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SHORT COMMUNICATION

Herbicidal effects of mixture of oxygenic terpenoids on paddy weeds

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ABSTRACT

To further explore the practical application of allelochemicals in rice production, the mixture of five oxygenic terpenoids [(–) carveol, (+) carvone, (–) menthone, (–) carvyl acetate, and (+) cedrol] identified in previous study, was evaluated in green house for their herbicidal activity on paddy weeds. This terpenoid mixture effectively reduced the growth (aboveground dry weight) of most paddy weeds by 90.0% in 2007 and 83.3% in 2009, respectively, over control. However, the growth of two tested hybrid rice cultivars (Shan-You 63 and Te-You-Hang II) was enhanced after the terpenoid mixture treatment. This study suggested that allelochemicals (including terpenoids), could be used as novel component of integrated weed management in rice production.

Key words: Allelopathy, herbicidal effect, paddy weeds, terpenoid mixture

INTRODUCTION

Since ancient times the weeds are major problem in cultivation of rice (*Oryza sativa* L.). These will become more problematic when labour (for hand-weeding) and water (for weed control in rice) will become scarce and expensive. Rice farmers are using the synthetic herbicides for weed control, but these cause problems of environment contamination, herbicide-resistant weeds and human health concerns. Many synthetic herbicides are hazardous due to crop injury, their long persistence, off-target toxicity and the pollutive, carcinogenic and mutagenic activities (5,6,7,11,12,14). This has prompted the scientists to search for alternative weed management strategies. Natural products have their unique merits: novelty and diversity of molecular structures, water-soluble and non-halogenated molecule composition, short half-life, potential unique mode of actions (4,6) and more environment-friendly than synthetic herbicides (3,12). Duke *et al*. (5,6) and Dayan *et al*. (4) summarized the advantages and weaknesses of natural products and have found the technologies for the developing natural products as herbicides. One of the

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common approaches is to identify the plant allelochemicals and related compounds from allelopathic species.

Potential use of terpenoids as herbicides is well known. Many monoterpenes containing an oxygen function severely inhibited germination of four annual weeds, i.e. *Lolium multiflorum* Lam., *Digiraria sanguinalis* L., *Amaranthus retroflexus* L., and *Abutilon theophrasti* Medik (21). Several benzyl ether derivatives of selected oxygenated monoterpenes and cinmethylin exhibited different selectivity and sensitivity than parent compounds (22). Four monoterpenes (citronellol, citronellal, cineole and linalool) reduced the germination and respiration in *Cassia occidentalis* (18). α-Pinene inhibited the radicle growth of *C. occidentalis* and caused oxidative damage in the root tissues through enhanced generation of reactive oxygen species (17). The phytotoxic monoterpenes (camphor, pulegone, and borneol) mixture showed synergistic action to inhibit the root elongation and germination of radish (*Raphanus sativus* L.), wheat (*Triticum vulgare* Vill.) and oats (*Avena sativa* L.) (1). The oxygenic monoterpenes have good solubility in water, which makes them potent biological inhibitors (24). The terpenoids are good candidates for natural herbicides, due to their bioactivity range between 10^{-5} and 10^{-7} mol L^{-1}. They were identified as having the highest potential among natural allelochemicals to be used as herbicides (12).

In our previous studies, some oxygenic terpenoids [carvone oxide, 5-isopropenyl-2-methyl-7-oxabicyclo [4,1,0] heptan-2-ol, menthol, 2-isopropyl-2,5-dimethyl-cyclohexanone, limonene dioxide, beta-necrodol, longiborneol, lanceol, cedrol, 3,7,11-trimethyl-1-dodecanol, 5,9,13-tirmethyl-4,8,12-tetradecatrienal and widdrol] were found in the diethyl ether extracts of root exudates from an allelopathic rice cultivar PI312777 by GC-MS determination (8,9). The phytotoxicity of five putative oxygenic cyclomonoterpenoids [(-)-carveol, (-)-carvyl acetate, (-)-menthone, (+)-carvone and (-)-carvone] with similar molecular formulae and molecular structures were assessed on barnyard grass [*Echinochloa crus-galli* (L.) Beauv.] in laboratory bioassays (9). Out of 36 combinations tested, mixture of 5 terpenoids [(–) carveol, (+) carvone, (–) menthone, (–) carvyl acetate, and (+) cedrol] was selected due to its optimal effects on germination and growth of *E. crus-galli* under laboratory conditions (10). The further greenhouse experiments showed that the mixtures inhibited the growth of *E crus-galli* but was not harmful to rice seedlings in a rice/weed mixed culture system (10). In this paper, we investigated that, (i) inhibitory rates of two hybrid rice cultivars on paddy weeds; (ii) inhibitory rates of two hybrid rice cultivars on paddy weeds with help of terpenoid mixture and (iii) the net inhibitory rates of terpenoid mixture on paddy weeds, to assess the actual herbicidal effects of terpenoid mixture in natural rice production.

MATERIALS AND METHODS

According to our previous results (10), the optimum combination of 5- terpenoids was prepared as under: 0.033 mM (–) carveol, 0.03 mM (+) carvone, 0.08 mM (–) menthone, 0.02 mM (–) carvyl acetate, and 0.001 mM (+) cedrol. All compounds were obtained from commercial sources (Sigma-Aldrich, China). Deionised water was used as the solvent for preparing solutions. Two experiments were done in green house as under.

