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Insecticidal Activities of *ar*-Turmerone Identified in *Curcuma longa* Rhizome against *Nilaparvata lugens* (Homoptera: Delphacidae) and *Plutella xylostella* (Lepidoptera: Yponomeutidae)

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Abstract The insecticidal activity of materials derived from the rhizome of turmeric, *Curcuma longa*, against four agricultural and four stored-product insects was examined using direct contact application method. The biologically active constituent of the *Curcuma* rhizome was characterized as the sesquiterpene ketone *ar*-turmerone by spectroscopic analysis. Potencies varied according to insect species and dose. In a test with *Nilaparvata lugens* female adults, *ar*-turmerone caused 100 and 64% mortality at 1,000 and 500 ppm, respectively. Against *Plutella xylostella* larvae, the compound gave 100 and 82% mortality at 1,000 and 500 ppm, respectively. Against *Myzus persicae* female adults and *Spodoptera litura* larvae, *ar*-turmerone at 2,000 ppm was effective but weak insecticidal activity was observed at 1,000 ppm. At a dose of 2.1 mg/cm^2, *ar*-turmerone was almost ineffective (<10% mortality) against adults of *Sitophilus oryzae*, *Callosobruchus chinensis* and *Lasioderma serricorne* as well as larvae of *Plodia interpunctella*. The naturally occurring *Curcuma* rhizome-derived *ar*-turmerone merits further study as potential insect-control agents or as lead compounds.

Key words natural insecticide, *Nilaparvata lugens*, *Plutella xylostella*, *Curcuma longa*, sesquiterpene ketone, *ar*-turmerone

Introduction

Control of insect pests have been through the development of synthetic insecticides. Although effective, their repeated use for decades has disrupted natural biological control systems and led to resurgence of some insect species, sometimes resulted in the development of resistance, had undesirable effects on non-target organisms, and fostered environmental and human health concerns (Georghiou and Saito, 1983; Hayes and Laws, 1991). These problems have highlighted the need for the development of new types of selective insect-control alternatives.

Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals and many of them are largely free from adverse effects. Much effort has, therefore, been focused on plant-derived materials as potential sources of commercial insect-control agents or as lead compounds (Jacobson and Crosby, 1971; Arnason *et al.*, 1989a; Isman, 1995; Hedin *et al.*, 1997). In East Asia, the rhizome from *Curcuma longa* L., belonging to the family Zingiberaceae, has long been considered to have natural medicinal properties such as an analgesic in the treatment of menstrual disorders, rheumatism, and traumatic diseases because it contains a number of monoterpenoids, sesquiterpenoids, and curcuminoids (Tang and Eisenbrand, 1992). Little work has been done from the basis to manage insect pests, although it has been noted that extractives of turmeric have insecticidal (Chander *et al.*, 1991; Chander *et al.*, 1992) repellent (Su *et al.*, 1982; Jilani and Su, 1983; Jilani *et al.*, 1988; Jilani and Saxena, 1990), growth-inhibiting (Jilani *et al.*, 1988) and antifeeding activities (Jilani and Saxena, 1990) against some stored-product insects as well as growth-inhibiting and insecticidal activity against nymphs of *Schistocerca gregaria* Forsk. and *Dysdercus koenigii*Walk (Chowdhury *et al.*, 2000). The insect repellent and growth-inhibiting constituents in turmeric are turmerone and *ar*-turmerone (Su *et al.*, 1982) and curcuminoids (Chowdhury *et al.*, 2000), respectively. Roh (2000) reported that methanol extract of the *Curcuma* rhizome has insecticidal activity against *Nilaparvata lugens* Stål female adults and *Plutella xylostella* L. larvae.

In the laboratory study described herein, we have examined the methanol extract of the rhizome from *C. longa* for insecticidal constituents against four agricultural

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insects and four stored-product insects.

Materials and Methods

Insects

The susceptible strains of *N. lugens*, *Myzus persicae* Sulzer, and *P. xylostella* were reared on rice plant (*Oryza sativa* L.) seedlings (7-10 days after germination), tobacco plant (*Nicotiana tabacum* L.), and chinese radish (*Raphanus sativus* L.) seedlings (5-6 days after germination) in acrylic cages, respectively. *Spodoptera litura* L. was reared on artificial diet (Im *et al.*, 1988) in plastic containers. They have been maintained in the laboratory without exposure to any insecticide at 25±1℃, 50-60% RH, and a photoregime of 16:8 (L:D) h.

