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Antifungal Activity Against *Colletotrichum* spp. of Curcuminoids Isolated from *Curcuma longa* L. Rhizomes

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Abstract Methanol extract of the rhizomes of turmeric, *Curcuma longa* L., effectively controlled the development of red pepper anthracnose caused by *Colletotrichum coccodes*. In addition three antifungal substances were identified from the methanol extract of *C. longa* rhizomes as curcumin, demethoxycurcumin, and bisdemethoxycurcumin using mass and ^{1}H-NMR spectral analyses. The curcuminoids in a range 0.4–100 μg/ml effectively inhibited the mycelial growth of three red pepper anthracnose pathogens, *C. coccodes*, *C. gloeosporioides*, and *C. acutatum*. The three curcuminoids inhibited mycelial growth of *C. coccodes* and *C. gloeosporioides* to an extent similar to the synthetic fungicide dithianon did, but the synthetic agent was a little more effective against *C. acutatum*. The curcuminoids also effectively inhibited spore germination of *C. coccodes*, and bisdemethoxycurcumin was the most active. Among the three curcuminoids, only demethoxycurcumin was effective in a greenhouse test in suppressing red pepper anthracnose caused by *C. coccodes*.

Key words: Antifungal activity, *Colletotrichum* species, *Curcuma longa*, curcuminoids, red pepper anthracnose

Red pepper, *Capsicum* spp., is one of the most important horticultural crops in Korea. Several species of plant pathogenic fungi in the genus *Colletotrichum* cause anthracnose in red peppers and many other fruits and vegetables [9, 16, 23]. The infected areas of the plant first appear bleached, but later become brown. In red peppers, as well as in other fruits and vegetables, anthracnose results in serious yield loss and occasionally in damage to the stems and foliage. Circular or angular sunken lesions develop on immature fruits of all sizes, and often multiple lesions are formed on individual fruits. When the disease is severe, lesions may coalesce. In older lesions, black structures called acervuli may form. The pathogen develops spores quickly and profusely, and can spread rapidly throughout a red pepper crop, resulting in up to 100% yield loss. Lesions may also appear on stems and leaves as irregularly shaped brown spots with dark brown edges [1].

Many species of *Colletotrichum* infect more than one host and, to confound identification, more than one *Colletotrichum* sp. may be present on one host. At least three species of *Colletotrichum* such as *C. gloeosporioides*, *C. acutatum*, and *C. coccodes*, cause the disease in red pepper crops in Korea [9, 16, 23]. Red pepper growers generally apply synthetic fungicides as a preventive measure to control anthracnose caused by *Colletotrichum* spp. The indiscriminate and overuse of a wide range of fungicides is prevalent, and many problems have emerged as a result: The increasing incidence or resistance to fungicides by *Colletotrichum* spp. and the loss of available agents for disease control are two main factors that drive the need to identify new plant protectants. In addition, the desire for safer pesticides with less environmental risk and mammalian toxicity is a major concern. The alternatives that have been pursued for the control of red pepper anthracnose include biological controls using antagonistic microorganisms and the use of safer chemicals such as food preservatives and plant-derived products [2, 4, 5, 7, 12, 17, 18, 21]. Meazza *et al.* [21] found that quinones occurring in higher plants have a good to moderate antifungal activity against *Colletotrichum* spp., and Bautista-Banos *et al.* [4] reported that the combination of 2.5% chitosan with aqueous extracts of custard apple leaves, papaya leaves, and papaya seeds had a fungistatic effect against the mycelial growth of *C. gloeosporioides*, a fungal agent of papaya anthracnose. The crude extract

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from rhizomes, leaves, and creeping branches of sweetflag (*Acorus calamus* L.), palmarosa (*Cymbopogon martini*) oil, *Ocimum sanctum* leaf extract, and neem (*Azadirachia indica*) oil reduce red pepper anthracnose caused by *Colletotrichum* spp. [11].

In a search for plant extracts with potent *in vivo* antifungal activity against red pepper anthracnose caused by *C. coccodes*, we found that treatment with an extract of *Curcuma longa* L. rhizomes reduced the development of red pepper anthracnose. In this study, three antifungal pigments were isolated from the extract, and their chemical structures were identified. We also evaluated both *in vitro* and *in vivo* antifungal activities of the purified substances against *Colletotrichum* spp.

