Original paper

Antifungal Activity of essential oil from *Cinnamomum zeylanicum* against onions Spoilage Fungi

Abstract:

This work aims to evaluation of the antifungal activity of dichloromethane essential oil of barks of *Cinnamomum zeylanicum* against black rot onions pathogenic fungi such as *Penicillium* sp and *Aspergillus niger*. The antifungal activity of essential oils of barks of *Cinnamomum zeylanicum* was tested *in vitro* by agar well diffusion method against plant pathogenic fungi strains vis *Penicillium sp*.and *Aspergillus niger*. The results showed that the essential oils of *Cinnamomum zeylanicum* exhibited the highest percentage of growth inhibition (100%) against *Aspergillus niger* and *Penicillium sp* at (1% v/v) and (2.5% v/v) minimum inhibitory concentration respectively. The ability of the extracts to inhibit the growth of the fungi is an indication of the antifungal potential of cinnamon, which make the candidate for production of antifungal agents. It can be applied in agricultural product for development of transgenic resistant to plant diseases.

Keywords: antifungal agents, fungi, *Cinnamomum zeylanicum, Aspergillus niger, Penicillium sp.*, microbial sensitivity.

Introduction

Cinnamomum is an important large genus of evergreen aromatic trees and shrubs belonging to the family *Lauraceae* (Sudmoon et. al. 2014), many of whose members are used as spices (Kumar et. al. 2019). Cinnamon is a common spice used by different cultures around the world for several centuries (Ranasinghe et. al. 2013). It has two main types, Ceylon cinnamon (*Cinnamomum zeylanicum* Blume) and Chinese Cassia (*Cinnamomum aromaticum* Ness) and which when dried, rolls into a tubular form known as a quill. Cinnamon has been used in food preparations and in traditional medicine by the Egyptians and the Chinese since ancient times to treat or relieve symptoms of dyspepsia, gastritis, diabetes, poor blood circulation, and

inflammatory diseases (Elshafie et. al. 2012). Some studies showed that extracts and its constituents from cinnamon possess antimicrobial (Zouheyr et. al. 2014), insecticidal (Fouad, 2013), anti-tyrosinase (Marongiu et al. 2007), antioxidant (Şimşek et. al. 2013), anti-diabetic (Sahib 2016), anticancer (Larasati, 2018), anti-inflammatory (Gunawardena et. al. 2015), hypotensive activities (Mahmoodnia et. al. 2017), and cholesterol-lowering effects (Alsoodeeri et. al. 2020). It contains several bioactive compounds that can be used against a wide range of microorganisms. Cinnamon bark crude extract has constantly been reported to have antifungal activity. This activity was attributed mainly to the presence of cinnamaldehyde and eugenol compounds (He et. al. 2005). Both cinnamon barks represent important source of compounds like flavonoids, tannins, glycosides, saponins, alkaloids and essential oils with biological activities such as bacteriostatic, fungistatic and anti-inflammatory. Many fungal species produce mycotoxins contaminating various foods and feed (Naeini et. al. 2010). *Aspergillus niger* can contaminate agricultural products at different stages including pre-harvest, harvest, processing and handling, thus causing considerable economic losses. For example, black rot of onions associated with *A. niger* is responsible for serious losses of onion bulbs in the field and in storage.

Onion is important vegetable crop grown in Algeria and can be used as mature and immature stages. Such an important vegetable crop is prone to many post harvest losses caused by bacterial and fungal pathogens, which in turn reduce the shelf life of onions. Onion suffers from many post harvest diseases like black mold, blue mold, brown rot and smudge among which, black mold, rots are the predominant diseases. *Aspergillus niger* is usually found in common mesophilic environments such as soil, plants, and enclosed air environments. However, natural products could potentially serve as effective alternatives of synthetic chemicals for the control of food

contamination by *Aspergillus* spp (Hyldgaard et. al. 2012). Among natural products, essential oils of aromatic plant are gaining interest as food additives and widely accepted by consumers because of their relatively low toxicity, high volatility, transient nature and biodegradability (Alizadeh et. al. 2010; Gupta et. al. 2011). Therefore, the food industry is now focused on finding solutions that fully satisfy the criteria of consumers while retaining food safety. For these reasons, there are currentstudies on the application of essential oils, extracts, oleoresins and their components extracted from spices and other aromatic plants, as alternative bio-preservatives (Kocić-Tanackov et. al. 2012).

