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Antifungal activity of clove (*Syzygium aromaticum*) essential oil

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Abstract

Phytopathogenic fungi are responsible for approximately 70% of cultivated plant diseases. These fungi, which are capable of infecting any tissue of the plant and at any stage of its growth, can cause both qualitative and quantitative damage. Currently, the main means of combating plant diseases are: Cultural struggle, genetic struggle, biological struggle, Physical struggle and Chemical struggle which are generally very specific to each group. These solutions are not effective in the long term and can lead to the appearance of new pathogenic races through mutations or random and spontaneous parasexualities in addition to environmental pollution as well as residues may be present in the water, the ground and also in the finished product. Hence the need to find alternative solutions to continue to fight against diseases by minimizing the different methods of control mentioned above and by reducing the use of chemicals. In addition, the innovation of biofungicides has become a necessity for the benefit of humans and the environment. Essential oils have a very broad spectrum of activity, mainly due to their nature. The aim of this work is to study the antifungal activity of the essential oil of cloves on three phytopathogenic fungi: *Fusarium oxysporum*, *Rhizoctonia solani*, *Alternaria alternata*. Clove essential oil showed strong antifungal activity against the fungi tested.

Keywords: Mushrooms, phytopathogenic, essential oil, clove, yield, bio fungicide, spectrum of activity

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1. Introduction

The clove tree is a tree 10 to 12m high. it refers to the leaves assembled in pairs where its Greek nomenclature Syzygium comes from. It is a tree native to the small volcanic islands of the Moluccas archipelago in Indonesia [1-2]. The clove tree grows in rich, fresh, acidic or neutral and well-drained soil, its flowering is from April to October depending on the variety, it is found in nurseries from the month of March and it is sown in the same month. It does not tolerate cold well, the temperature of which should not drop by 15-18°C during the cold season. Under a clove tree nothing grows because it heats the soil too much. It prefers a sunny or semi-shaded exposure. Cloves are harvested and dried buds from the clove tree, they are the oldest spices and drugs known to history. Clove is known in China before the Christian era, it is used to freshen the breath “hi-sho-hiang” before speaking to the emperor [3], it was reported in Europe in the 4th century when Emperor Constantine offered it as a luxury product to Pope Sylvester [4]. It was the Portuguese who discovered its country of origin: The Moluccas Islands. In the 12th century, cloves arrived in Rome and France, but always as a luxury product [5]. Its consumption as a spice became widespread in the 16th century because of its high price. Indonesia remains the world's leading producer of cloves chaired by Zanzibar which is one of the largest producers of cloves followed by Madagascar which comes third. [6]. Clove is a very expensive spice, it is useful in many oriental and western cuisines, in addition to its antiseptic and anesthetic properties, it is known in dentistry, cosmetics and perfumery [7]. Few people know that clove warms, stimulates, is recommended against nausea, protects against intestinal parasites, strengthens the kidneys and a few drops of clove essential oil soothe toothache. Most of the world's production is intended for the manufacture of kreteks, which are traditional Indonesian cigarettes made from tobacco and cloves [8]-[9]. In the kitchen, clove flavors meat, fish and carp broths. In industry, cloves flavor canned vegetables, applesauce and other fruits. It is introduced in the manufacture of vermouth for the beverage industry and also its powder is used in curry with a harmonious taste. The yield of clove essential oil is the highest of plants. It can reach 19%. [10]. Essential oils are viscous liquids extracted from plant matter, generally of quite complex composition [11]. They

are products containing volatile principles contained in plants and which can be more or less modified depending on the harvest season, drying and the extraction procedure. The French Standardization Association (AFNOR) defines essential oils as products extracted from natural raw materials by distillation with water or steam or from citrus fruits by mechanical processes. These products are separated from the aqueous phase by physical processes. Essential oils can be found in any part of the plant such as fruits (citrus), flowers (rose, jasmine), seeds (nutmeg), leaves (eucalyptus), bark (blackcurrant), wood (cedar) or roots (iris) [12] [13]. Essential oils are germination inhibitors in the field of plant interactions and protect plants against predators, insects and fungi, in addition they are attractors of pollinators in the field of plant-animal interaction.

2. Materials and Methods

This experimental work was divided into three parts:

- Extract clove essential oil using 2 types of assembly
- Calculation of the yield of clove essential oil
- Evaluation of the antifungal activity of clove essential oil against the fungi tested.

