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การควบคุมโรคเน่าราสีเทาของผลมะเขือเทศโดยการรมด้วยน้ำมันระเหยของเทียนเยาวภาณี

Control of Gray Mold Rot of Tomato Fruit by Fumigation with Ajowan Volatile Oil

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Abstract

The application of natural products for preservation of fresh produce after harvest is receiving more attention since it is safer and more sustainable than the application of synthetic chemicals. In this study, the essential oil extracted from mature seeds of ajowan (*Trachyspermum ammi* (Linn.) Sprague) was evaluated for its potential as a biofumigant against gray mold decay of tomato caused by *Botrytis cinerea*. The results from *in vitro* assay showed that volatile headspace of the essential oil had strong fungicidal activity against the pathogen with minimum fungicidal quantity of 0.33 µl/cm^3. The *in vivo* experiment showed that fumigation of tomato with the vapor of ajowan oil reduced the incidence of the disease significantly. These results support the potential of ajowan oil as a biofumigant for the control of gray mold decay of tomato during storage.

Keywords: ajowan, *Trachyspermum ammi*, tomato, fumigation, *Botrytis cinerea*

บทคัดย่อ

การใช้สารธรรมชาติในการถนอมรักษาพืชผลหลังเก็บเกี่ยวกำลังได้รับความสนใจมากขึ้น เพราะมีความปลอดภัยและยั่งยืนกว่าการใช้สารเคมีสังเคราะห์ การวิจัยนี้ ได้นำน้ำมันระเหยที่สกัดจากเมล็ดแก่ของเทียนเยาวภาณี (*Trachyspermum ammi* (Linn.) Sprague) มาศึกษาศักยภาพการใช้ประโยชน์ในรูปของสารรมฆ่าเชื้อ เพื่อยับยั้งโรคราสีเทาบนผลมะเขือเทศที่เกิดจากเชื้อรา *Botrytis cinerea* ผลการศึกษาในอุปกรณ์ทดลองพบว่า ไอระเหยของน้ำมันเทียนเยาวภาณีมีฤทธิ์สูงในการฆ่าเชื้อรา *B. cinerea* โดยมีค่าปริมาณต่ำสุดในการฆ่าเชื้อราเท่า 0.33 ไมโครลิตรต่อลูกบาศก์เซนติเมตร และจากการทดลองกับผลมะเขือเทศพบว่า การรมผลมะเขือเทศด้วยไอระเหยของน้ำมันเทียนเยาวภาณีสามารถลดการเกิดโรคเน่าได้อย่างมีนัยสำคัญ นอกจากนั้น ผลจากการวิจัยนี้สนับสนุนแนวคิดในการใช้น้ำมันเทียนเยาวภาณีเป็นสารรมฆ่าเชื้อ เพื่อควบคุมโรคเน่าราสีเทาของมะเขือเทศระหว่างเก็บรักษา

คำสำคัญ: เทียนเยาวภาณี, *Trachyspermum ammi* , มะเขือเทศ, การรมไอระเหย, *Botrytis cinerea*

Introduction

Botrytis cinerea or ‘‘gray mold’’ is one of the most devastating phytopathogens of commercial crops, and widely distribute throughout the world. Several fungicides have been used to mitigate the damage caused by gray mold. However, the control of gray mold by using synthetic fungicides was found to be ineffective and needs to be avoided due to the emergence of resistant strains of the fungus and the harmful toxicity of the chemicals itself (Elad *et al*., 1992). During the past decades, efforts in searching of alternative pesticides have been focused on natural compounds from plants because they are less toxic and more environmental compatible (Lee *et al*., 2007). 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 (Soliman and Badeaa, 2002), bactericidal (Dorman and Deans, 2000), insecticidal (Isman, 2000), and/or nematocidal (Pandey *et al*., 2000) activities. To the most of our knowledge, essential oils can be utilized in different ways as a pest or disease control agent according to their properties and formulation such as spray, dipping solutions or fuming agent. In this study, the essential oil extracted from mature seeds of ajowan, an aromatic plant of the family Umbelliferae, was evaluated as a biofumigant against *Botrytis cinerea*.

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Materials and Methods

1. Fungal strains

Three virulent strains of *Botrytis cinerea* (N01-01, N02-03 and N03-01) were obtained from the plant pathogen collection of the Applied Biology Program, Phranakhon Si Ayutthaya Rajabhat University, Thailand. Fresh cultures were prepared by transferring conidium suspension of each strain from deep freeze culture (under glycerol) to new potato dextrose agar (PDA) plates. The working cultures were monthly sub-cultured and maintained on PDA at 4 ºC.

2. Plant materials and extraction of essential oil

In this study, mature and dried seeds of ajowan (*Trachyspermum ammi* (Linn.) Sprague) were used for essential oil extraction. The plant material was procured from a local market of Phranakhon Si Ayutthaya, Thailand. Essential oil was extracted from the plant material based on hydrodistillation in a modified Clevenger type-apparatus (Sahaf and Moharramipour, 2008). Firstly, the plant material was ground into fine powder using mortar and pestle, and then 500 g powder was weighed and filled in a round-bottomed flask. An aliquot volume of distilled water was added to cover the material. The distillation was performed at temperature around 100 ºC for 4 hours. Oil distillate assembled in the reservoir was collected, dried with saturated sodium sulfate anhydrous and stored in a refrigerator at 4 ºC.