This research was aimed at evaluating the antifungal activity of dichloromethane essential oil of barks of *Cinnamomum zeylanicum* against black rot onions pathogenic fungi such as *Penicillium sp* and *Aspergillus niger in vitro* in order to determine their antifungal inhibition activity.

Materials and Methods

The experimental was a completely randomized design with two factors essential oil dose with five levels (0.25, 0.5, 1, 2.5 and 5% v/v) and three volumes of red onions (small, medium and big) with three replicates

Plant material

The spice cinnamon bark (originating from Indonesia) was purchased from local market in Mascara city (Algeria). The spice was authenticated by Dr Zehafi. Bachir at the department of biology- university of Mustapha Stambouli, Mascara, Algeria. Cinnamon was identified as *Cinnamomum zeylanicum.* Three volumes of red onions (small, medium and big), were selected for this investigation. Onions were cultivated in Mascara region. The spoiled samples of red onions were collected from local

markets in plastic bags. They were subjected to microbiological analysis to isolate spoilage organisms by standard dilution plate.

Fungi material

The strains of *Penicillium sp* and *Aspergillus niger* used in this study were isolated from spoiled onions. The samples of onions with signs spoilage were bought at vegetable market in Mascara city Algeria. The samples were transported to the microbiology laboratory for fungal identification.

Extraction method

The *C. zeylanicum* essential oil was extracted from the bark rolls. The extraction of essential oil (EO) of *C. zeylanicum* was performed by steam distillation of Clevenger-type. A mass of 60 g *C. zeylanicum* was mixed with 200 ml of distilled water (20) and the distillation time was 5 hours. The aqueous essential oil distillate was transferred into 125ml separating funnel. Dichloromethane (10 ml) was added to the separating funnel, which was then capped tightly and shaken vigorously with occasional venting. Layers were separated, and the lower CH_2Cl_2 layer was transferred to a clean beaker. The extraction process was repeated with two more 10ml portions of CH_2Cl_2 and all of the fractions were collected. The combined CH_2Cl_2 layers were washed with 10-20ml distilled water and separated into a dry container (Senhaji et. al. 2004).

Isolation and identification of Fungi

The spoilages samples were cut with a sterile knife and crushed. 1 ml of the crushed bulb there after serially diluted using sterile distilled water. The spread plate technique was employed. Aliquots 1ml of the serially diluted sample 10^3 was spread on the surface of potato dextrose agar contained in a sterile petri dish. The petri dish also had two percent Chloramphenicol added to inhibit bacterial growth. Incubation was carried out in an inverted position at 28 °C for five days. The fungal colonies that

developed were purified by repeated sub-culturing on sterile malt agar. The final concentration $1x10^6$ CFU/ml was done by counting the microorganism in a neubauer chamber (CLSI, 2002). The fungal colonies growing on the culture plates were identified morphologically on the basis of their color, type of spores, the presence of sporodochia or appressoria, colony texture, and other growth characteristics of the fungi (CLSI, 2002).

Antifungal activity

Fungal cultures of *Penicillium* sp. and *A. niger* were cultured on MA. Cultures were incubated at 25°C ± 2 for 3-10 days. The mycelial growth test with malt agar was used to investigate the antifungal activity of the essential oil (Ishii, 1995). Agar well diffusion method (Bauer et. al. 1996) was used to test for the inhibition activity of the essential oils against *Penicillium sp.* and *A. niger*. The fungi were cultured on 20 ml MA in petri-dishes. An inoculum of 0.1 ml fungal suspension $1x10^6$ CFU/ml was spread uniformly over this medium by using the spreader and allowed to solidify on the agar medium for15 min. Wells of 5 mm in diameter were made on the surface of cultured medium by using sterilized cork borer and each well was filled with 50 µl of certain concentration (0.25, 0.5, 1, 2.5 and 5% v/v) of essential oil. Well*s* were distributed evenly on the medium in the petri-dish, the medium were allowed to stand on the bench for one hour for proper diffusion. Plates were incubated at 25º ±2 C for (3-10) days. Inhibition activities of the extracts were determined by measuring the inhibition zones formed around the wells in millimeter. The minimum inhibitory concentration (MIC) of oil necessary for inhibition of mycelial growth of the fungal strain was determined in MA medium and as control MA without the essential oil were used.