2.1. Raw material

Clove (Syzygium aromaticum) are dried flower buds of an aromatic tree (Singh et al,2015), it belongs to the genus Eugenia, one of the 75 genera, family Myrtaceae [14]. Originally from the Moluccas island "Indonesia" and brought at the beginning of the 18th century to different parts of the world: Zanzibar, India, Madagascar, etc. It is known in food preparation thanks to its benefits as an anti-cancer agent and as a traditional remedy against asthma, digestive system disorders, respiratory and dental disorders, head and throat aches. In addition to its antimicrobial, antifungal and antiviral properties, clove essential oil has anti-inflammatory, cytotoxic and anesthetic activities.

2.2. Fungal strains

The three fungi used in this work are virulent fungal species, they are chosen for the significant damage they cause to crops. They are regularly maintained by transplanting onto PDA (Poteto Dextrose Agar) medium.

2.2.1. Fusarium oxysporum

The genus Fusarium was discovered by Link in 1809. This genus brings together a large number of very diverse species from a morphological point of view. Each of its species is present in nature by a majority of saprophytic strains or parasite forms of more or less specialized and endowed with real virulence [15]. *Fusarium oxysporum* is the most widespread species; it contains the most frequent and most important phytopathogenic forms of the fungal microflora of cultivated soils.

2.2.2. Rhizoctonia solani

Rhizoctonia is a species of the genus Rhizoctonia [16], a soil pathogen that attacks many crops around the world, it is known for its significant diversity in terms of cultural morphology, host range and aggressiveness.

2.2.3. Alternaria alternata

Alternaria alternata is a very common and virulent species of fungus [17], it lives in symbiosis as an endophyte of several plants [18]. Currently its definition is based on morphological, genetic and geometric analyses.

2.3. Culture medium used

During our experiment, we used PDA (Poteto Dextrose Agar). The choice of culture medium is linked to its suitability for good pathogen development. The composition of the PDA medium is as follows:

- 200g potato
- 20g of glucose
- 20g of agar agar
- 100ml of distilled water

2.4. Methods

The extraction of the essential oil was carried out by hydro distillation. In the present work the assembly was done by 2 methods.

2.4.1. Extraction by hydro distillation: simple assembly

The extraction of the essential oil was carried out by hydro distillation with a simple setup (figure 1). We introduced 40 g of cloves into a 2-liter flask impregnated with 1 liter of distilled water. Everything is then brought to the boil at atmospheric pressure for 2 and a half hours. The heat allows the bursting and release of the odorous molecules contained in the plant cells. These molecules form an azeotropic mixture with water vapor. The vapors loaded with essential oil, passing through a refrigerant, condense and chat in a beaker. Then the essential oil separates from the water by difference in density.

2.4.2. Extraction by hydro distillation: Clevenger

The extraction of the essential oil was also carried out by hydro distillation in a Clevenger apparatus (figure 2). Three distillations were carried out by boiling for 2 and a half hours 40 g of cloves with 1 liter of water in a 2-liter flask topped with a 60 cm long column connected to a condenser [19].

2.4.3. Calculation of extraction yield

The essential oil yield is defined as the ratio between the mass of EO obtained and the mass of plant material to be treated. The extraction yield is expressed as a percentage, it is calculated by the following formula

$$Y\ eo\ (\%) = (M\ eo/Md) * 100$$

Or
Y eo: EO yield expressed as a percentage

M eo: Mass of the EO in grams
Md: Mass of dried plant material in grams

2.4.4. Antifungal test methodology

The microbiological part was evaluated by two following methods: The disc method, which targets the sensitivity of fungal strains, referenced via an essential oil and which determines the inhibition diameter. The minimum inhibitory concentration (MIC) which determines the minimum concentration of the essential oil that inhibits the growth or kills fungal strains. The disk method is a method of diffusing the products to be tested from a paper disk, which makes it possible to qualitatively measure the sensitivity of strains to the antimicrobial effects of essential oils [20]. Based on the diameter of the inhibition zone, we can classify the strains tested according to Belaich et al (1979) [22] as follows:

- Diameter = 0 mm: resistant strain
- Diameter = 5mm: not very sensitive strain
- Diameter = 10mm: sensitive strain
- Diameter=20-30mm: fairly sensitive strain
- Diameter >30mm: very sensitive strain

