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Essential Oil Prepared from *Cymbopogon citrates* Exerted an Antimicrobial Activity Against Plant Pathogenic and Medical Microorganisms

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Essential oils are mixtures of volatile, lipophilic compounds originating from plants. Some essential oils have useful biological activities including antimicrobial, spasmolytic, antiplasmodial, and insect-repelling activities. In this study, we tested the antimicrobial activity of essential oil prepared from the aromatic plant, *Cymbopogon citrates*, against three important plant pathogenic and medical microorganisms, *Pectobacterium carotovorum*, *Colletotrichum gloeosporioides*, and *Aspergillus niger*. It effectively inhibited the growth of the bacterium, *Pectobacterium carotovorum*, in a dose-dependent fashion, and 0.5% of the oil inhibited the growth of bacteria completely. Similarly, the essential oil inhibited the growth of plant pathogenic fungus, *Colletotrichum gloeosporioides*, and the addition of 1% of essential oil completely inhibited the growth of fungus even after 5 days of culture. Finally, it effectively inhibited the growth of the medically and industrially important fungal species, *Aspergillus* spp. These results suggest that the essential oil from *Cymbopogon citrates* may be an environmentally safe alternative to inhibit antimicrobial agents for various uses.

KEYWORDS : Antimicrobial activity, Essential oil, Microorganism, Pathogenic, Plant

The expanded use of synthetic chemicals in industry negatively impacts environmental and public health due to their slow decomposition and accumulation in the environment. Consequently, there is a growing interest in natural products for use as alternative pesticides and as antimicrobial agents because they have a lower impact on the environment and they meet consumer demand for greener products. Some plant extracts and essential oils are alternatives to synthetic chemicals.

Essential oils are mixtures of volatile compounds obtained by distilling an essence prepared from natural materials (Delgado Ayza, 2005). Some essential oils have useful activities, including antimicrobial, spasmolytic, and insect-repelling properties (Kumaran *et al*., 2003; Cha *et al*., 2005a, b). Consequently, there have been attempts to use essential oils for food preservation, pharmacological purposes and aromatherapy (Crowell, 1999; Oussalah *et al*., 2006).

The genus *Cymbopogon* belongs to the Family Poaceae (Gramineae) and is an important aromatic herbal species. *Cymbopogon citrates* (DC.) Stapf. (Gramineae), also known as lemon grass, is a plant cultured in most of the tropical and subtropical countries as a source of essential oil. *Cymbopogon citrates* is a source of essential oil and is widely used as a component of ethnopharmaceuticals in tropical and subtropical countries. For example, *Cymbopogon citrates* is used in Peru for preparing soft drinks and is used as an aromatic, pleasant-tasting herbal tea throughout its distribution area (Di Stasi *et al*., 1989; Duke, 1989; Tortoriello and Romero, 1992). The aerial parts of the plant are widely used in folk medicine to treat various health problems, such as digestive disorders, inflammation, diabetes, nerve disorders, and fever (Olaniyi *et al*., 1975; Puatanachokchai *et al*., 2002; Sheweita *et al*., 2002; Nogueira *et al*., 2008; Blanco *et al*., 2009).

The biological activity of the essential oil prepared from *Cymbopogon citrates* is dependent mainly on citronellal and citral, which are the mixture of geranial and general (Paranagama *et al*., 2003). Essential oils from various species of *Cymbopogon* are used in the perfumery, cosmetic, and soap industries and have a remarkable commercial value. In addition, the essential oil of *Cymbopogon citrates* has antifungal and insecticidal activities (Irkin and Korukluoglu, 2009). In this study, we tested the antimicrobial activity of essential oil prepared from *Cymbopogon citrates* against the growth of three important plant pathogenic and medical microorganisms, *Pectobacterium carotovorum*, *Colletotrichum gloeosporioides*, and *Aspergillus niger*, to confirm whether the essential oil from aromatic plants can be used as agricultural and health-related chemicals.

Materials and Methods

Chemicals and laboratory wares. Unless otherwise specified, all the chemicals and laboratory wares used in

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this study were purchased from Sigma Chemical Co. (St. Louis, MO) and SPL Co. (Seoul, Korea), respectively.