The fungi toxicity of the oils in terms of percentage of growth inhibition of mycelia was calculated by using the formula:

$$\text{Growth inhibition \%} = dc - dt/dc\ (100)$$

Where:

dc = Average increase in mycelial growth in control.

dt = Average increase in mycelial growth in treatment (Venkatesh et. al. 2017).

Statistical analysis

To determine the significant differences among the percentage mycelia inhibition of the extracts and the control against the isolated pathogen, the experiments were performed in triplicate. Data reported are the mean and standard deviation values calculated from the replicates. The results were analyzed using the variance (ANOVA). Differences ($P \leq 0.05$) were considered to be significant.

Results

Essential oils obtained from Cinnamon bark was checked for their antifungal potentials against *Penicillium sp and Aspergillus niger*, causal agent of black mold disease in some vegetables by disc diffusion method. These medicinal plants were chosen based on either traditional usage. The essential oils were checked in different concentrations of 0.25, 0.5, 1, 2.5 and 5% v/v for their ability to inhibit the mycelial growth of the test fungi. The results in (Fig. 1) showed that the essential oils of cinnamon have the significant potential to inhibit mycelial growth of test fungi completely on *A. niger* and *Penicillium* sp. at concentrations of 5% v7v. ($p< 0.05$). Therefore, lower concentrations (2.5, 1,0.5 and 0.25% v/v) of essential oils were used to determine the MIC on these fungi (Figure 1). *Aspergillus niger* was the most affected with growth inhibition average of 100, 100, 70 and 27% (Fig. 2) respectively

and *Penicillium* sp was the minor affected with growth inhibition average of 100, 64, 52 and 11% (Fig. 3).

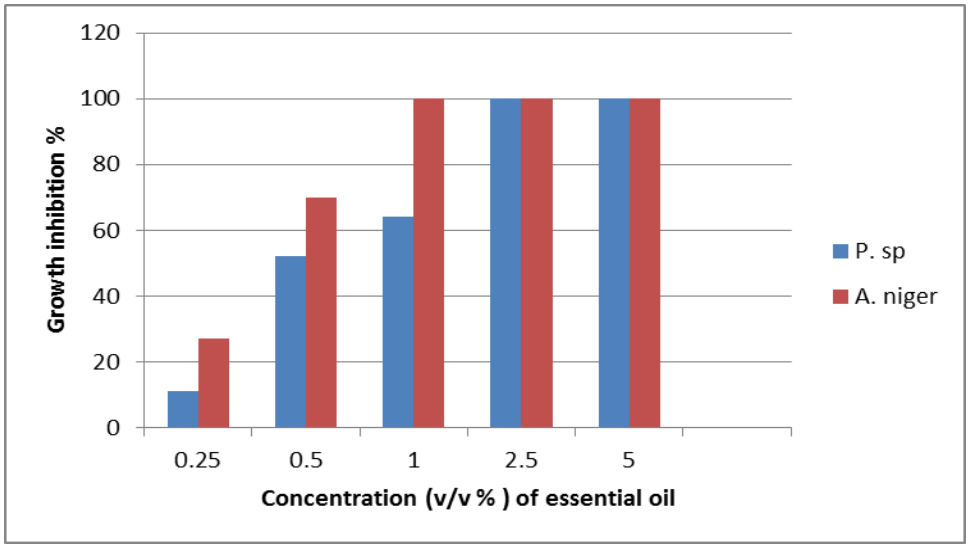

Fig. 1. Average growth inhibition of *Cinnamomum zeylanicum* essential oil at different concentrations on *Aspergillus niger* and *Penicillium sp.*

Values were mean ± standard error of the mean for bioassay conducted in triplicate.