MATERIALS AND METHODS

Isolation of Antifungal Substances

Dried and finely powdered rhizomes (800 g) of *C. longa* were purchased from Fine Korea Co. (Seoul, Korea). The powder was extracted twice with methanol (6 l) at room temperature for 1 day and then filtered. The two filtrates were combined and then concentrated to dryness *in vacuo* at 35°C using a rotary evaporator to yield about 61 g. The extract was redissolved in 3 l of 80% methanol and then partitioned with equal volumes of *n*-hexane. Both layers were concentrated to dryness. The aqueous layer was redissolved in 3 l of distilled water and then extracted twice with equal volumes of ethyl acetate and butanol. The organic and aqueous layers were concentrated to dryness under reduced pressure. The four fractions were bioassayed for *in vivo* antifungal activity against *C. coccodes* on red pepper plants: The ethyl acetate extract (35 g) was the most active, followed by the butanol extract (about 3.0 g; Table 2). A portion (10 g) of ethyl acetate extract was applied to a silica gel column (5 cm i.d.×60 cm) containing 500 g of Kiesel gel 60 (70–230 mesh; E. Merck, Darmstadt, Germany). The column was eluted with *n*-hexane-ethyl acetate (2:1, v/v). The fractions were monitored by TLC (Kiesel gel GF 254, 0.25-mm film thickness; Merck) and reduced into a fraction, F1 (4.5 g), which showed an *in vivo* antifungal activity against red pepper anthracnose caused by *C. coccodes*. Fraction F1 was subjected to silica gel column chromatography [200 g, Kiesel gel 60 (230–400 mesh), 3.6 cm i.d.×60 cm, chloroform-methanol (99:1, 98:2, 97:3, 95:5, v/v)]. The fractions were monitored by TLC and reduced into three fractions, F11, F12, and F13. These three fractions contained yellow pigments as a major compound, which were thought to be structurally related to each other. F11 (2.3 g) was subjected to Sephadex LH-20 column chromatography (70 g, 3.6 cm×60 cm) using methylene chloride:*n*-hexane:methanol (5:5:1, v/v/v) to yield 750 mg of compound **1**, fraction F12 (504 mg) was also chromatographed over Sephadex LH-20 [35 g, 3.2 cm i.d.×45 cm, methylene chloride:*n*-hexane: methanol (5:5:1, v/v/v)] to yield 450 mg of compound **2**, and fraction F13 (610 mg) was further chromatographed on a Sephadex LH-20 column [35 g, 3.2 cm i.d.×45 cm] with 100% methanol as the eluent to yield 798 mg of compound **3**.

Spectral Measurements

Structural determination of the three substances was made by spectroscopic analyses. Mass spectra were recorded on a mass spectrometer (JEOL JMS-DX303; JEOL Ltd., Tokyo, Japan), and ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Bruker AMX-500 (500 MHz for ^{1}H) NMR spectrometer (Bruker Analytische Messtechnik GmbH, Rheinstetten, Germany). Spectra were referenced to tetramethylsilane (TMS).

In Vivo Antifungal Activity

Both the purified substances and the fractions obtained during the purification of active substances from *C. longa* rhizomes were tested for *in vivo* antifungal activity against *C. coccodes* on red pepper plants. Thus, red pepper plants (*Capsicum annuum* L., cv. Hyangchon) were grown in vinyl pots (4.5 cm in diameter) in a greenhouse at about 25(±5)°C for 4 weeks. At the sixth leaf stage, the potted plant seedlings were sprayed until run-off with Tween 20 solution (250 μg/ml), which contained samples of either the fractions or the purified substances. Control plants were treated with Tween 20 solution containing 10% acetone. In addition, dithianon (10 and 50 μg/ml) was applied as a positive control. After 24 h, the treated plant seedlings were inoculated with spores (5×10^5 spores/ml) of *C. coccodes*. After incubation for 2 days at 25(±2)°C and 100% RH, the inoculated plants were kept in a growth chamber for 2 days at 25(±2)°C and 70–80% RH with 8 h of daylight per day. Disease severity was assessed 4 days after inoculation. The pots were arranged as a randomized complete block with three replicates per treatment. Experiments were conducted twice in a growth chamber, and the six estimates for each treatment were converted into percentage fungal control as compared with the control plants.