Laboratory-reared strains of *Sitophilus oryzae* L., *Callosobruchus chinensis* Lucas, and *Plodia interpunctella* Hübner were reared on rice grain, adzuki bean (*Phaseolus angularis* L.), and peanuts (*Arachis hypogaea* L.), respectively, in plastic containers at 28 ±1℃, 50-60% RH, and a photoregime of 16:8 (L:D) h. A susceptible strain of *Lasioderma serricorne* F. was reared in 0.5-liter masonry rearing jars containing 150 g of sterilized diet (wheatfeed/yeast, 95:5, w/w) at 28 ± 1℃, 70-75% RH, and a photoregime of 16:8 (L:D) h.

Isolation and identification

The dried rhizome (2 kg) from *C. longa* was purchased from Boeun medicinal herb shop, Kyungdong Market, Seoul, Korea. It was finely powdered, extracted twice with methanol (3 liter) at room temperature for 2 days and filtered. The combined filtrate was concentrated under vacuum at 35°C to yield about 10% (based on the weight of the dried rhizome). The extract (20 g) was sequentially partitioned into hexane 12.6 g (2× 800 ml), chloroform 3.8 g (2×800 ml), ethyl acetate 0.8 g (2×800 ml), and water 2.8 g (800 ml) for subsequent bioassay. The organic solvent portions were concentrated to dryness by rotary evaporation at 35°C, while the water portion was freeze-dried.

Because of its good insecticidal activity against *N. lugens* female adults and *P. xylostella* larvae, the hexane fraction (10 g) was chromatographed on a silica gel column (Merck 70-230 mesh, 500 g, 6 cm i.d.×67 cm), and successively eluted with chloroform/methanol (50:1, v/v). Column fractions were analyzed by TLC (silica gel G), and fractions with a similar TLC pattern were pooled. The active fraction (1.6 g) was successively rechromatographed on a silica gel column, using hexane/ethyl acetate (30:1, v/v). A prep HPLC (Spectra System P2000, Thermo Separation Products) was used for further separation of the constituents. The column was a μ Porasil Silica (19 mm i.d.×300 mm, Waters) using hexane/ethyl acetate (200:1, v/v) at a flow rate of 4 ml/min and detected at 242 nm. Finally, an insecticidal principle (86 mg) was isolated.

Structural determination of the active isolate was made by spectroscopic analysis. ^{1}H- and ^{13}C-NMR spectra were recorded in deuterochloroform with a BRUKER AM-500 spectrometer at 400 and 100 MHz, respectively. Mass spectra were obtained on a JEOL GSX 400 spectrometer.

Bioassay

Spray method was used for the bioassay of *N. lugens*. Ten female adults (3- to 5-day-old) were transferred onto a test tube (3×20 cm) containing five rice plant seedlings (7-10 days after germination) wrapped with cotton and water (20 ml). Each *Curcuma* rhizome-derived fraction and isolate in 4 ml methanol was suspended in distilled water with Triton X-100 (Junsei, Osaka, Japan) added at the rate of 0.1 ml/liter. Controls received methanol-Triton X-100 solution. Test material solutions were applied at a rate of 0.1 ml per test tube by a glass spray unit connected to a forced air supply (Pacific Chemical, Seoul), as previously described (Ahn *et al.*, 1995).

The toxicity of the *Curcuma* rhizome-derived fractions and isolate to the aphid and two lepidopteran larvae used was examined by leaf dipping assay. Cabbage (*Brassica oleracea* L, 25-day-old) leaves for 3rd instar larvae of *P. xylostella* and *S. litura*, and tobacco leaves for *M. persicae* female adults from each plant species grown in glasshouse were collected, and disks (5.5 cm diameter) were punctured from each leaf. Leaf disks were dipped in each test solution described above for 30 s. Controls received methanol-Triton X-100 solution. After drying in a fume hood for 30 min, 10 individuals of *P. xylostella*, *S. litura*, and *M. persicae* were placed separately onto the treated and the control leaf disks in petri dishes (6×1.5 cm).