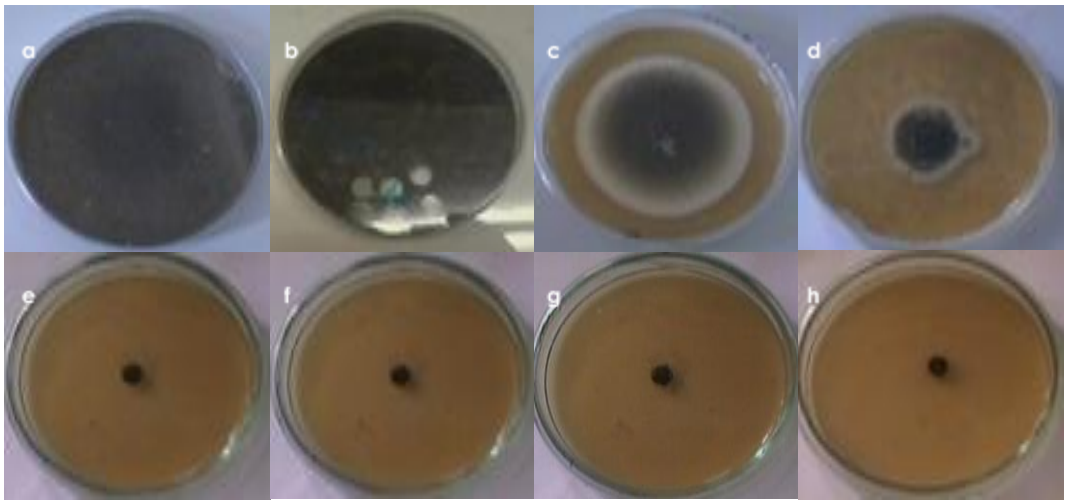

Fig. 2. Influence of *Cinnamomum zeylanicum* essential oils on mycelium growth of *Aspergillus. Niger.* (a) *Aspergillus niger;* (b) effect of solvent (dichloromethane); (c) Control (no essential oil); (d) effect of essential oil (0.25 v/v %); (e) effect of essential

oil (0.5 v/v %); (f) effect of essential oil (1 v/v %); (g) effect of essential oil (2.5 v/v %); (h) effect of essential oil (5 v/v %)

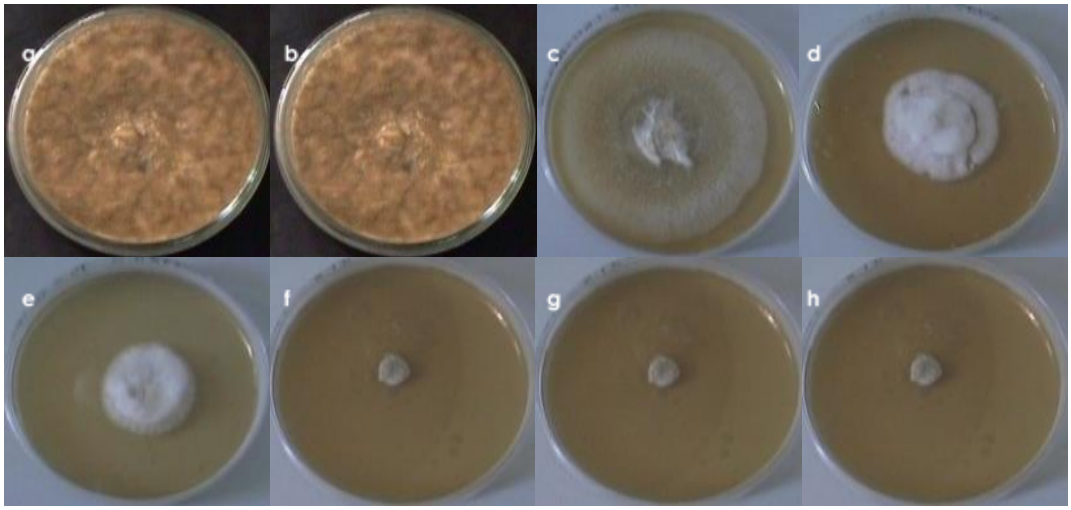

Fig. 3. Influence of *Cinnamomum zeylanicum* essential oils on mycelium growth of *Penicillium. Sp*. (a) *Penicillium sp;* (b) effect of dichloromethane; (c) control (no essential oil); (d) effect of essential oil (0.25 v/v%); (e) effect of essential oil (0.5 v/v%); (f) effect of essential oil (1 v/v%); (g) effect of essential oil (2.5 v/v%); (h) effect of essential oil (5 v/v%)

