The Potential of Plant Essential Oils as Biofumigant Against Fungal Pathogens of Postharvest Tomato

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Abstract

However recognized as more sustainable and safer way for crop production, organic agriculture is still facing many technical difficulties. One of the problems is concerning the management of posthavest disease caused by fungal pathogens. Since the applications of synthetic fungicides are prohibited, thus organically produced crops are more vulnerable to be attacked by pathogens. Therefore, the development of novel, reliable and more compliant technologies for diseases management are required. The aim of this study was to demonstrate the potential use of plant essential oils as biofumigant to control of fungal diseases of tomato during storage. We selected three essential oils, including clove oil, cinnamon oil and ajowan oil, for this study. The *in vitro* test for antifungal activity showed that the volatile headspace of the three oils exhibited potent effect on the test pathogens which includes *Botrytis cinerea*, *Fusaruim oxysporum* and *Aspergillus niger*. Then ajowan oil, which showed highest inhibitory effect, was further evaluated for its effectiveness when applied as biofumigant. Results from the experiment indicated that fumigation with ajowan oil can reduce the occurrence gray mold decay of tomato (caused by *B. cinerea*) significantly. The rate of control was up to 100 % during fifteen days of storage at 20 ºC. These results support the idea of using the essential oil as biofumigant for protection of postharvest tomato from fungal diseases. However, further comprehensive studies are still required especially on the issues concerning the reliability and eco-efficiency of this method at trial and commercial level.

Key words: Biofumigant, antifungal activity, postharvest diseases

1. Introduction

However has been valued as a more sustainable approach, organic agriculture is still facing challenges. One of the greatest barriers is related to the global requirements or guidelines of organic farming practice those prohibit the application of any types of synthetic chemicals, especially pesticides and fertilizers, throughout the chain of production [1]. This restriction posed a great risk to organic crops to be attacked by destructive pests and may lead to a massive loss of crop yield. Therefore, in order to realize the effective organic farming systems, it is necessary to develop alternative strategies for the control of crop pests those better comply with the requirements.

During the past decades, efforts in searching of alternative pesticides have been focused on natural compounds from plant due to their less toxic and more environmental compatible in nature [2]. Many plants have been extracted and screened for their biocidal activities against certain crop pests.

Essential oils are among the plant derived natural products that have been reported to possess desirable bioactivities such as fungicidal [3], bactericidal [4], insecticidal [5], and/or nematocidal [6] activities. To the most of our knowledge, essential oils can be utilized in different ways as pest or disease control agent according to their properties and formulation such as spray, dipping solutions or fuming agent. In this report, we demonstrate the potential use of selected plant essential oils as biofumigant in the control of fungal diseases of fresh cut tomato during storage.

2. Materials and Methods

2.1 Fungal strain

The test fungi, *Aspergillus niger*, *Botrytis cinerea*, and *Fusarium oxysporum*, were obtained from the plant pathogen collection of the Applied Biology Program, Phranakhon Si Ayutthaya Rajabhat University (Thailand). Fresh cultures were prepared by transferring of conidia suspension of each strain from deep freeze culture (under glycerol) to new potato dextrose agar plates. The working cultures were monthly sub-cultured and maintained on PDA at 4 ºC.

2.2 Plant materials and extraction of essential oils

Plant materials including cinnamon bark, clove bud and ajowan seed were procured from local market. The essential oils were extracted by hydro-distillation using a modified Clevenger type-apparatus [7]. Firstly, the plant materials were ground to powder, then 500 g of each were weighed and filled in a round bottom flask. An aliquot volume of distilled water was added to cover the material. The distillations were performed at a temperature around 100 ºC for 4 hours. Oil distillates assembled in the reservoir were collected, dried with saturated sodium sulfate anhydrous and stored in a refrigerator at 4 ºC.