Filter paper diffusion method was used for the stored-product insects as previously described (Kim and Ahn, 2001). A dose (2.1 mg/cm^2) of each *Curcuma* rhizome-derived fraction and isolate dissolved in 100 μl methanol was applied to filter papers (Whatman No. 2, 5.5 cm diameter). Controls received 100 μl methanol. After drying in a fume hood for 2 min, each paper was placed in the bottom of a petri dish (5.5×1.2 cm), and 10 adults of *S. oryzae* (7- to 10-day-old), *C. chinensis* (4- to 6-day-old) and *L. serricorne* (6- to 8-day-old), and 10 larvae of *P. interpunctella* (2nd instar) were placed in each petri

Table 1. Effectiveness of *C. longa* rhizome-derived materials on various insects using direct contact application

Material	Mortality, %			
	N. lugens[a]	*P. xylostella*[b]	*S. oryzae*[c]	*P. interpunctella*[c]
Methanol	100a	100a	0a	0a
Hexane	100a	100a	0a	0a
Chloroform	0b	0b	0a	0a
Ethyl aceate	0b	0b	0a	0a
Water	0b	0b	0a	0a

Means within a column followed by the same letter are not significantly different at $P = 0.05$ (Scheffe's test). Mortalities were transformed to arcsine square-root before ANOVA. Means (±SE) of untransformed data are reported.
[a]Spray method, 5000 ppm.
[b]Leaf dipping method, 5000 ppm.
[c]Filter paper diffusion method, 2.1 mg/cm^2.

Table 2. Insecticidal activity of *C. longa* rhizome-derived *ar*-turmerone against agricultural insects using direct contact application

Conc., ppm	Mortality (mean±SE), %			
	N. lugens[a]	*M. persicae*[b]	*P. xylostella*[b]	*S. litura*[b]
2000	100±0.0a	80±4.5a	100±0.0a	90±3.2a
1000	100±0.0a	50±4.5b	100±0.0a	30±4.5b
500	64±2.4b	10±3.2c	82±3.7b	0±0.0c
250	40±3.5c	0±0.0d	52±3.7c	0±0.0c
125	20±2.2d	0±0.0d	24±2.4d	0±0.0c

Means within a column followed by the same letter are not significantly different at $P = 0.05$ (Scheffe test). Mortalities were transformed to arcsine square-root before ANOVA. Means (±SE) of untransformed data are reported.
[a]Spray method.
[b]Leaf dipping method.

dish and covered with a lid.

Treated and control insects were held at the same conditions mentioned earlier. Mortalities were determined 48 h after treatment. Test insects were considered dead if appendages did not move when prodded with a camel-hair brush. All treatments were replicated ten times.

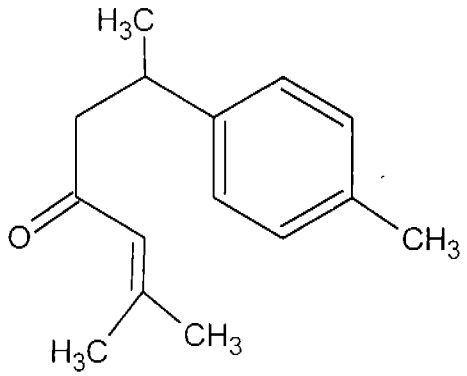

Fig. 1. Structure of the sesquiterpene ketone *ar*-turmerone, an insecticidal constituent of *Curcuma longa* rhizome.

Statistical analysis

The percentage of mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test at $P = 0.05$ (SAS Institute, 1990). Means (±SE) of untransformed data are reported.

Results and Discussion

Identification

When fractions obtained from the methanol extract of the *Curcuma* rhizome were laboratory assayed according to direct contact application, significant differences were observed in the toxicity to the insects used (Table 1). The hexane fraction at 5,000 ppm showed good insecticidal activity against *N. lugens* females and *P. xylostella* larvae but was almost ineffective against *S. oryzae* adults and *P. interpunctella* larvae at a dose of 2.1 mg/cm^2. There was no mortality in the untreated controls.

Purification of the biologically active constituents from the hexane fraction was done by silica gel chromatography and HPLC.

Bioassay-guided fractionation of the *Curcuma* rhizome extract afforded an active constituent identified by spectroscopic analysis, including MS and NMR. The active constituent was characterized as the sesquiterpene ketone *ar*-turmerone (Fig. 1). The compound was identified on the basis of the following evidence: $C_{15}H_{20}O$; EI-MS (70eV), *m/z* (% rel intensity) M^+ 216 (100, base peak), 201 (30), 132 (21), 119 (65), 117 (14), 83 (81), 55 (17); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.20 (d, 3H, J = 7Hz), 1.84 (d, 3H, J = 2 Hz), 2.03 (d, 3H, J = 2 Hz), 2.25 (s, 3H), 2.63 (d, 2H, J = 2 Hz), 3.22 (m, 1H), 6.10 (m, 1H), 7.07 (s, 4H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.8, 21.0, 22.6, 27.6, 36.9, 53.5, 125.1, 127.7, 130.0, 136.6, 144.6, 157.0, 202.5.