Mycelial Growth Inhibition Assay

The three substances dissolved in dimethyl sulfoxide (DMSO) were tested for an *in vitro* antifungal activity against *C. gloeosporioides*, *C. acutatum*, and *C. coccodes*, which are fungal agents that cause anthracnose on red pepper plants. Potato dextrose agar (PDA) was used as a basal medium for the three fungi. The media incorporating the antifungal substances at concentrations of 100, 33.3, 11.1, 3.7, 1.2, and 0.4 μg/ml were inoculated at the center with agar discs of the test fungi (5 mm in diameter). Five replicate plates of each concentration of fungus were incubated at 25°C for all test fungi. Control plates containing

media mixed with DMSO (10 μl/ml) were included. After incubation for 2–5 days, the radial growth was measured. This experiment was conducted twice, and the activity is expressed as inhibition activity (%) of radial growth.

Spore Germination Inhibition Assay

The effects of the three substances on spore germination of *C. coccodes* were investigated. A spore suspension of *C. coccodes* in distilled water was prepared from 14-day-old cultures grown on oatmeal agar medium. Three dilutions of each of the three substances in DMSO were made to obtain 20, 4, and 0.8 mg/ml. Test compound solution (10 μl) was added to the spore suspension (1 μl; containing 10^5 spores), and samples (50 μl) were loaded onto three hole-slide glasses, which were then placed on moistened papers in a sealed case. The spores of *C. coccodes* were incubated for 7 h at 25°C. Approximately 100 spores from each of three replicates were examined under a light microscope to determine the percentage of germinated spores. This experiment was repeated once, and the results are presented as mean values with standard deviation of the mean.

Results and Discussion

Structure Determination

As shown in Table 1, the methanol extract of *C. longa* rhizomes showed *in vivo* antifungal activity against *C. coccodes* on red pepper plants, which was not detected in the water extract, indicating that the antifungal substances in *C. longa* rhizomes may not be polar, but nonpolar. Further solvent fractionation showed the strongest antifungal activity against *C. coccodes* in the ethyl acetate fraction with 92% control values at 2,000 μg/ml, followed by the butanol fraction with 50% control values at 2,000 μg/ml (Table 2). However, little antifungal activity was shown in the other fractions, such as the *n*-hexane and aqueous fractions.

Table 1. *In vivo* antifungal activities of methanol and water extracts of *Curcuma longa* L. rhizomes against *Colletotrichum coccodes* on red pepper plants[a].

Sample	Conc. (μg/ml)	Control value (%)[b]
Methanol extract	3,000	88±5.0
	1,000	50±4.3
	333.3	25±13
Water extract	3,000	25±10
	1,000	5±11
	333.3	0
Dithianon	50	98±0.8
	10	58±8.7

[a]Seedlings were inoculated with spores of *Colletotrichum coccodes* 1 day after solutions of the plant extracts were sprayed onto the leaves.
[b]Each value represents the mean±standard deviation of two runs with three replicates each.

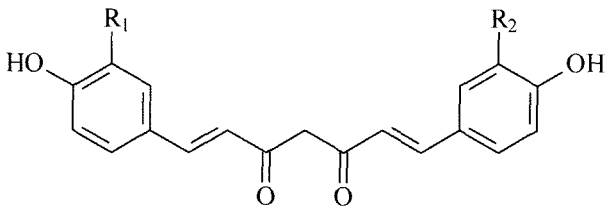
Table 2. *In vivo* antifungal activities of the four fractions obtained by solvent partitioning of a methanol extract of *Curcuma longa* L. rhizomes against *Colletotrichum coccodes* on red pepper plants[a].

Fraction	Conc. (μg/ml)	Control value (%)[b]
n-Hexane layer	2,000	5±4.6
Ethyl acetate layer	2,000	92±2.4
Butanol layer	2,000	50±7.3
Aqueous layer	2,000	0
Dithianon	50	94±1.2
	10	55±9.3

[a]Seedlings were inoculated with spores of *Colletotrichum coccodes* 1 day after solutions of the plant extracts were sprayed onto the leaves.
[b]Each value represents the mean±standard deviation of two runs with three replicates each.

Three antifungal substances, compounds **1** (750 mg), **2** (450 mg), and **3** (798 mg), were purified from the ethyl acetate fraction by repeated silica gel and Sephadex LH-20 column chromatographies. The structures of the compounds were elucidated as curcumin (**1**), demethoxycurcumin (**2**), and bisdemethoxycurcumin (**3**) by electron impact mass and ^{1}H-NMR spectra (Fig. 1) [15, 24, 26].

