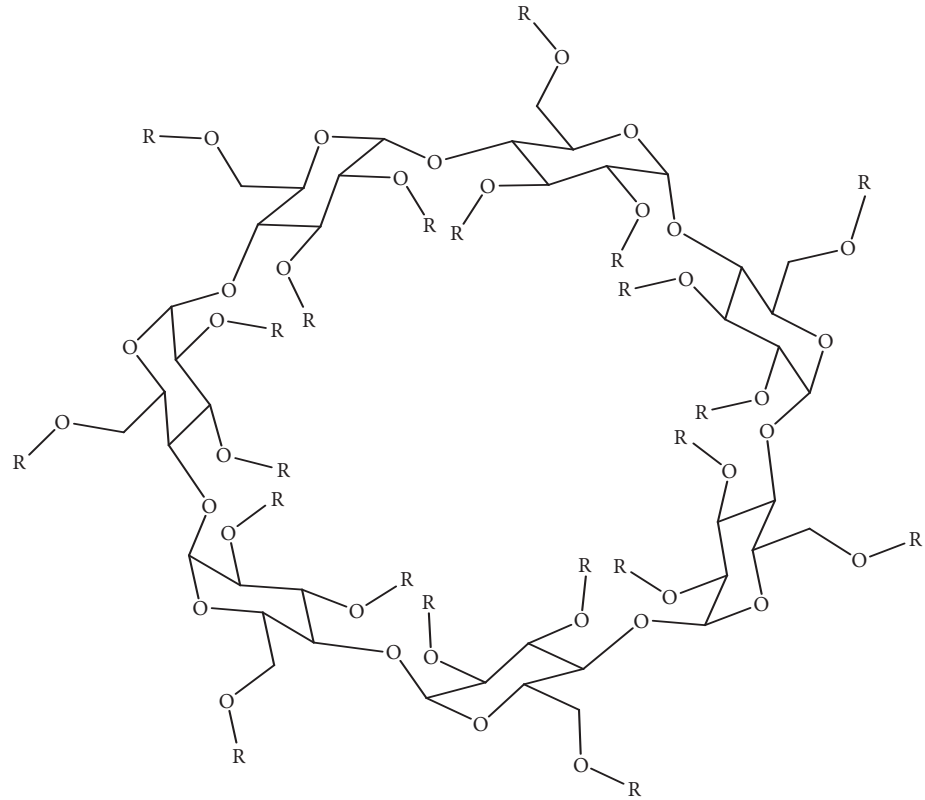


Figure 1: The chemical structure of β-cyclodextrin (R=H) and succinic acid-modified β-CD (R=CO-CH_2-CH_2-COOH).

and encapsulated EO was conducted on LINSEIS STA PT1600 between 28 and 550°C (10°C/min) in an air atmosphere.

2.7. Docking Study. The optimum structure of the inclusion complex (HAEO/SACD) was identified using AutoDock 4.2 software [23]. The major compound of HAEO (α- thujone), as the guest molecule, was obtained from PubChem. The structure of SACD, as the host molecule, was drawn using ChemDraw and then optimized in Gaussian 09W on the 6-311G (d, p) basis [24]. The docking process was carried out by AutoDock Tools 1.5.7 as follows: the guest was allowed to be flexible, the docking grid with dimension of 34 Å × 50 Å × 36 Å, and Lamarckian genetic algorithm (LGA) was applied [25].

2.8. Antifungal Assay against B. cinerea. The antifungal activity was investigated by evaluating the inhibition of *B. cinerea* growth through direct contact (DC) into the culture medium of PDA (potato dextrose agar). The HAEO/SACD and 0.05% (v/v) tween 80 were incorporated into 100 ml of PDA and then poured into a petri dish of 80 mm diameter. *B. cinerea* was placed in the center of a petri dish, from a mycelial disk with a diameter of 4 mm. The plates were incubated at 25 ± 1°C for 7 days in the dark. HAEO (100 μl) and SACD were also tested, in order to investigate the encapsulation process through SACD on the antifungal activity of HAEO. Each treatment was carried out three times, and the antifungal activity (AA) was determined according to the following equation:

$$\text{AA } (\%) = \frac{\text{Dc} - \text{Dt}}{\text{Dc}} \times 100, \quad (1)$$

where Dc and Dt represent the diameters (mm) of the colony zone in the control and the test, respectively. The data from the antifungal activity were examined statistically through analysis of variance (ANOVA) using SPSS software. All the values were presented as average value ± standard deviation (SD), with $p \leq 0.05\%$ considered statistically significant.