Insecticidal activity

The insecticidal activity of the *Curcuma* rhizome-derived *ar*-turmerone against four agricultural insects was examined by direct contact application (Table 2). Responses varied according to insect species and dose. In a test with *N. lugens* females, *ar*-turmerone caused 100 and 64% mortality at 1,000 and 500 ppm, respectively. Against *P. xylostella* larvae, this compound gave 100 and 82% mortality at 1,000 and 500 ppm, respectively. Against *M. persicae* females and *S. litura* larvae, *ar*-turmerone at 2,000 ppm was effective but weak insecticidal activity was obtained in application of 1,000 ppm. There was no mortality in the untreated controls.

Toxic effects of direct contact application of *ar*-turmerone on stored-product insects used were assessed. At a dose of 2.1 mg/cm^2, *ar*-turmerone was almost ineffective (<10% mortality) against adults of *S. oryzae*, *C. chinensis*, and *L. serricorne*, as well as larvae of *P. interpunctella* (Data are not shown).

It has been well recognized that plant extracts and phytochemicals could be developed into products suitable for integrated pest management because many of them are selective to pests, have no or little harmful effects on non-target organisms and the environment, act in many ways on various types of pest complex such as neem extracts, and may be applied to the plant in the same way as other agricultural chemicals (Arnason *et al.*, 1989a; Schmutterer, 1992; Hedin *et al.*, 1997). For example, derivatives of neem (*Azadirachta indica* A. Juss), belonging to the family Meliaceae, are found to have a variety of biological activities including insecticidal activity against nearly 200 species of insects without any adverse effects on most non-target organisms (Saxena, 1989; Lowery and Isman, 1995). Additionally, some plant-derived materials are found to be highly effective against insecticide-resistant insect pests (Arnason *et al.*, 1989b; Ahn *et al.*, 1997). Much concern has been focused, therefore, on the distribution, nature, and practical use of chemical substances having the insecticidal activity for insects in plants. In our present study, the *Curcuma* rhizome-derived materials exhibited insecticidal activity against *N. lugens* females and *P. xylostella* larvae but were almost ineffective against *S. oryzae* adults and *P. interpunctella* larvae. The insecticidal constituent of the *Curcuma* rhizome was identified as the sesquiterpene ketone *ar*-turmerone with species selective activity. This compound revealed good insecticidal activity against *N. lugens* females and *P. xylostella* larvae. These results indicate that *ar*-turmerone confirms its usefulness as a good insect-control agent or as a lead compound. Turmeric powder is found to have repellency to *Sitophilus granarius* L., *Rhizopertha dominica* F. and *T. castaneum* Herbst (Jilani and Su, 1983), insecticidal activity against *S. oryzae* (Chander *et al.*, 1991) and *T. castaneum* (Chander *et al.*, 1992), whereas turmeric oil has repellent and growth-inhibiting effects on *T. castaneum* (Jilani *et al.*, 1988), repellent and antifeeding effects on *R. dominica* (Jilani and Saxena, 1990), and insecticidal activity against nymphs of *S. gregaria* and *D. koenigii* (Chowdhury *et al.*, 2000). Turmeric rhizome extract has been reported to show insecticidal activity against *N. lugens* females and *P. xylostella* larvae (Roh 2000) and *Aedes aegyptii* L. larvae (Roth *et al.*, 1998) and growth-inhibiting activity against nymphs of *S. gregaria* and *D. koenigii* (Chowdhury *et al.*, 2000). *ar*-Turmerone, a constituent of *Curcuma* spp. such as *C. longa*, *C. amada*, *C. domestica*, and *C. xanthorrhiza* (Tang and Eisenbrand, 1992; Uehara *et al.*, 1992), possesses repellent activity against *T. castaneum* (Su *et al.*, 1982) and larvicidal activity against *A. aegyptii* (Roth *et al.*, 1998).

Results of this and earlier studies indicate that *C. longa* rhizome-derived materials could be useful products for developing new types of insect-control agents for managing field populations of *N. lugens* and *P. xylostella* on crops because this plant species is used for medicinal purposes and as spices in food.

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