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Vanillin enhances the antifungal effect of plant essential oils against *Botrytis cinerea*

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KEYWORDS
Essential oil;
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Synergistic effect

Summary Antifungal activity against *Botrytis cinerea* of lavender, rosemary, peppermint, sweet basil, rose, ginger, and thyme extracts alone at different concentrations (0.04, 0.19, and 0.40 mg/mL) or in combination with vanillin was investigated. Comparatively, 0.04 and 0.19 mg/mL concentrations of essential oil extracts were less inhibitory than that of 0.40 mg/mL concentrations. However, with rosemary extracts, high inhibitory activity was observed for all tested concentrations. On the other hand, the combination of essential oil extracts with vanillin exhibited a marked antifungal activity for *B. cinerea* with thyme, lavender, and peppermint extracts. These inhibitory effects are interesting in connection with the prevention of gray mold rot in many agricultural products and these antifungals could be used instead of synthetic products.
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Introduction

The protection of agricultural products from plant pathogens is necessary and has been achieved by various physical and chemical methods. Antimicrobial chemicals such as benzimidazoles, aromatic hydrocarbons, and sterol biosynthesis inhibitors are often used in control of plant disease in agriculture (Moorman and Lease, 1992). However, there are some problems in utilizing the chemicals, for example, the high risk of toxic residues in the products and adapted fungi resistance to the chemicals (Sholberg and Conway, 2004). In addition, using synthetic antimicrobial chemicals could cause harm to agricultural workers when used continuously for a long period. Thus, research in the use of natural antimicrobials for disease control in agriculture is increasing. A number of naturally occurring compounds, including essential oils extracted from herbal plants, have been considered as natural antifungicides for controlling pathogens. There are many reports of the antifungal activity of

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essential oils from herbal plants. The activity varies widely, depending on the type of essential oil, test method, and microorganism (Giese, 1994). Moreover, the application of essential oils as antifungals in agricultural products is limited because of the high level concentrations required when compared with classical antifungicides and a potential loss of antifungal action due to their volatility (Cassella et al., 2002). In order to enhance the efficacy of plant essential oils, the combined use of essential oils with synthetic antifungals has been evaluated and reported to have potential synergistic effects (Shin and Lim, 2004).

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a useful flavor and is often used as a background note or flavor enhancer to achieve the flavor profiles of many food products (Brandt, 1996). In addition, antifungal activity of vanillin has been reported against some fungi (Lopez-Malo et al., 1995). However, synergistic effects between essential oils and vanillin against fungi growth have not yet been described.

In this study, extracts from lavender, peppermint, rose, rosemary, sweet basil, ginger, and thyme alone and in combination with vanillin were tested for activity against *Botrytis cinerea* in vitro as the first step towards the development of a biological control system for the control of *Botrytis* rot of agricultural products.

Materials and methods

Plant materials

Seven types of plants, lavender (*Lavandula angustifolia*), rosemary (*Rosmarinus officinalis*), peppermint (*Mentha* × *piperita*), sweet basil (*Ocimum basilicum*), rose (*Rosa* × *hybrida*), ginger (*Zingiber officinale*), and thyme (*Thymus vulgaris*) were used for this investigation. Lavender, rosemary, and peppermint samples were freshly harvested from a greenhouse in the Faculty of Agriculture, Meijo University (Nagoya, Japan). Sweet basil, rose, ginger, and thyme samples were purchased and used fresh from a local supermarket.

Extraction

Extraction was carried out using *n*-hexane, and all operations were at room temperature. Fresh samples of corollas of lavender, petals of rose, leaves with stems of rosemary, peppermint, sweet basil, and thyme, and rhizomes of ginger were chopped into small pieces before *n*-hexane extraction (Wako Pure Chemical Industries, Osaka Japan) at the ratio of 1:10 (w/v) for 2 days. The extracts were filtrated using filter paper (Advantec No. 2, Toyo Roshi, Tokyo Japan) containing anhydrous Na_2SO_4 (Kanto Chemical Co., Tokyo, Japan) to separate the plant sample and eliminate moisture content. Thereafter, the extracts were evaporated under a gentle vacuum in a rotary evaporator at 30 °C. Each resulting extract was dissolved at a suitable concentration in dimethyl sulphoxide (DMSO) and used for antifungal tests.

The concentration of each extract was analyzed by using gas chromatography (CE Instruments EL980, Yanaco, Japan) equipped with a flame ionization detector (FID) and DB-WAX capillary column (60 m × 0.25 mm i.d.) (J&W Scientific, CA USA). Injector and detector temperatures were set at 240 and 280 °C, respectively. Column temperature was gradually increased from 80 to 220 °C at a rate of 2 °C/min and maintained at 220 °C for 60 min. Helium was used as carrier, at a flow rate of 20 mL/min. Samples in hexane (2.0 μL) were injected manually. Ethyl heptanoate was used as an internal standard (Tsuro et al., 2001).

Fungal species and antifungal activity tests

Botrytis cinerea isolate, obtained from the Shizuoka Prefecture Institute of Citrus culture collection (Japan), was originally isolated from orange tissue in Shizuoka prefecture, Japan, in September 2005.

Antifungal activity was examined in vitro in plastic petri dishes containing potato dextrose agar (PDA) (Cakir et al., 2005). The assays for essential oil antifungal activity were prepared at 0.04, 0.19, and 0.40 mg/mL concentrations of PDA. The combination effect of essential oil and vanillin was assessed at 0.40 mg/mL of essential oil with 0.625 mg/mL of vanillin in PDA. The petri dishes were inoculated with fungi immediately after their preparation by placing in the centre of each plate a 7 mm diameter mycelial mass of the cultivated test fungi cut with a sterile cork borer from the periphery of a 1-week-old culture growing on a PDA plate. The petri dishes were sealed with paraffin film and incubated in the dark at 22 ± 2 °C, and the diameter (mm) of the extension of hyphae from the centre to the sides of dishes was measured every 24 h for 4 days. The incubation was stopped when the mycelial mass of control petri dishes had almost filled the petri dish (ca. 4 days). The diameter of the growth mass was determined by averaging the radial growth of the mycelial mass in four orthogonal directions. Mean growth measurements are

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