3. Results and Discussion

3.1. Chemical Composition of HAEO. In essential oils isolated from *A. herba-alba*, 30 components were identified (Table 1), corresponding to 96.3% of the total oil. α-Thujone (65.0%) was the most abundant compound, followed by three other main constituents: β-thujone (14.4%), camphor (6.0%), and α- phellandrene (2.3%).

Regarding previous investigations on the chemical composition of Moroccan *A. herba-alba* essential oils, Boudalia et al. [26] obtained 1, 8-cineole, the major component of *A. herba-alba* (Boulemane, west of Morocco), whereas Paolini et al. [27] found three main groups: camphor, chrysanthenone, and (α or β) thujone from *A. herba-alba* essential oils harvested in eight locations (East Morocco). Other studies have shown that thujone and chrysanthenone were the main compounds in *A. herba-alba* essential oil [28].

Table 1: Chemical composition of *A. herba-alba* essential oil.

Compound	Retention index	Retention time (min)	Area (%)
Monoterpene hydrocarbons			4.4
α- pinene	939	7.63	0.1
Camphene	953	8.30	0.7
β- myrcene	991	10.58	0.1
α- phellandrene	1005	9.59	2.3
(+)-4-carene	1018	12.13	0.2
p-cymene	1026	12.66	0.5
γ- terpinene	1062	15.23	0.5
Oxygenated monoterpenes			3.4
Eucalyptol	1033	13.08	0.6
Cis-verbenol	1142	24.03	0.4
Terpinen-4-ol	1177	26.36	0.9
Trans-carveol	1217	30.84	0.1
Cis-carveol	1229	31.10	0.6
Bornyl acetate	1285	33.64	0.1
Thymol	1290	34.75	0.1
Myrtenyl acetate	1235	41.19	0.1
Lilac alcohol A	1240	43.42	0.4
Xanthoxylin	1625	45.47	0.1
Ketones			87.4
α- thujone	1001	20.02	65.0
β- thujone	1112	20.74	14.4
Chrysanthenone	1123	20.80	0.4
Camphor	1140	22.83	6.0
Pinocarvone	1162	24.51	0.3
Carvone	1242	31.39	0.1
Carvenone	1252	31.93	0.3
2-undecanone	1293	34.14	0.9
Aldehydes			0.5
α- thujanol	1182	27.01	0.2
Myrtenal	1193	27.97	0.3
Sesquiterpene hydrocarbons			0.4
β- copaene	1432	37.23	0.2
β- ylangene	1438	39.78	0.2
Oxygenated sesquiterpenes			0.2
Spathulenol	1640	43.51	0.2
Total			96.3

In Morocco, *A. herba-alba* essential oil (HAEO) is generally characterized by substantial levels of ketones such as thujones and camphor [10]. Thujones and thujone-containing essential oil have been demonstrated to possess significant pharmacological and agroalimentary activities [29].

3.2. Encapsulation Process

3.2.1. SEM Analysis. The topography of SACD and HAEO/SACD are shown in Figure 2, which displays the SEM photographs of SACD in 2000 and 4000 magnifications, respectively, in Figures 2(a) and 2(b). The morphology of SACD showed various asymmetric shapes, and the large particles had smooth surfaces. Conversely, as shown in Figures 2(c) and 2(d), HAEO/SACD showed small particles of irregular size that were agglomerated; similar findings were obtained previously [21]. These differences in the morphology of SACD before and after the encapsulation process may serve as evidence that the inclusion complex has indeed formed.

3.2.2. FTIR Analysis. HAEO mainly contained ketones with higher percentages (87.4%), which *α*-thujone significatively presented (65.0%). Therefore, the FTIR spectrum of HAEO (Figure 3) consisted of the sharp deep absorption bands of carbonyl C=O (1741 cm^{-1}), also exhibiting characteristic bands at 2857, 2931 and 2956 cm^{-1}, which can be assigned to the stretching of C-H. The SACD spectrum showed a broad band at 3300 cm^{-1} corresponding to hydroxyl bonds (O-H) and consisted of prominent ester bands at 1722 cm^{-1}. In contrast, it was evident to observe a decrease in the intensity of the C=O ester bands as well as the disappearance of the characterized HAEO peaks in the IC (HAEO/SACD) spectra as a result of the incorporation of HAEO into the SACD. Similar observations were indicated previously [30], elucidating the disappearance of characteristic peaks of the guest due to its incorporation into the host molecule.