Oleoresin, a rhizome extract from turmeric (*C. longa* L.), consists of a volatile oil fraction and a heavy fraction of a yellowish-brown color. Mathi [19] reported that the amounts of oleoresin in the rhizomes range from 3% to 6% of the total dry weight. It is composed predominantly of sesquiterpenic ketones [13] and 2–8% of curcuminoids such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin [22]. Curcuminoids possess various biological properties in various animal and cell cultures, including antimutagenic activity. Kim *et al.* [14] found that curcumin, demethoxycurcumin, and bisdemethoxycurcumin isolated from *C. longa* protected PC12 rat pheochromocytoma



1: R_1=R_2=OCH_3
2: R_1=H, R_2=OCH_3
3: R_1=R_2=H

Fig. 1. Chemical structures of curcuminoids isolated from *Curcuma longa* rhizomes (**1**, curcumin; **2**, demethoxycurcumin; **3**, bisdemethoxycurcumin).

and normal human umbilical vein endothelial cells from βA (1-42) insult, and the antioxidant activity of these compounds, determined by DPPH radical trapping experimentation, was superior to that of a-tocopherol. In addition, the antileishmanial activity of curcuminoids has also been reported [8]. Huang [10] demonstrated that curcumin inhibited the epidermal metabolism of arachidonic acid via lipoxigenase and cycloxigenase. Curcumin is an HIV-1 integrase inhibitor, a natural antioxidant with anticancer activity, and a satisfactory anti-inflammatory agent [20]. Curcuminoids also inhibit herbivory by some insects such as *Schistocera gregaria* Forsk and *Dysdercus koenigiin* Walk [6].

As for the antibacterial and antifungal activities of curcuminoids, Ramprasad and Sirsi [25] reported that curcumins show bacteriostatic activity against *Staphylococcus*, whereas Aspisariyakul *et al.* [3] found no *in vitro* antifungal activity of curcumin against all tested isolates of dermatophytes, pathogenic molds, and yeasts. However, Kim *et al.* [15] reported that curcumin showed *in vivo* antifungal activity against *Phytophthora infestans, Puccinia recondita*, and *Rhizoctonia solani*. In this study, we demonstrated that the curcuminoids exhibited both *in vitro* and *in vivo* antifungal activity against *C. coccodes*.

In Vitro Antifungal Activity

The three curcuminoids isolated from *C. longa* rhizomes inhibited the mycelial growth of three red pepper anthracnose pathogens: *C. coccodes*, *C. acutatum*, and *C. gloeosporioides*. The curcuminoids showed similar mycelial growth inhibitory activity against each of the three *Colletotrichum* species (Fig. 2). *C. gloeosporioides* was relatively less sensitive to the chemicals than *C. coccodes* and *C. acutatum*. It is noteworthy that the activity of curcuminoids against mycelial growth of the two *C. coccodes* and *C. gloeosporioides* was comparable to that of the synthetic fungicide dithianon. The synthetic agent was a little more effective against *C. acutatum* than the curcuminoids.

As mentioned earlier, Ramprasad and Sirsi [23] found *in vitro* antibacterial activity of curcumin, especially against *Staphylococcus*, whereas Apisariyakul *et al.* [3] reported that curcumin rhizomes did not inhibit the growth of dermatophytes such as *Microsporum gypseum*, *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, and *T. rubrum* and some pathogenic molds such as *Exophiala jeanselmei*, *Sporothrix schenckii*, *Fonsecaea pedrosoi*, and *Scedosporium apiospermum*. In our study, curcuminoids were shown to have a good to moderate antifungal activity against the three *Colletotrichum* species tested. To our knowledge, this is the first report on the mycelial growth inhibitory activity of curcuminoids against plant-pathogenic *Collectotrichum* species.

Curcuminoids also inhibited spore germination of *C. coccodes*. In contrast to its inhibition of mycelial growth of the three *Colletotrichum* species, bisdemethoxycurcumin showed the most potent inhibitory activity against spore germination (Fig. 3): Its inhibitory activity was 87% at 0.8 μg/ml, 99% at 4 μg/ml, and 100% at 20 μg/ml. Curcumin, demethoxycurcumin, and dithianon inhibited the spore germination of *C. coccodes* to a similar extent. Although the three compounds at 0.8 μg/ml concentration showed only weak activity of less than 18% inhibition, they almost completely inhibited spore germination of *C. coccodes* at doses ≥4 μg/ml.