3. *In vitro* antifungal assay

The *in vitro* efficacy of Ajowan oil was valuated against *B. cinerea* based on volatile activity assay in invert petri plate (90 mm in diameter) (Tripathi *et al*., 2009). An aliquot of 20 ml PDA was poured into each petri plates. After solidification, a 5 mm well was made on the medium at the center of each plate using a sterile cork borer. A 5 mm mycelium disc of the test fungi was inserted into the well and the plates were inverted up-side-down. A sterile paper disc was placed on the lid of each inverted plate. Different quantities of ajowan oil (6.25, 12.5, 25.0, 50.0, 100.0, and, 200 µl) were dispensed on to the paper disc to obtain the final dosage of 0.08, 0.17, 0.33, 0.66, 1.32 and 2.65 µl/cm^3 (of air space inside the petri plate) respectively [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 the control set, an equal volume of dimethyl sulfoxide (DMSO) was applied on the paper disc instead of ajowan oil. All the inverted plates were incubated at 20 ºC in an environmental control chamber for 7 days. The antifungal activity was expressed in terms of the percentage of mycelium growth inhibition and calculated according to the following equation:

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

Where Δd_o is the average diameter of the fugal colonies in the control set and Δd is the average diameter of the fugal colonies in the treatment sets. The lowest dose of the essential oil which caused 100 % inhibition is referred to as minimum inhibitory dose (MID) and lower MID indicates higher inhibitory activity.

4. *In vivo* experiment

The *in vivo* efficacy of ajowan oil was evaluated against *B. cinerea* on tomato (*Lycopersicon escalentum* Mill. cv. Tho). Early ripening tomatoes were obtained from local growers. The fruits were surface-sterilized by dipping for 15 minute in 0.5 % commercial bleach and then washed three times with sterile distilled water. After air dried, a uniform wound (5 mm wide and 3 mm deep) was made at the equator of each fruit using a sterile cork borer. Wounded fruits were left in a laminar flow cabinet till the wounds were well dried. An aliquot of 50 µl spore suspension of *B. cinerea* (10^4 CFU/ml) was injected into each wound. The inoculated fruits were arranged in 2.5

liter plastic boxes, 6 fruits per box. Three dosages of ajowan oils (1xMID, 2xMID and 4xMID) were applied separately into each box by dispensing on to 1x1 cm cellulose sponges "Scotch-Brite" placed inside the box. For the control boxes, DMSO was applied instead of ajowan oil. The boxes were sealed and stored at 20 ºC. After 5 days of storage, diameters of decay lesions were recorded. The experiment was conducted thrice.

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 study, essential oil extracted from ajowan seeds was evaluated *in vitro* and *in vivo* against gray mold fungus, *Botrytis cinerea*.

According to the *in vitro* assay under modified atmosphere condition in invert Petri plates, it is clear that headspace vapor of ajowan oil possesses antifungal activity against *B. cinerea*. The efficacies of the essential oil on each isolate of *B. cinerea* were quantified and showed in Table 1.

Table 1 *In vitro* efficacy of ajowan oil against *B cinerea*.

Dosage of ajowan oil	Inhibitory efficacy against *B. cinerea* (%)		
($\mu l/cm^3$)	N01-01	N02-03	N03-01
0.08	66.19 ± 3.60^{b}	48.10 ± 6.44^{a}	68.24 ± 5.64^{b}
0.17	75.24 ± 5.02^{c}	72.38 ± 8.24^{bc}	73.68 ± 7.48^{bc}
0.33	100^{d}	100^{d}	100^{d}
0.66	100^{d}	100^{d}	100^{d}
1.32	100^{d}	100^{d}	100^{d}
2.65	100^{d}	100^{d}	100^{d}

Means followed by different letters are significantly different (Fisher's LSD test, $P < 0.05$).

In Table 1, 0.33 $\mu l/cm^3$ (or 0.33 ml/l) was considered as the minimum inhibitory dose (MID), since all isolates of *B. cinerea* were totally inhibited at this dosage. Thus we use this data as the basis in the design of further *in vivo* experiment.

In the *in vivo* experiment, tomato fruits inoculated with *B. cinerea* were fumigated with three different dosages of ajowan oil, which are one, two and four fold of MID value (Table 2). In theory, fumigation of tomato with ajowan oil at the dosage of 1xMID should completely inhibit gray mold development. However, the result from our *in vivo* experiment was found to deviate from the assumption, since gray mold decay of tomato was still observed in the 1xMID (0.33 ml/l) treatment. This may be due to the effect of other influential factors such as type of material used as an oil adsorbent that may affect the release of volatile constituents. This finding also suggested that, to achieve the best result, the essential oil should be applied at a dose higher than the MID level.

Conclusions

In broader perspective, the results from this work should be scientific evidence supporting the potential use of certain plant essential oils as biofumigants for the control of postharvest pathogens of fresh-cut produce. For this study, ajowan oil should be a promising biofumigant for the control of gray mould decay of fresh-cut tomato. However, further comprehensive studies are still required especially on the issues concerning the reliability of this proposed method at trial and commercial levels.

Table 2 The *In vivo* efficacy of ajowan oil against tomato gray mold during 5 days of storage.

Dosage of ajowan oil (ml/l)	Lesion diameter of infected fruit (mm)		
	N01-01	N02-03	N03-01
0 (control)	23.5±1.5[a]	24.2±1.5[a]	21.3±2.9[a]
0.33 (1xMID)	7.1±1.9[b]	8.3±3.6[b]	0
0.66 (2XMID)	0	0	0
1.32 (4xMID)	0	0	0

Means followed by different letter are significantly different (Fisher's LSD test, $P < 0.05$)

Figure 1 *In vivo* experiment on the fumigation of tomato with different dosages of ajowan oil.

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