3.2.3. TGA Analysis. The thermogravimetric analysis (TGA) is an effective analytical tool for examining changes in physicochemical properties [31] and also providing additional information about the thermal behavior of EO during the inclusion process. As shown in Figure 4, the HAEO has already begun to evaporate from room temperature up to 179°C, demonstrating the unstable nature of HAEO in free form. Moreover, two weight losses were noticed in the case of SACD; the first one below 115°C accounted for the dehydration process, and the rest decreased above 230°C attributed to the decomposition process. The thermogram of HAEO/SACD revealed significant differences from the TGA obtained for SACD. Along with the processes of dehydration and decomposition, TGA of the inclusion complex revealed an additional weight loss between 210°C and 270°, explained by the essential oil's volatilization. These findings did not provide more proof for the formation of the inclusion complex (HAEO/SACD), but they also suggest that the EO in its encapsulated form is stable from a thermal viewpoint.

3.2.4. Molecular Docking Analysis. The optimal binding mode of the inclusion complex between *α*-thujone with SACD is shown in Figure 5(a). In the docking pose, the function C=O (red color) of *α*-thujone was fully incorporated into the inner cavity of SACD, in contrast to the carbon backbone (green color), which was slightly sequestered. The binding energy value was −5.3 kJ mol^{-1}, illustrating that the inclusion complex formation was stable and the formation of hydrogen bonds (Figure 5(b)) may account for this stability. Nevertheless, the outcomes of molecular modeling reflected the results of our earlier experiments.

3.3. Antifungal Activity.

After the characterization of the inclusion complex, we performed a comparison of the antifungal activity of essential oil before and after the inclusion complex.