The minimum inhibitory concentration (MIC) of essential oils was determined according to the method of Remmal et al; (1993) and Satrani et al; (2001) [19] [22]. Due to the non-miscibility of essential oils with water and therefore with the culture medium, an emulsion was carried out using a 0.2% agar solution. It makes it possible to obtain, in the medium, a homogeneous distribution of essential oils and to maximize germ/compound contact. Dilutions are prepared at 1/10, 1/25, 1/50, 1/100, 1/200, 1/300 and 1/500 in this agar solution. In test tubes each containing 13.5 ml of solid medium PDA (Poteto Dextrose Agar), sterilized in an autoclave for 20 min at 121°C and cooled to 45°C, 1.5 ml of each of dilutions to obtain final concentrations of 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000 and 1/5000 (v/v). The tubes are shaken to properly disperse the essential oil in the culture medium before pouring them into the Petri dishes. Controls, containing the culture medium and the 0.2% agar solution alone, are also prepared. A mycelial disk 4 mm in diameter, taken from the active growth zone of a one-week culture, is placed in the center of a Petri dish, incubation is done in the dark for 7 days at 25°C. Each test is repeated three times.

3. Results and discussion

3.1. Yield

The essential oils were extracted from cloves marketed widely in Morocco. The essential oil yield is expressed as a percentage % (W/W). The average yield of clove essential oil by hydro distillation with a simple setup provided a rate of 11.36% compared to 13.004% for hydro distillation with a Clevenger apparatus. Guan et al, (2007), obtained similar rates [23] who reported rates of 11.5%, 19.6% which are the highest rates. However, the lowest rates were noted by Alitonou et al, (2012) [24], Rodriguez et al (2014) [25] and Saeed and Shahwar (2015) [26] with percentages of 0.18%, 2.2% and 2.1% respectively. The difference in yield can be attributed to the harvest season and geographic origin [27], drying conditions [28] and extraction technique [22].

3.2. Antifungal activity

3.2.1. Determination of sensitivity

The sensitivity test results of the fungal strains tested towards clove essential oils extracted by hydro distillation by simple assembly and by the Clevenger apparatus are represented in Table 1. The two essential oils tested showed their effectiveness against the reference fungal strains of Fusarium oxzsporum, *Rhizoctonia solani* and *Alternaria alternata*. These strains are very sensitive to essential oils of clove (EO simple assembly and EO Clevenger apparatus) according to the interpretation of Belaich et al, (1979) [22]. Clove essential oil from simple assembly has the highest antifungal activity for all strains with an inhibition diameter ranging from 55mm to 62mm. While the essential oil of clove from Clevenger assembly presents less fungal activity compared to that of the simple assembly EO with diameters of the inhibition zone ranging from 35mm to 45mm.

3.2.2. Minimum inhibitory concentration

In order to better evaluate the antifungal power of clove essential oils, a study is carried out by determining the minimum inhibitory concentration. The results of this study are summarized in Table 2 and 3. The results indicated in Table 2 show a very strong antifungal power of clove EO against all the strains tested. Clove essential oil extracted by simple assembly displays significantly lower MICs of 1/500 against *Alternaria alternata* and *Rhizoctonia solani*. According to the results it seems that *Fusarium oxysporum* is less sensitive with an MIC = 1/300. According to the results illustrated in Table 3, the essential oil of cloves extracted by the Clevenger apparatus exerts a significant inhibitory action with an MIC=1/200 against *Fusarium oxysporum*, while *Rhizoctonia solani* and *Alternaria alternata* have a MIC =1/300. The analysis of the results obtained for the essential oils of cloves from simple assembly or from the Clevenger device allows us to note that the two oils have a significant antifungal activity on the fungal strains suckled (*Fusarium oxysporum*, *Rhizoctonia solani* and *Alternaria alternata*), despite the remarkable difference in the inhibition diameter for the 3 fungal strains (for example 55mm versus 35mm for *Fusarium oxysporum*, 56mm versus 43mm for *Rhizoctonia solani* and 62mm versus 45mm for *Alternaria alternata* [table 1]) the MIC recorded for the two oils remain nearby. The high antifungal activity of clove essential oil is linked to the presence of certain chemical functions; they conclude that phenols (eugenol) are antifungal knowing that eugenol presents 92.94% of the constituents of clove essential oil clove. The antifungal action decreases depending on the type of chemical function as follows:

Phenols>alcohols>aldehydes>ketones>ethers>hydrocarbons

Figure 1. Hydro distillation by simple assembly

Figure 2. Hydro distillation by the Clevenger apparatus

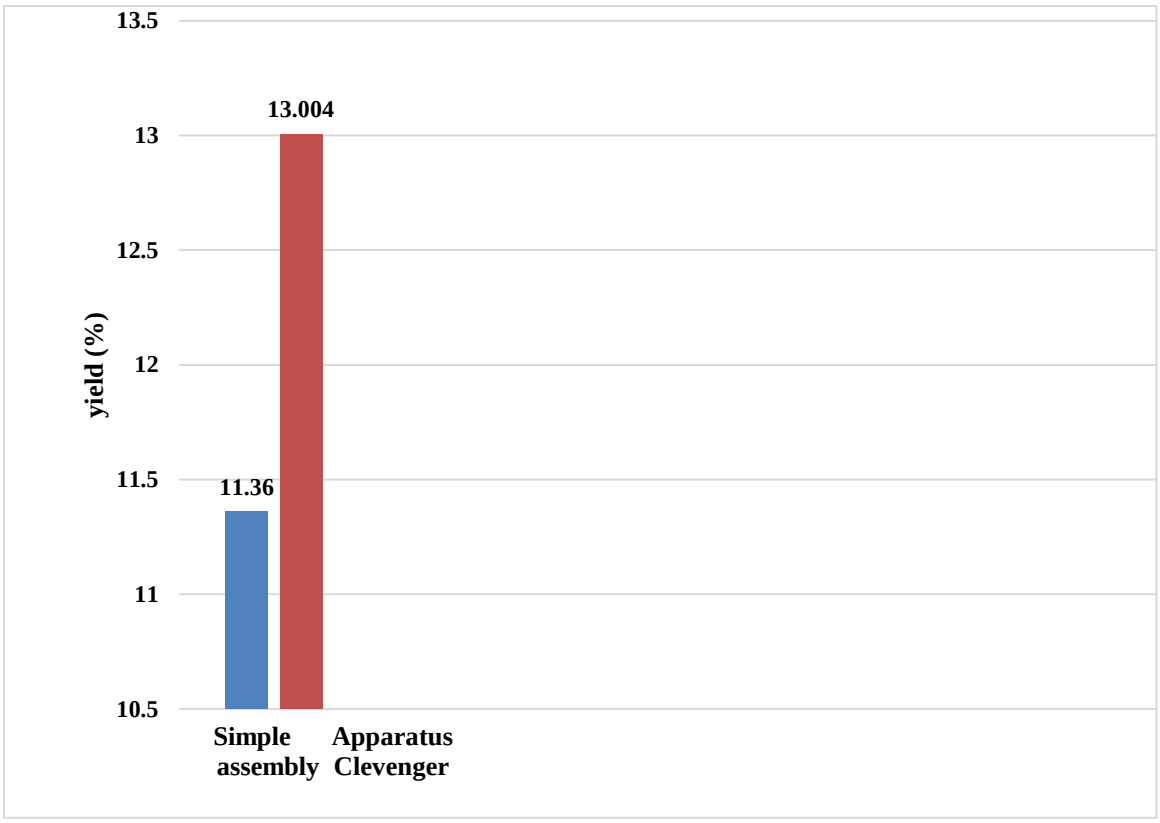
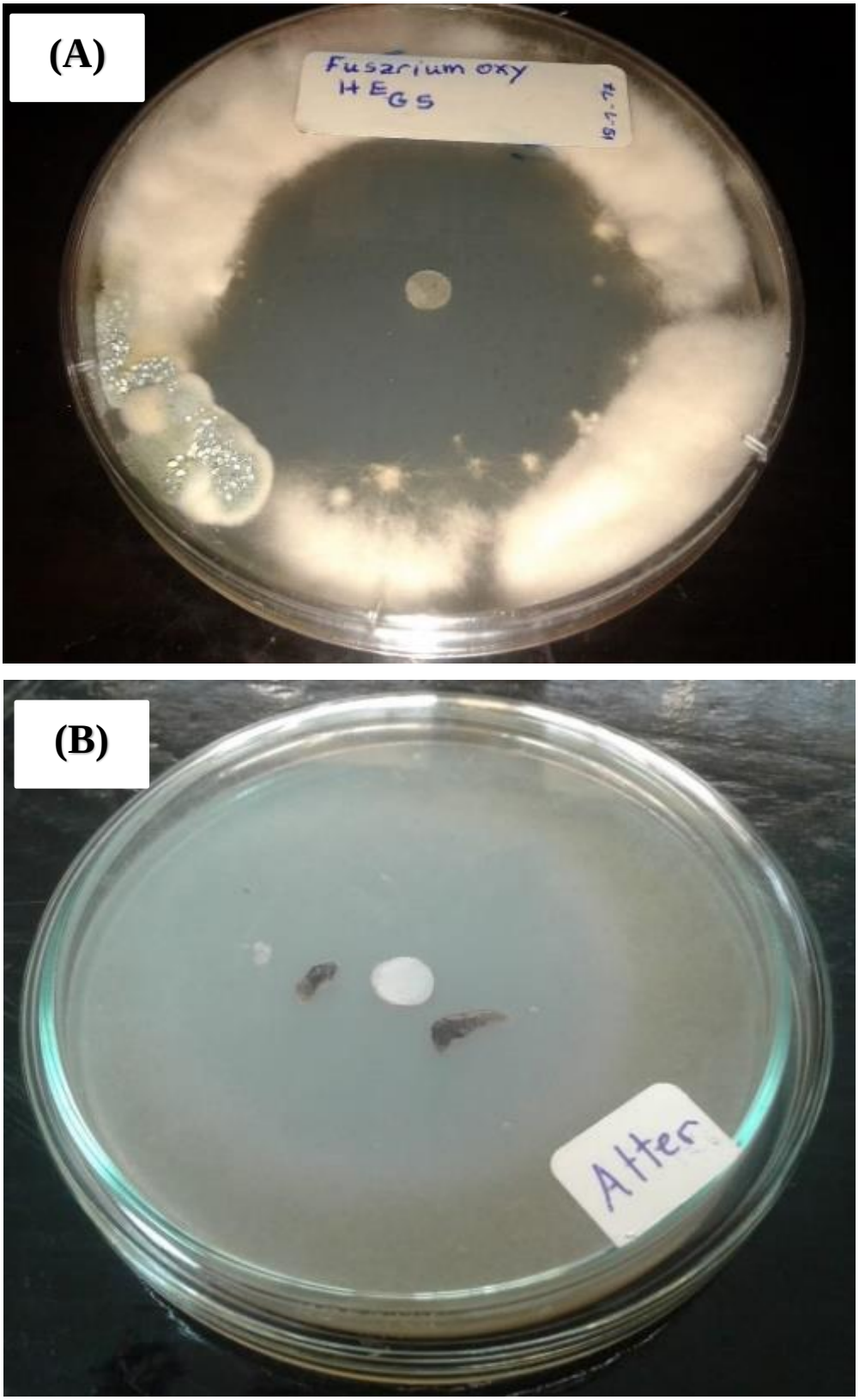