Discussion

The antifungal activity of certain bioactive compounds from medicinal plants has attracted a lot of attention within the scientific community largely as a result of the growing problem of multidrug resistance among pathogenic fungi. In addition, medicinal plant oils are the promising sources of antifungal drugs. The vegetables pathogens microorganisms such as *Penicillium sp and Aspergillus niger* causal agent of black mold disease onions collected from one location in Mascara (Algeria). Cinnamon oil had completely activity against the two pathogens fungi such as *Penicillium sp and Aspergillus niger* with the MIC of 2.5 and 1 % respectively. However, the antifungal activity of crude essential oil varied among the test

pathogens. According to the results of present study, dichloromethane extract of cinnamon can prevent mold growth on onions at dose of 2.5 % (v/v). The findings of the current study concur with reports from previous studies on different levels of antifungal activity of essential oil of cinnamon of varied chemical profiles against a diverse group of vegetables pathogenic fungi. The efficacy of cinnamon oil as an antifungal agent was also reported in various studies (Velluti et. al. 2003; Lopez-Malo et. al. 2007). Mau (Mau et al. 2001) reported the effects of cinnamon oils on different fungal species *Aspergillus flavus, Aspergillus niger, Penicillium italicum* CCRC 30567 and *Aureobasidium pullulans* CCRC 31981, indicating that cinnamon is a natural antifungal agent. Boniface (Boniface et. al. 2012) described the fungicidal activity of essential oils of cinnamon species against *C. albicans* (MIC = 0.40mg/ml), fungistatic activities against *Aspergillus ochraceus, Aspergillus parasiticus* and fungicidal against *Fusarium oxysporum* and *Penicilium digitatum*. A study from Gupta (Gupta et. al. 2008) indicated that cinnamon oils have potential action against *Rhizomucor* sp. amongst the test fungi (*Alternaria* sp., *Aspergillus fumigatus, Aspergillus niger, Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp. and *Rhizomucor* sp).

Xing et. al. (2010) studied the in vitro and in vivo antifungal efficacy of cinnamon oil against *Rhizopus nigricans, Aspergillus flavus* and *Penicillium expansum,* the minimum inhibitory concentrations of cinnamon oil were 0.64%, 0.16% and 0.16% v/v, respectively. The antifungal activity of cinnamon oil against *A. flavus* and *P. expansum* was stronger than that against *R. nigricans*. In an *in vivo* study, cinnamon oil with concentrations of 2.0% and 3.0% v/v showed complete control the growth of fungi in wound-inoculated lingwu long jujube and sand sugar orange fruits.

Sukatta et. al. (2008) tested the activity of the essential oil from clove and cinnamon against 6 fungi causing postharvest decay of grapes: *Aspergillus niger, Alternaria*

alternata, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Phomopsis viticola* and *Rhizopus stolonifera*. Overall, the essential oil from cinnamon is more potent than clove, except on *Rhizopus stolonifer*. In other study, El-Baroty (El-Baroty et. al. 2010) reported stronger antifungal activity of cinnamon oil against tested fungi (*Aspergillus niger*, *Penicillium notatum*, *Mucor hiemalis* and *Fusarium oxysporum*) than that of ginger essential oils. Another report Singh (Singh et. al. 2007) found that the leaf and bark volatile oils were highly effective against all the tested fungi (*Aspergillus niger, Aspergillus flavus, Aspergillus ochraceus, Aspergillus terreus, Fusarium moniliforme, Fusarium graminearum, Penicillium citrinum* and *Penicillium viridicatum*) except *Aspergillus ochraceus*. It is seen that the inhibiting effect of the oil corresponds to their concentration, the inhibitory effect of the essential oils was proportional to its concentration (Rasool et. al. 2006; Amini et. al. 2012). Whereas increase in concentrations the susceptibility of fungi increases as well.

The effects of growth inhibitory of plant essential oils depend on species of fungi (Amini et. al. 2012). The main constituent of cinnamon oil is cinnamaldehyde, which is the compound containing an aldehyde group and conjugated double bond outside the ring. This compound possesses much stronger antifungal activity (Wang et. al. 2005) and it may be a potential lead compound for the development of antifungal drugs (Bang et. al. 2000). Essential oils have two prominent features; low toxicity for people and environment due to their natural properties and low risk for resistance development by pathogenic microorganisms (Daferera et. al. 2000). For these reasons and considering the results, we recommend the use of cinnamon oils for development of new and safe antifungal agent. As a result of these finding and opportunities, we suggest cinnamon oils as a potential source of safe botanical food preservative that inhibits *Aspergillus* and *Penicillium* mycelial growth. However, further studies should

be conducted to explore large scale utilization and also exploring the efficacy of cinnamon oils using other toxigenic organisms that contaminate food commodities.