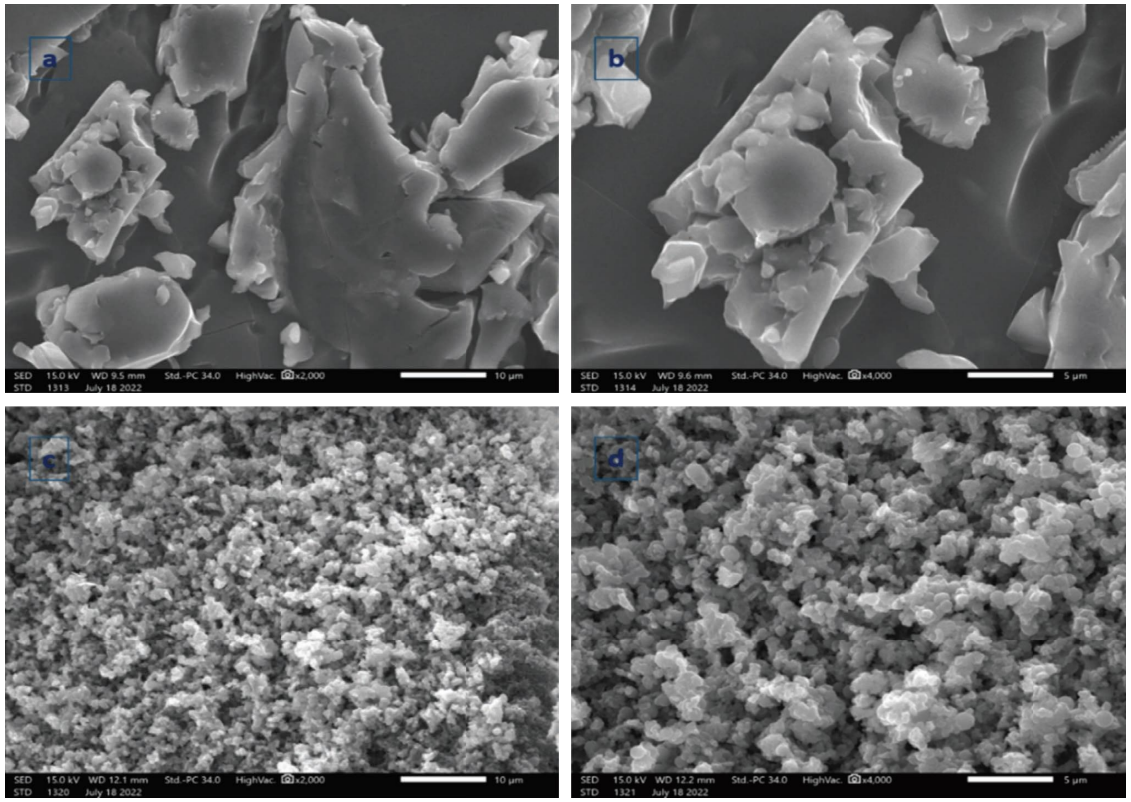


Figure 2: The SEM microscopes of SACD (a, b) and HAEO/SACD (c, d) at 2,000 and 4,000 magnifications, respectively.

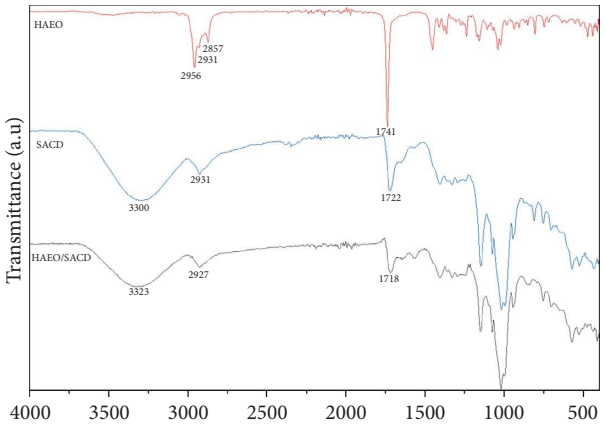


Figure 3: The FTIR spectra of HAEO, SACD, and inclusion complex.

Figure 6 illustrates the antifungal activity of HAEO, SACD, and HAEO/SACD complexes against *B. cinerea* after incubation for 7 days. Concerning free essential oil, HAEO demonstrated a remarkable inhibiting property with 12.00 ± 0.75% (Table 2), which was supported by the previous studies [13, 32]. While SACD exhibited a certain relative activity (8.16 ± 0.09%), this inhibited behavior can be explained by the presence of carboxylic and hydroxyl groups [19, 33]. Additionally, the hydrophobic cavity of cyclodextrin may affect the growth of *B. cinerea*.

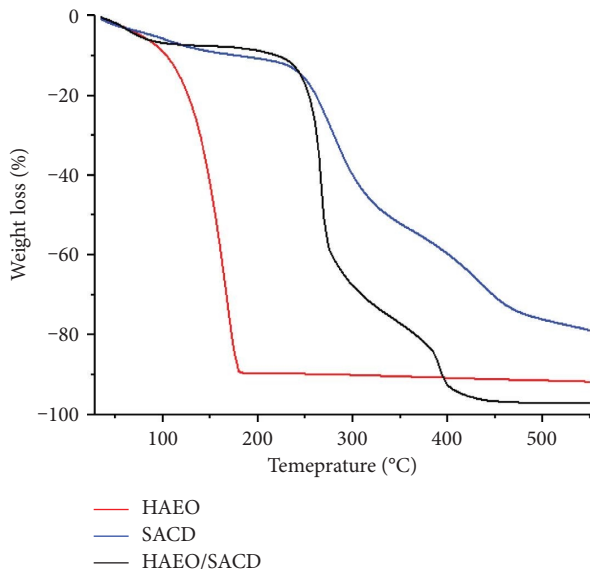
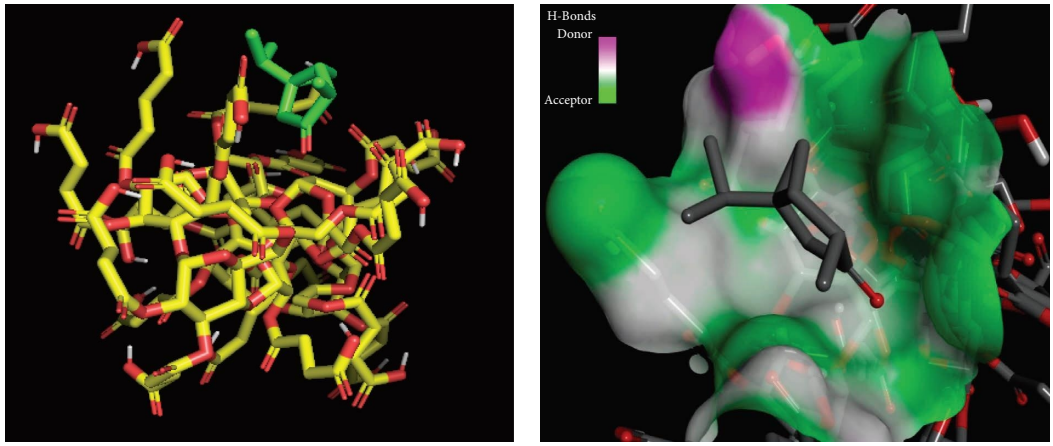