Figure 3. Average yield of clove essential oil depending on type of assembly

Table 1. The diameter of inhibition caused by clove EO

Strains	EO simple assembly	EO Clevenger apparatus
Fuzarium oxysporum	55 mm	35 mm
Rhizoctonia solani	56 mm	43 mm
Alternaria alternata	62 mm	45 mm


(A)
Fusarium oxy
HEGS
(B)
After

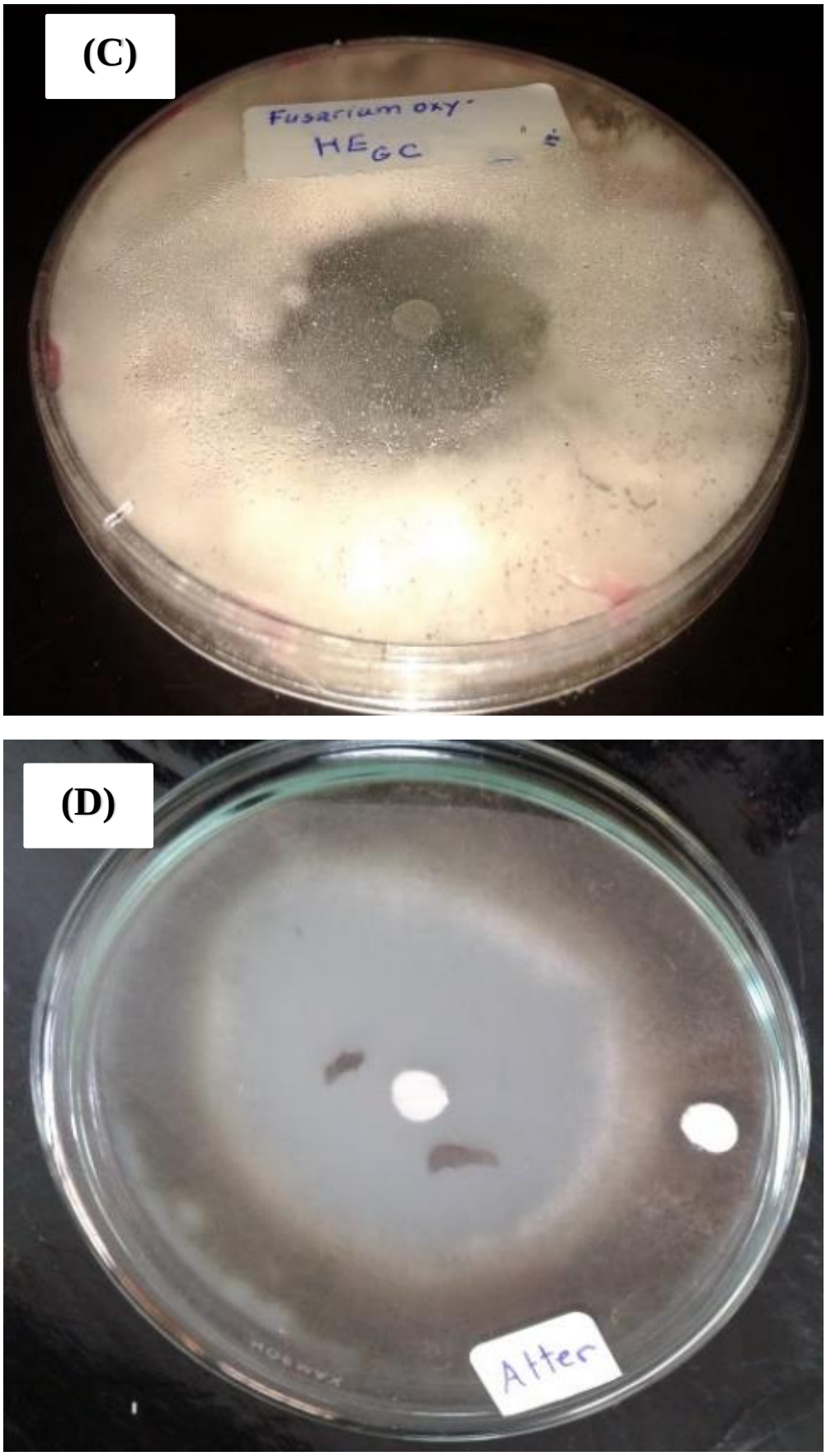


Figure 4. Growth inhibition effect against fungal to clove EO by simple assembly (A, C) and by Clevenger (B, D). A, B: *Fusarium oxysporum* and C, D: *Alternaria alternata* showed the very sensitive effecte of clove EO

Table 2. MIC of clove essential oil extracted by simple assembly on fungal strains tested

Petri dish	T	1	2	3	4	5	6	7
EO concentration	+	**1/500**	**1/300**	**1/200**	**1/100**	**1/50**	**1/25**	**1/10**
Fusarium oxysporum	+	+	+	-	-	-	-	-
Rhizoctonia solani	+	+	-	-	-	-	-	-
Alternaria alternata	+	+	-	-	-	-	-	--

Table 3. MIC of clove essential oil extracted by Clevenger apparatus on the fungal strains tested

Petri dish	T	1	2	3	4	5	6	7
EO Concentration	+	**1/500**	**1/300**	**1/200**	**1/100**	**1/50**	**1/25**	**1/10**
Fusarium oxysporum	+	+	+	+	-	-	-	-
Rhizoctonia solani	+	+	+	-	-	-	-	-
Alternaria alternata	+	+	+	-	-	-	-	--

4. Conclusions

Cloves are harvested and dried buds from the clove tree. Its essential oil yield is very high, in the present work; it is 11.36% for hydro distillation with a simple setup and 13.004% for hydro distillation with a Clevenger apparatus. The antifungal activity of clove essential oil is very strong against the fungal strains tested: *Fusarium oxysporum*, *Rhizoctonia solani*, *Alternaria alternata*. These strains are very sensitive to clove essential oil even with minimum concentrations of 1/300 for Fusarium oxzsporum and 1/500 for *Rhizoctonia solani* and *Alternaria alternata*.

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