Conclusion

In conclusion**,** significantly higher broad-spectrum of antifungal activity was observed in cinnamon oil because it showed highest percentage of growth inhibition at lowest inhibitory concentration. Therefore, it could be used for the development of new environmentally friendly antifungal agent for the preservation of food. These results support the plant oils can be used as natural antimicrobial agent in the fight against molds species responsible for vegetables contamination.

Acknowledgments

The authors thank the Management of University of Mustapha Stambouli, Mascara for providing laboratory of bioconversion, microbiology engineering and food security facilities and constant encouragement for this research.

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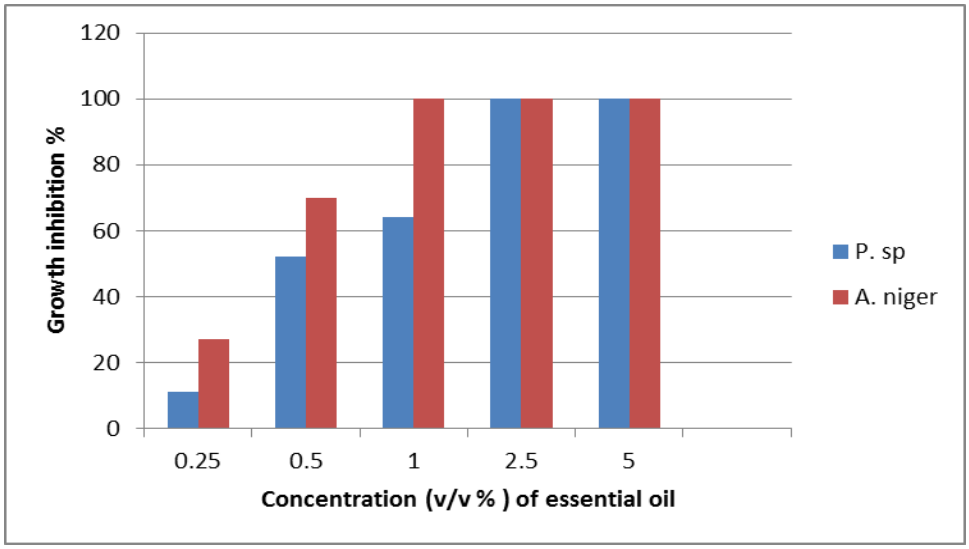

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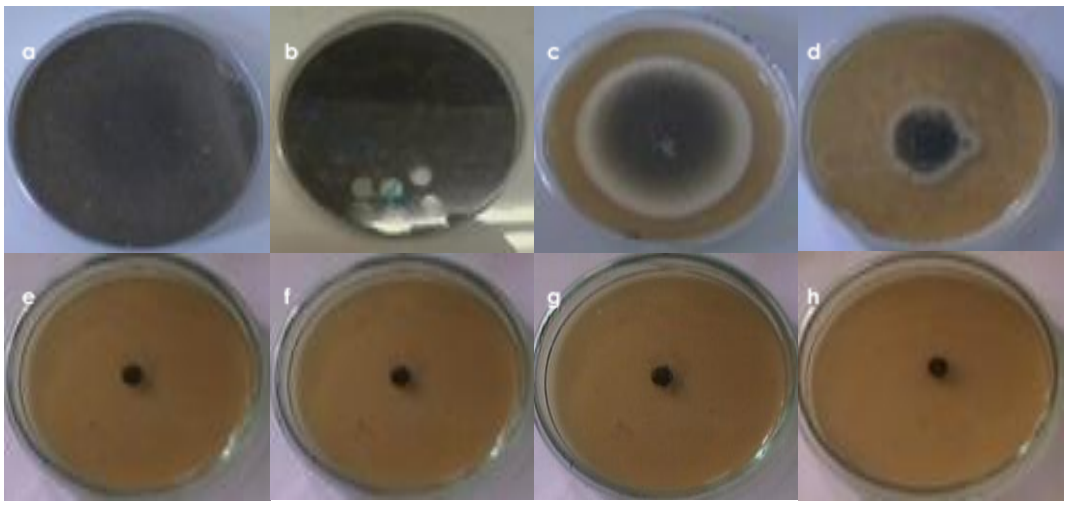