Essential oil and preparation of culture media. Essential oil from *Cymbopogon citrates* was purchased from NBM Inc. (Jeonju, Korea) and used with the emulsifying agent provided by the supplier. The oil was filtered through a 0.45 μm membrane filter (Sartorius, Goettingen, Germany) and applied either directly onto sterilized LB liquid culture medium (BD Bioscience, Sparks, MD) or into molten potato dextrose agar (PDA, BD Bioscience) before solidification.

Microorganisms. In this study, three microorganisms, two plant pathogens and one health-related microorganism, were used. Three strains (KCTC 10225, KCTC 10458, KCTC 10057) of *Pectobacterium carotovorum*, a causative agent of soft rot in cabbage, were kindly provided by Dr. S. G. Heo (National Institute of Agricultural Science, Suwon, Korea). Ten different variants (PER-10, PER-23, PER-24, PER-25, PER-30, PER-35, PER-38, PER-43, PER-45, and PER-46) of *Colletotrichum gloeosporioides*, a causative agent of anthracnose in persimmon, were kindly provided by Dr. T. H. Lim (Samhoub Inc., Kimje, Korea). *Aspergillus niger* ATCC 9642, an important industrial and health-related microorganism, were purchased from American Type Culture Collection (Manassas, VA).

To prepare the inoculums for *Pectobacterium carotovorum*, bacterial stock culture was grown in LB broth for 18 to 24 h at 28°C to a concentration of 10^8 cells/*ml*. To prepare the inoculums for *Colletotrichum gloeosporioides*, the fungi were cultured initially on PDA media, then a disc of 0.5 cm diameter was cut with a cork borer. To prepare the inoculums for *Aspergillus niger*, spores were produced initially on PDA media, then a conidial suspension was prepared in Tween 20 (0.02%) with vigorous shaking to break conidial chains and to reduce conidial aggregation. The conidial suspension was filtered through glass wool to remove residual mycelial fragments and used to inoculate with a concentration of 80 spores/*ml*.

Determination of minimal inhibitory concentration. The minimum inhibitory concentration of the essential oil against *Pectobacterium carotovorum* was determined by broth dilution method such that the lowest concentration of essential oil resulting in complete inhibition of visible growth of the bacteria was determined after 24 h culture at 28°C. Confirmation of the complete inhibition of bacterial growth was performed by plating and growing the culture medium on agar plates.

Assessment of inhibition of mycelia growth. The degree of inhibition of mycelia growth of *Colletotrichum gloeosporioides* by the addition of essential oil was determined by measuring the growth of mycelia after inoculation of disc agar containing inoculums. The diameter of mycelial growth was measured after 48 h culture at 28°C.

Results and Discussion

Essential oils are the odorous and volatile products of plant secondary metabolisms and have a wide range of application in folk medicine, food flavoring and preservation, and fragrance industries. In this study, we tested antimicrobial activity of essential oil prepared from *Cymbopogon citrates* against the growth of three important plant pathogenic and medical microorganisms, *Pectobacterium carotovorum*, *Colletotrichum gloeosporioides*, and *Aspergillus niger*.

We tested the inhibitory activity of essential oil from *Cymbopogon citrates* on the growth of *Pectobacterium carotovorum*, a causative agent of soft rot in cabbage (Table 1). It effectively inhibits the growth of plant pathogenic bacteria, *Pectobacterium carotovorum*. When we added serially diluted essential oil into medium, essential oil inhibited the growth of the bacteria dose-dependently. The minimal inhibitory concentration of essential oil was 1/400 (v/v). The dose-dependent inhibition on the growth of the bacteria and the minimal inhibitory concentration was the same for all 3 strains of *Pectobacterium carotovorum*.

We tested antimicrobial activity of *Cymbopogon citrates* against *Colletotrichum gloeosporioides*, a causative agent of anthracnose and one of the most important genera of plant pathogens worldwide. Anthracnose, caused by *Colletotrichum* or *Glomerellar*, is very common and destructive disease in numerous crops and ornamental plants and is the main post-harvest disease in cereals, grasses, legumes, fruits, vegetables, and perennial crops (Kim *et al*., 2003). As shown in Fig. 1, addition of essential oil into culture medium inhibited the growth of *Colletotrichum gloeosporioides* dose-dependently. However, the inhibitory activity of the essential oil decreases as the culture period increases. Nevertheless, the addition of 1% essen-

Table 1. Inhibitory effect of the essential oil prepared from *Cymbopogon citrates* on the growth of *Pectobacterium carotovorum*

P. carotovorum strains	Minimal inhibitory concentration (v/v)				
	Control	1/800	1/400	1/200	1/100
KCTC 10225	+	+	+	–	–
KCTC 10458	+	+	+	–	–
KCTC 10057	+	+	+	–	–

Stock culture of *Pectobacterium carotovorum*, prepared as described in the Materials and methods, was inoculated into culture medium containing different concentrations of essential oil from *Cymbopogon citrates* for 48 hr and the growth of the bacteria was reported.