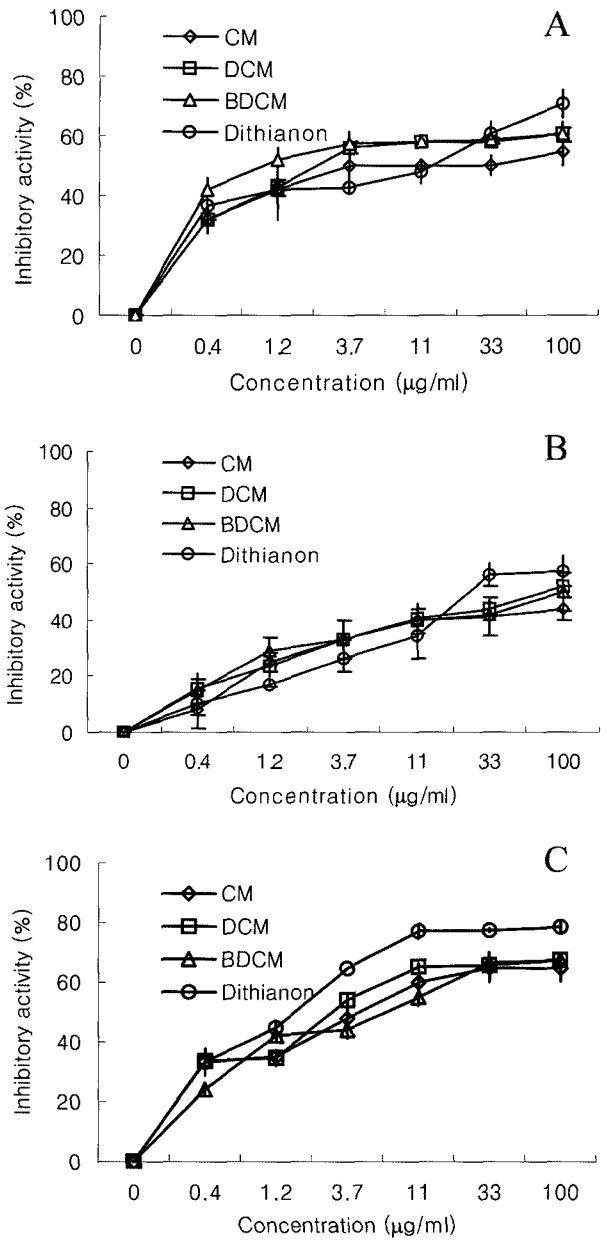


Fig. 2. Inhibitory activities of curcuminoids and dithianon against mycelial growth of *Colletotrichum coccodes* (A), *C. gloeosporioides* (B), and *C. acutatum* (C) (CM, curcumin; DCM, demethoxycurcumin; BDCM, bisdemethoxycurcumin).

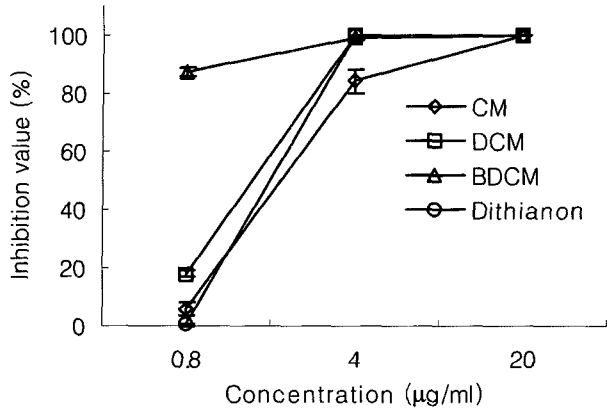


Fig. 3. Inhibitory activities of curcuminoids and dithianon against spore germination of *Colletotrichum coccodes* (CM, curcumin: DCM, bisdemethoxycurcumin).