2.3 *In vitro* antifungal assay

The essential oils obtained from 2.2 were preliminary valuated for their *in vitro* efficacies against the test fungi based on volatile activity assay in invert Petri plate (90 mm diameter) [8]. An aliquot 20 ml of PDA were poured into each Petri plates. After solidified, a 5 mm well was made on the medium at the center of each plate using sterile cork borer. A 5 mm mycelia disc of the test fungi was placed into the well and the plates were then inverted up-side-down. A sterile paper disc was placed on the lid of each inverted plate. Different dose of essential oil (0.1, 0.2, 0.4, 0.8, 0.16, 0.32, 0.64 μl/cm^3) were applied on to the paper disc. The dose of essential oil was defined as the volume of essential oil per the air space inside the container [Total volume of 90 mm Petri plate was 95.5 ml, thus the air space of plate containing 20 ml medium was 75.5 cm^3]. For

each control set, an equal volume of Dimethyl sulfoxide (DMSO) was applied on the paper disc instead of essential oil. All the inverted plates were incubated at 20 ºC in an environmental control chamber for 7 days. The antifungal activity was expressed in term of the percentage of mycelium growth inhibition and calculated according to following equation:

$$\% \text{ inhibition} = \frac{\Delta d_O - \Delta d}{\Delta d_O} \times 100$$

Where Δd_o and Δd are the average diameter of the fugal colonies in the control and treatment sets, respectively

2.4 *In vivo* experiment

From the *in vitro* test, the most efficacious oil was selected for further *in vitro* test against tomato gray mold. Early ripening tomatoes were obtained from local grower. The fruits were surface sterilized by dipped for 15 minute in 0.5 % commercial bleach and then washed three times with sterile distil water. After air dried, the fruits were inoculated with *B. cinerea*, the causal agent of tomato gray mold, by dipped in conidia suspension of the fungus. The inoculated fruits were arranged in 8 liter plastic boxes, 25 fruits for each box. The paper discs were placed in the center of the boxes and then ajowan oil was applied on discs. Each essential oil was applied at two dosages of 40 and 80 µl/1. For control, DMSO was applied instead of essential oil. The boxes were sealed and stored at 20 ºC. The occurrence of gray mold was periodically recorded at 5, 10 and 15 days. The experiment was conducted thrice.

3. Results and discussion

The antimicrobial potential of plant essential oil has long been recognized and numerous research papers had reported that aromatic medicinal plants are good sources of essential oils. In present work, clove bud, cinnamon bark and ajowan seed were selected for the extraction of essential oils based on hydro-distillation method**.** The essential oils obtained (as show in Fig. 1) were rich of aroma and volatile in nature thus we hypothesized that their headspace vapor should possess antimicrobial activity and might be applied as biofumigant against fungal pathogens of tomato.

3.1 *In vitro* antifungal assay

To confirm our hypothesis, the essential oils were preliminary checked for their activities against *A. niger*, *B. cinerea*, and *F. oxysporum*, the predominant rotting pathogens of tomato. Results from the volatile activity assay were showed in Fig. 2 and Fig. 3. It is clear that headspace vapor of each essential oils tested possess antifungal activities.

Fig. 1 Essential oils obtained by hydro-distillation;
(A) Clove oil
(B) Ajowan oil