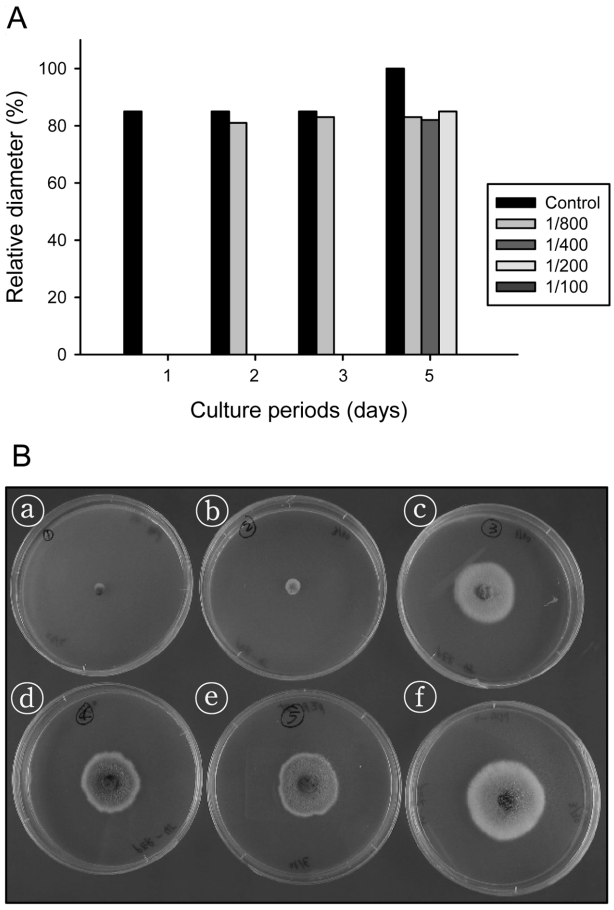

Fig. 1. Inhiitory effects of essential oil from prepared from *Cymbopogon citrates* on the growth of *Colletotrichum gloeosporioides*. A, mycelial blocks prepared as described in the Materials and methods were placed onto medium containing different concentrations of essential oil and the relative diameter of mycelia growth was measured for 5 days; B, pictures of culture plates taken on the 5th day of culture. ⓐ through ⓕ represents the pictures of plates containing 0.2% 0.1%, 0.05%, 0.125%, 0.0625%, and 0% essential oil, respectively.

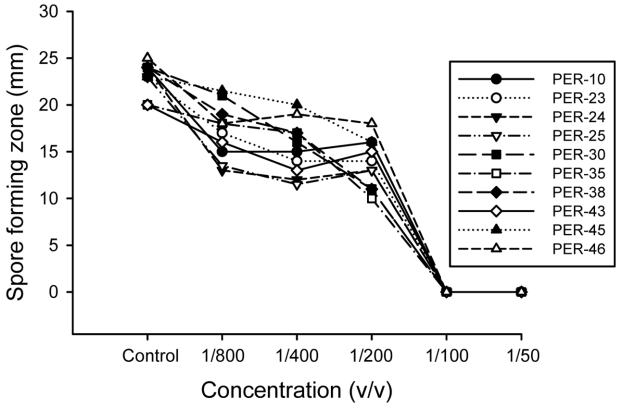

Fig. 2. Inhibitory effect of essential oil prepared from *Cymbopogon citrates* on the growth of different strains of *Colletotrichum gloeosporioides*. Mycelial blocks prepared as described in the Materials and methods were placed onto the medium containing different concentrations of essential oil and the relative diameter of spore forming zone was measured on the 5th day of culture.