In Vivo Antifungal Activity

The results of *in vivo* antifungal activity of the curcuminoids against *C. coccodes* causing red pepper anthracnose are shown in Table 3. In contrast to the *in vitro* antifungal activities, only demethoxycurcumin showed potent *in vivo* antifungal activity: This compound almost inhibited the development of red pepper anthracnose at concentrations of 500 and 1,000 μg/ml, whereas curcumin and bisdemethoxycurcumin did not exhibit any *in vivo* antifungal activity against *C. coccodes* on red pepper plants. Whether such differences between *in vivo* and *in vitro* antifungal activities of demethoxycurcumin and the other two curcuminoids are influenced by the lipophilicity of the compounds or their persistence on the surfaces of red pepper plants is not clear. Further study is needed to elucidate the mechanism. Nevertheless, these results suggest that the *in vivo* antifungal activities of crude extracts of *C. longa* rhizomes are due to demethoxycurcumin, even if the other compounds cannot completely be ruled out. On the other hand, the synthetic fungicide dithianon is approximately ten times more active than demethoxycurcumin.

Table 3. *In vivo* antifungal activities of three curcuminoids isolated from *Curcuma longa* L. rhizome against *Colletotrichum coccodes* on red pepper plants[a].

Chemical	Conc. (μg/ml)	Control value (%)[b]
Curcumin	1,000	0
	500	0
	250	0
Demethoxycurcumin	1,000	97±2.0
	500	92±2.5
	250	4±4.3
Bisdemethoxycurcumin	1,000	0
	500	0
	250	0
Dithianon	50	99±0.5
	10	53±11

[a]Seedlings were inoculated with spores of *Colletotrichum coccodes* 1 day after the chemical solutions were sprayed onto the leaves.
[b]Each value represents the mean±standard deviation of two runs with three replicates each.

The three curcuminoids, including demethoxycurcumin, showed no phytotoxicity against red pepper plants even at 2,000 μg/ml concentration. Rhizomes of *C. longa* (turmeric) are widely used as a seasoning, a coloring ingredient in curry powder, and a medicine. This indicates no biotoxicity of the three compounds to humans. Demethoxycurcumin from rhizomes of *C. longa* has not only a potent *in vitro* antifungal activity against three *Colletotrichum* species, but also an *in vivo* control efficacy against red pepper anthracnose caused by *C. coccodes*. The results in this study suggest that turmeric powder, turmeric extracts, or demethoxycurcumin could be used as an agricultural fungicide for the control of anthracnose on various crops. In addition, demethoxycurcumin could be an important lead compound for chemical synthesis.

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References

1. Agrios, G. N. 1978. *Plant Pathology*, pp. 298–301. Academic Press, New York, U.S.A.
2. Almada-Ruiz, E., M. A. Martinez-Tellez, M. Hernandez-Alamos, S. Vallejo, E. Primo-Yufera, and I. Vargas-Arispuro. 2003. Fungicidal potential of methoxylated flavones from citrus for *in vitro* control of *Colletotrichum gloeosporioides*, causal agent of anthracnose disease in tropical fruits. *Pest Manag. Sci.* **59:** 1245–1249.
3. Apisariyakul, A., N. Vanittanakom, and D. Buddhasukh. 1995. Antifungal activity of turmeric oil extracted from *Curcuma longa* (Zingiberaceae). *J. Ethnopharmacol.* **49:** 163–169.
4. Bautista-Banos, S., M. Hernandez-Lopez, E. Bosquez-Molina, and C. L. Wilson. 2003. Effects of chitosan and plant extracts on growth of *Colletotrichum gloeosporioides*, anthracnose levels and quality of papaya fruit. *Crop Prot.* **22:** 1087–1092.
5. Cho, S.-J., S. K. Lee, B. J. Cha, Y. H. Kim, and K.-S. Shin. 2003. Detection and characterization of the *Gloeosporium gloeosporioides* growth inhibitory compound iturin A from *Bacillus subtilis* strain KS03. *FEMS Microbiol. Lett.* **223:** 47–51.
6. Chowdhury, H., S. Walia, and B. S. Saxena. 2000. Isolation, characterization and insect growth inhibitory activity of major