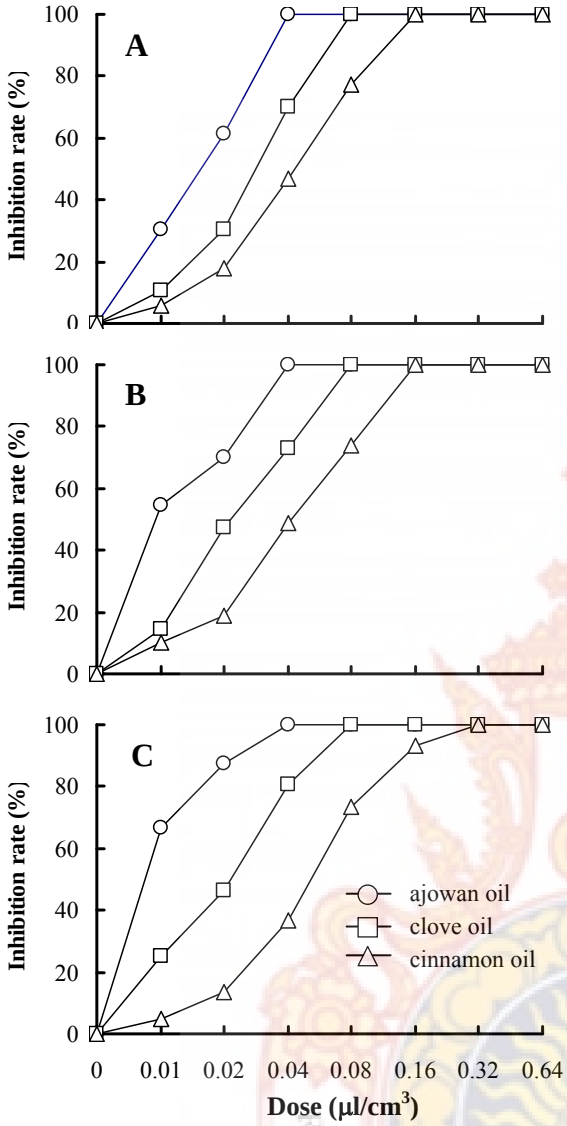

Fig. 2 Inhibitory effects of essential oils on the mycelial growth of test fungi.
(A) *Fusarium oxysporum*
(B) *Botrytis cinerea*
(C) *Aspergillus niger*

The lowest dose of each essential oil which caused 100 % inhibition may refer to as **Minimum Inhibitory Dose (MID)** and lower MID indicated higher inhibitory activity. Therefore, accordind to data in Fig. 2, ajowan oil was considered as the most potent followed by clove and cinnamon oil since their MID (*against F. oxysporum and B.cinerea*) were 0.04, 0.08, 0.16 µl/cm^3 respectively.

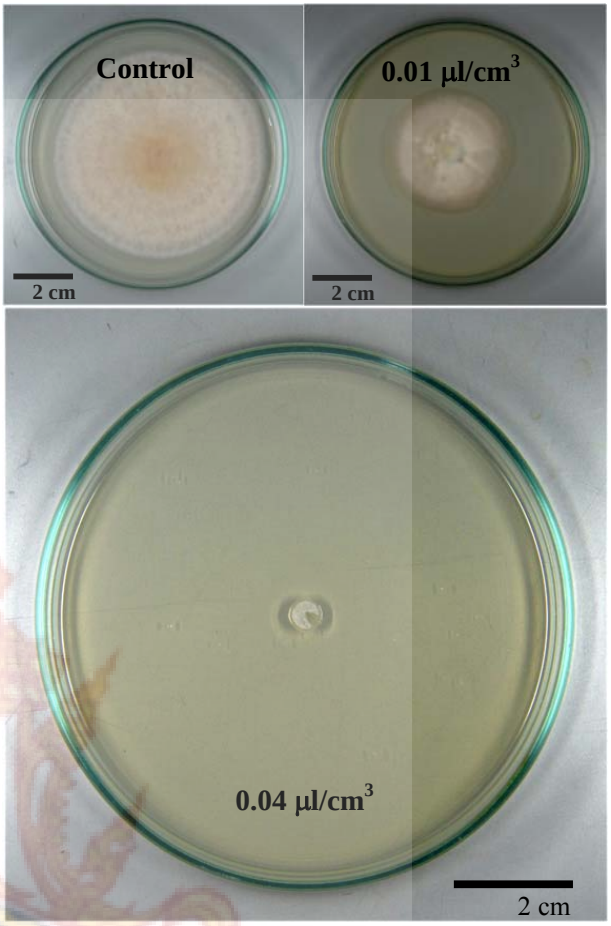

Fig. 3 Photographs comparing the radial growth of *F. oxysporum* treated with different doses of ajowan oil. Size of the fungal colony reflects inhibitory effect of the volatile head space of essential oil. At the dosage of 0.04 µl/cm^3 of ajowan oil the fungus was completely inhibited so the radial growth was not observed**.**

3.2 *In vivo* experiment

In this step, ajowan oil, which showed highest *in vitro* activity, was selected for further *in vivo* experiment against tomato gray mold. In the experiment, tomato fruits were artificially inoculated with conidia of *B. cinerea* and were fumigated with ajowan oil at the dosage of 40 and 80 µl/1. A control treatment was set by application of DMSO instead of ajowan oil. Result of the experiment was showed in Fig. 4 and Fig. 5.