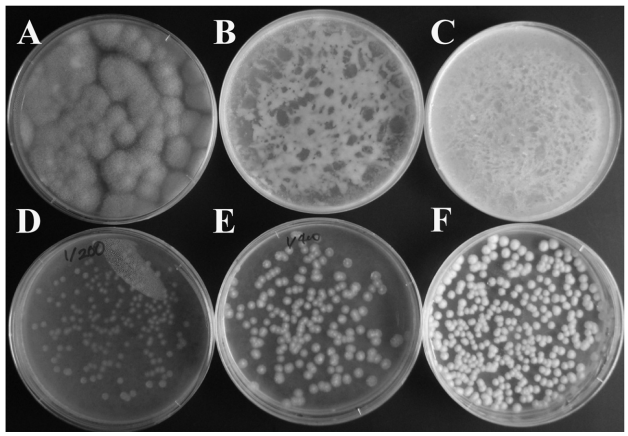

Fig. 3. Inhibitory effect of essential oil prepared from *Cymbopogon citrates* on the growth of *Aspergillus niger*. *Aspergillus niger* spores were inoculated onto the medium containing different concentrations of essential oil and the pictures were taken on the 3rd day of culture. A through E represents pictures of plates containing 0.2% 0.1%, 0.05%, 0.125%, 0.0625%, and 0% essential oil, respectively.

tial oil from completely inhibited the growth of *Colletotrichum gloeosporioides* even 5 days after inoculation. The inhibitory activity was similar in all 10 strains tested and 1% essential oil from *Cymbopogon citrates* completely inhibited the growth of all the tested strains of *Colletotrichum gloeosporioides* (Fig. 2). More interestingly, when the spores from *Colletotrichum gloeosporioides* were pretreated with a low concentration of essential oil, there was a decrease in spore germination rate. Untreated spores had an 80% germination rate, but the rate went down to 50% after 0.25% essential oil treatment for 48 h (data not shown). Essential oil prepared from *Cymbopogon citrates* effectively inhibits the growth of plant pathogenic fungus, *Colletotrichum gloeosporioides*, although the efficiency may be slightly weaker than against bacteria.

Aspergillus spp. are capable of growing in a wide range of organic substrates. *Aspergillus* spp. are very useful microorganisms in industry, although the same fungus can be harmful in some health-related product such as cosmetics. We tested the inhibitory activity of the essential oil prepared from *Cymbopogon citrates* against the growth of *Aspergillus* spp. using *Aspergillus niger* (Fig. 3). *Aspergillus niger* grew very well and the spores were formed normally after three days of culture in control culture plates. However, the addition of essential oil inhibited the growth of the fungi very efficiently. Even 0.125% of essential oil,

the lowest essential oil concentration tested, inhibited the growth of *Aspergillus niger*. Spore formation was clearly inhibited by addition of essential oil. These results showed that the essential oil prepared from *Cymbopogon citrates* inhibited the growth of *Aspegillus* spp. very efficiently.

The physical nature of essential oils, low molecular weight substances with pronounced lipophilic tendencies, is believed to allow them to penetrate cell membranes quickly. The high efficiency of essential oils to penetrate tissue has been proven in other studies and essential oils penetrate tissue roughly 100 times faster than water and 10,000 times faster than salts (Rommelt *et al*., 1974; Burt, 2004; Burt *et al*., 2005; Edris, 2007). Also, essential oils themselves have been known to contain various compounds with antimicrobial activity. For example, essential oils contain a number of small terpenoids and phenolic compounds, such as thymol, carvacrol, and eugenol, which exert high antimicrobial activity in their pure forms (Didry *et al*., 1993; Juneja and Friedman, 2007). In addition, the antimicrobial activities of oregano, savory, and thyme are likely due to their high contents of thymol and carvacrol, which are one of the most efficient volatile antibacterial agents known so far (Nevas *et al*., 2004). Hence, the antimicrobial components contained within essential oils, together with their high efficiency in penetrating microbial cell membrane, are believed to exert their antimicrobial activity against the plant pathogenic and health-related microorganisms tested in this study (Burt, 2004; Trombetta *et al*., 2005; Edris, 2007).

Development of plant essential oils as natural antimicrobial agents has enormous potential for profit. The volatile compounds, which are the main components of antimicrobial activity of essential oil, could be developed for agricultural applications. For example, natural products are usually less toxic than synthetic chemicals. In addition, natural products are easily bio-degraded and the harmful effects on the environment and public health are, therefore, lower (Kurita *et al*., 1981, Kalemba and Kunicka, 2003). This speculation is confirmed by the fact that citral oil is used as a plant protectant and its active components are normally used in the plant cultivation. In addition, essential oil from *Cymbopogon citrates* has been traditionally used in Asia for a long time.

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