turmeric constituents and their derivatives against *Schistocerca gregaria* (Forsk) and *Dysdercus koenigii* (Walk). *Pest Manag. Sci.* **56:** 1086–1092.
7. Gamagae, S. U., D. Sivakumar, R. S. Wilson Wijeratnam, and R. L. C. Wijesundera. 2003. Use of sodium bicarbonate and *Candida oleophila* to control anthracnose in papaya during storage. *Crop Prot.* **22:** 775–779.
8. Gomes, D. C., L. V. Algegrio, M. E. De Lima, L. L. Leon, and C. A. Araujo. 2002. Synthetic derivatives of curcumin and their activity against *Leishmania amazonensis*. *Arzneimittelforschung* **52:** 120–124.
9. Hong, J. K. and B. K. Hwang. 1998. Influence of inoculum density, wetness duration, plant age, inoculation method, and cultivar resistance on infection of red pepper plants by *Colletotrichum coccodes*. *Plant Dis.* **82:** 1079–1083.
10. Huang, M. T. 1998. Inhibitory effect of curcumin, chlorogenic, caffeic acid and ferulic acid on tumor promotion in mouse skin by 12-O-tetradecanoyl phorbol-13-acetate. *Cancer Res.* **48:** 5941–5946.
11. Jeyalakshmi, C. and K. Seetharaman. 1998. Biological control of fruit rot and die-back of chili with plant products and antagonistic microorganisms. *Plant Dis. Res.* **13:** 46–48.
12. Kang, S. W., S. I. Hong, and S. W. Kim. 2005. Identification of *Aspergillus* strain with antifungal activity against *Phytophthora* species. *J. Microbiol. Biotechnol.* **15:** 227–233.
13. Kelkhar, N. C. and B. S. Rao. 1934. Studies in Indian essential oils from rhizomes of *Curcuma longa* Linn. *J. Indian Inst. Sci.* **17A:** 7–24.
14. Kim, D. S. H. L., S.-Y. Park, and J. Y. Kim. 2001. Curcuminoids from *Curcuma longa* L. (Zingiberaceae) that protect PC12 rat pheochromocytoma and normal human umbilical vein endothelial cells from βA(1-42) insult. *Neurosci. Lett.* **303:** 57–61.
15. Kim, M.-K., G. J. Choi, and H.-S. Lee. 2003. Fungicidal property of *Curcuma longa* L. rhizome-derived curcumin against phytopathogenic fungi in a greenhouse. *J. Agric. Food Chem.* **51:** 1578–1581.
16. Kim, W. G., E. K. Cho, and E. J. Lee. 1986. Two strains of *Colletotrichum gloeosporioides* Penz. causing anthracnose on red pepper fruits. *Kor. J. Plant Pathol.* **2:** 107–113.
17. Kuk, J. H., S. J. Ma, J. H. Moon, K. Y. Kim, S. H. Choi, and K. H. Park. 2002. Antibacterial and antifungal activities of a naphthoquinone derivative isolated from the fruits of *Catalpa ovata* G. D_{ON}. *J. Microbiol. Biotechnol.* **12:** 858–863.
18. Lee, C.-H. and H.-S. Lee. 2005. Antifungal property of dihydroxyanthraquinones against phytopathogenic fungi. *J. Microbiol. Biotechnol.* **15:** 442–446.
19. Mathi, C. K. 1979. The pattern of rhizome yield and their accumulation of commercially important chemical constituents in turmeric during growth and development. *Qual. Plant Plant Foods Hum. Nutr.* **36:** 215–219.
20. Mazumber, A. 1995. Inhibition of human immunodeficiency virus type-1 integrase by curcumin. *Biochem. Pharmacol.* **49:** 1165–1170.
21. Meazza, G., F. E. Dayan, and D. E. Wedge. 2003. Activity of quinones on *Colletotrichum* species. *J. Agric. Food Chem.* **51:** 3824–3828.
22. Montaldo, A. 1972. *Cultivo de raices y tubérculos tropicales*, Instituto Interamericano de Ciencias Agricolas de la OEA, Lima, Peru, p. 257.
23. Park, K. S. and C. H. Kim. 1992. Identification, distribution, and etiological characteristics of anthracnose fungi of red red pepper in Korea. *Kor. J. Plant Pathol.* **8:** 61–69.
24. Pedersen, U., P. B. Rasmussen, and S.-O. Lawesson. 1985. Synthesis of naturally occurring curcuminoids and related compounds. *Liebigs Ann. Chem.* 1557–1569.
25. Ramprasad, C. and M. Sirsi. 1956. Studies on Indian medicinal plants: *Curcuma longa* Linn - *in vitro* antibacterial activity of curcumin and the essential oil. *J. Sci. Res. Inst.* **15:** 239–242.
26. Syu, W.-J., C.-C. Shen, M.-J. Don, J.-C. Ou, G.-H. Lee, and C.-M. Sun. 1998. Cytotoxicity of curcuminoids and some novel compounds from *Curcuma zedoaria*. *J. Nat. Prod.* **61:** 1531–1534.