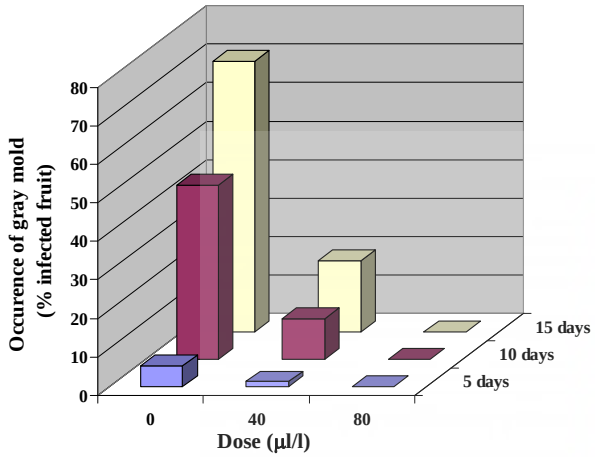

Fig. 4 The development of tomato gray mold in different treatments of ajowan oil compared with the untreated control.

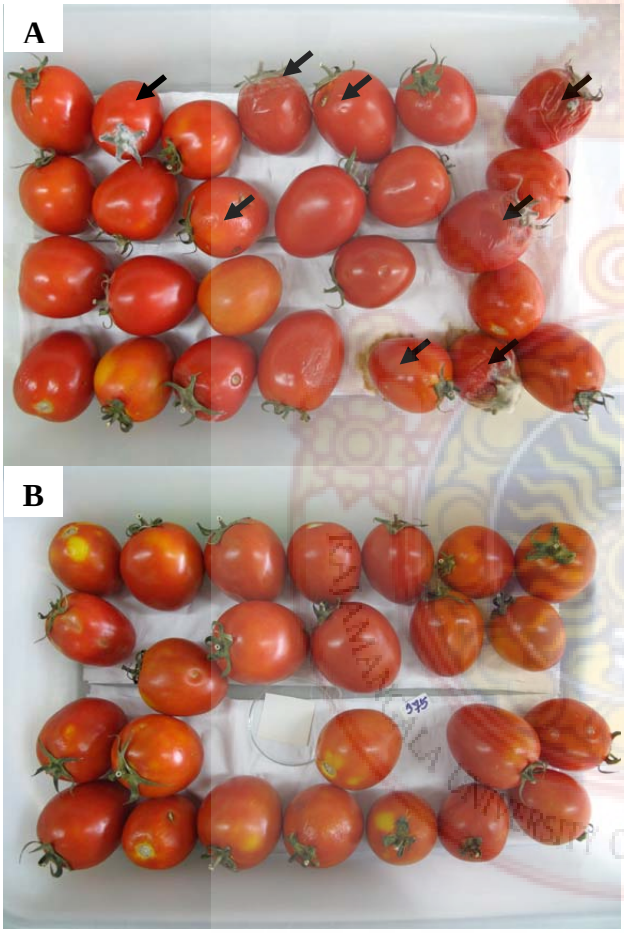

Fig. 5 Photographs showing experimental set of tomato after 10 days storage;
(A) Control set
(B) Fumigated with 80 µl/l of ajowan oil.

In Fig. 4, the occurrences of tomato gray mold in each treatment were graphically compared. It was apparent that the development of gray mold was significantly suppressed under the treatment of ajowan oil. For the dosage of 80 µl/l, the development of gray mold was found to be suppressed completely throughout the period of experiment so no infected fruit was found at this dosage. We have attempted to recover the fungal conidia from the tomato surface by using standard plate count technique, but there was no revival of the fungal conidia. This may be due to fungicidal effect of the oil that completely killed the fungal conidia at the first time.

At the lower dose, 40 µl/l, some infected fruits were found. This finding should be referred to the *in vitro* assay, where the 40 µl/l (0.04 µl/ml) dose of ajowan oil was recorded as MID. But in the *in vivo* experiment, the application of ajowan oil at this dosage was not fully effective since the occurrence of tomato gray mold was still observed. This finding suggested that the essential oil should be applied over the MID in order to achieve the best result.

4. Conclusions

In broader perspective, the results from this work provide scientific evidences that support the potential use of certain plant essential oils as biofumigant for the control of postharvest pathogens of fresh cut produces. And for this study, ajowan oil should be the best biofumigant for the control of fugal pathogens of fresh cut tomato as compared to the other. However, further comprehensive studies are needed, especially on the issues concerning the reliability and eco-efficiency of this method at trial and commercial level.

5. Acknowledgements

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