Fig. 2. Influence of *Cinnamomum zeylanicum* essential oils on mycelium growth of *Aspergillus. Niger.* (a) *Aspergillus niger;* (b) effect of solvent (dichloromethane); (c) Control (no essential oil); (d) effect of essential oil (0.25 v/v %); (e) effect of essential oil (0.5 v/v %); (f) effect of essential oil (1 v/v %); (g) effect of essential oil (2.5 v/v %); (h) effect of essential oil (5 v/v %)

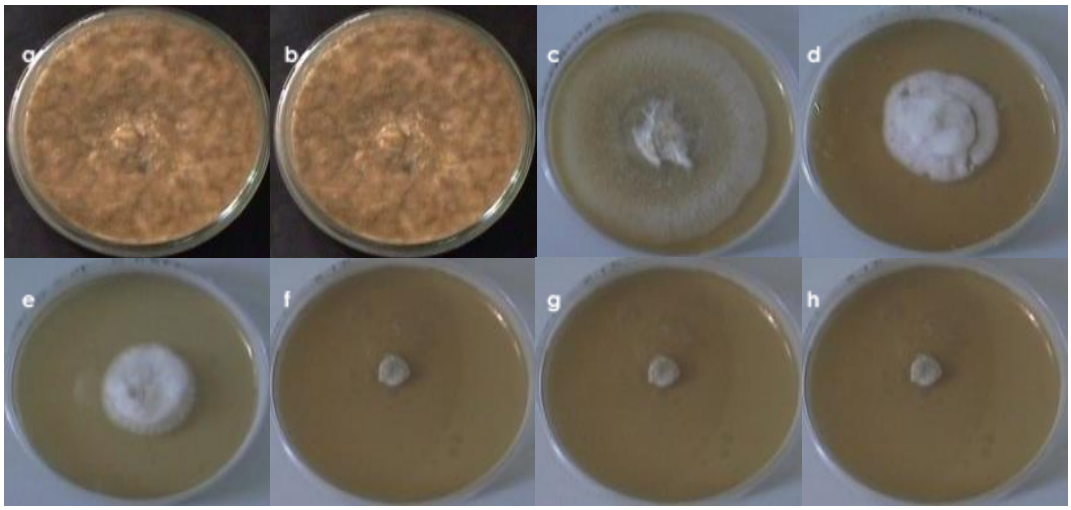

Fig. 3. Influence of *Cinnamomum zeylanicum* essential oils on mycelium growth of *Penicillium. Sp*. (a) *Penicillium sp;* (b) effect of dichloromethane; (c) control (no essential oil); (d) effect of essential oil (0.25 v/v%); (e) effect of essential oil (0.5 v/v%); (f) effect of essential oil (1 v/v%); (g) effect of essential oil (2.5 v/v%); (h) effect of essential oil (5 v/v%)

Fig. 1. Average growth inhibition of Cinnamomum zeylanicum essential oil at different concentrations on Aspergillus niger and Penicillium sp.

Fig. 2. Influence of Cinnamomum zeylanicum essential oils on mycelium growth of Aspergillus. Niger. (a) Aspergillus niger; (b) effect of solvent (dichloromethane); (c) Control (no essential oil); (d) effect of essential oil (0.25 v/v %); (e) effect of essential oil (0.5 v/v %); (f) effect of essential oil (1 v/v %); (g) effect of essential oil (2.5 v/v %); (h) effect of essential oil (5 v/v %)

Fig. 3. Influence of Cinnamomum zeylanicum essential oils on mycelium growth of Penicillium. Sp. (a) Penicillium sp; (b) effect of dichloromethane; (c) control (no essential oil); (d) effect of essential oil (0.25 v/v%); (e) effect of essential oil (0.5 v/v%); (f) effect of essential oil (1 v/v%); (g) effect of essential oil (2.5 v/v%); (h) effect of essential oil (5 v/v%)