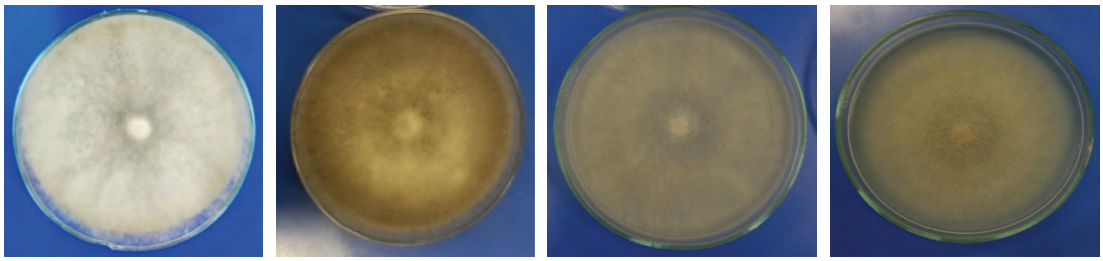
Figure 4: The TGA thermogram of HAEO, SACD, and inclusion complex.

After the encapsulation process, the essential oil improved its inhibited characteristic against *B. cinerea* to 38.34 ± 0.58%. According to our findings, the encapsulation of essential oil using SACD promotes antifungal activity, while the host and guest act in synergy to limit the growth of *B. cinerea*. Furthermore, this inclusion complex becomes



(a) (b)

Figure 5: The optimal structure for α-thujone inside the cavity of SACD (a) and the hydrogen bonds (b).



Control (PDA) HAEO SACD HAEO/SACD

Figure 6: Growth of *B. cinerea* on PDA medium containing HAEO, SACD, and HAEO/SACD.

Table 2: The inhibition zone of HAEO, SACD, and HAEO/SACD.

Samples	The inhibition zone (%)
HAEO	12.00 ± 0.75
SACD	8.16 ± 0.09
HAEO/SACD	38.34 ± 0.58

a promising alternative biopesticide and can be useful for the development of new options for limiting the growth of *B. cinerea* because of its following characteristics: nontoxic, biodegradable, and economically feasible.

4. Conclusions

In this present work, the inclusion complex of *A. herba-alba* essential oil using the SACD increases its thermal stability and antifungal activity. The SEM, FTIR, and TGA were adopted to understand the changes in particle morphology, molecular structure, and thermal behavior during the encapsulation process. As well, the docking study allows us to visualize the incorporation of the major component of HAEO (α-thujone) into the SACD. Therefore, the encapsulation of EOs in SACD is a potential novel formulation for inhibiting the growth of *B. cinerea* due to its features: an environmentally safe alternative to chemical fungicides and higher stability, solubility, and antifungal activity than free EO. Indeed, in order to be exploited for industrial purposes and to further understand its mechanism of action against phytopathogens, additional in vivo investigations and cytotoxicity assessments were recommended.

Data Availability

The data used to support the findings of this study have not been made available because of legal and ethical concerns.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Ez-zoubi Amine wrote the original draft, investigated the study, conceptualized the study, and proposed the methodology; Yassine Ez zoubi visualized the study, validated the study, and provided the software; Fatiha Bentata supervised and visualized the study; Ayoub El-Mrabet provided the software; Chaymae Ben Tahir validated the study; Mustapha Labhilili performed data curation; and Abdellah Farah reviewed and edited the manuscript, was responsible for resources, and conceptualized the study.

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