Experiment I.

This study consisted of 3 treatments: TR1, TR2 and control. TR1 (30 rice seedlings planted in paddy soil without terpenoid mixture), TR2 (30 rice seedlings planted in paddy soil with terpenoid mixture) and control (CK) (paddy soil only, without rice plants and without the terpenoid mixture). Experiments were conducted in greenhouse of Agroecological Institute, Fujian Agriculture and Forestry University, Fuzhou, China, during August and September, 2007. The temperature ranged from 25 °C to 35 °C.

Thirty seedlings (2 leaf stage, hybrid rice Shan-You 63) were transplanted in a plastic pot (45 cm length × 35 cm width × 15 cm height) filled with 10 cm deep paddy field soil. The seedlings were spaced 5-6 cm apart from each other. The soil was sandy loam (pH: 5.7 and available nitrogen, phosphorus, and potassium were 28.1 mg kg^{-1}, 119.7 mg kg^{-1}, and 372.4 mg kg^{-1}, respectively). At 7-days after planting, 1.0-L of prepared terpenoid mixture was added to the first pot (denoted as TR2); 1.0-L distilled water was added to the second pot (denoted as TR1) and to the third pot (denoted as CK). The pots were kept in the greenhouse. Urea fertilizer was added @ 0.5 g per pot. Additional distilled water was added daily to each pot to maintain 1.0 cm water level above the soil. At 30 days after planting, all rice and weeds plants were harvested (cut near to soil surface) and separated species-wise. The heights of rice plants were measured. Rice and weed plant materials were oven-dried at 120 °C for 30 min and then at 80 °C for 48 h, and thereafter the aboveground dry weights of rice and weed plants were recorded, separately.

Experiment-II. (Repeat trial)

The above trial was repeated between May 1 to May 30, 2009 with hybrid rice, Te-You-Hang II (most popular and high-yielding rice cultivar in southeast China). The culture method, experimental conditions, treatments and Pot maintenance and measurements were same as in 2007. The temperature ranged from 19 °C to 27 °C.

The inhibitory rate (IR) was used to evaluate the effects of treatments on plant growth and was calculated as under:

IR1 (%) = [(Control – Treatment 1)/Control] × 100.

It shows inhibitory rates of 2 hybrid rice cultivars on paddy weeds

IR2 (%) = [(Control – Treatment 2)/Control] × 100.

It shows inhibitory rates of 2 hybrid rice cultivars and terpenoid mixture on paddy weeds.

IR3 (%) = (IR2 – IR1).

It shows the net inhibitory rates of terpenoid mixture on paddy weeds

IR4 (%) = [(Treatment 2 – Treatment) /Control] × 100.

It shows inhibitory rates of 2 hybrid rice cultivars and terpenoid mixture on paddy weeds, when TRI was used as control.

Statistical analysis:

All experiments were done in randomized design with three replications. Data are presented as mean ± standard error (SE). They were subjected to a one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test at $P < 0.05$ or $P < 0.01$ where appropriate. All statistical analyses were performed using DPS (Data processing system) (20).

RESULTS AND DISCUSSION

The terpenoids mixture effectively reduced the paddy weeds (Figure 1.). There were 11 main weed species identified in the untreated control pots (CK, paddy soil only without rice plants and without the terpenoid mixture treatment) in the 2007 experiment (Table 1). These species were from the families of Gramineae, Cyperaceae, Compositae, Scrophulariaceae, Pontederiaceae, Lemnaceae, Polygonaceae as well as some unidentified weeds, including *E. crus-galli* (L.) Beauv., *E. crus-galli* (L.) Beauv. Var. *zelayensis* (H.B.K.) Hithc., *Leptochloa chinensis* (L.) Nees., *Leptochloa panacea* (Retz.) Ohwi., *Cyperus difformis* L., *Cyperus rotundus* L., *Eclipta prostrata* L., *Mazus japonicus* (Thunb.) O. Kuntze., *Monochoria vaginalis* (Burm. f.) Presl ex Kunth, *Lemna minor* L., *Polygonum lapathifolium* L.. The total aboveground dry weight of these weeds was 13.278 g in the CK pots. All the weed species were also found in TR1 pots. The presence of rice seedlings in TR1 resulted in the significant reduction of the aboveground dry weight of paddy weeds, compared to the untreated control (CK). The total aboveground dry weight of weeds decreased from 13.278 g (CK) to 5.634 g (TR1). The inhibitory rate of paddy weeds (IR1) was 57.57% for TR1, compared to the untreated control. However, treatment of the terpenoid mixture in TR2 caused a complete suppression of the growth of *E. crus-galli*, *C. rotundus*, *E. prostrata*, and *P. lapathifolium*. It also significantly suppressed the growth of other weed species. The total aboveground dry weight of these weeds significantly decreased from 13.278 g (CK) to 1.333 g (TR2). The inhibitory rate of paddy weeds (IR2) was 89.97% for TR2, compared to the control (CK) (Table 1). The difference in inhibitory rates (IR3) between IR2 and IR1 was 32.40%. The inhibitory rate of paddy weeds (IR4) was 76.36% for TR2, when TRI was used as control.

In the repeat trial in May 2009, six main weed species were identified in all the CK, TR1 and TR2 pots. These species included *E. crus-galli* (L.) Beauv., *C. difformis* L., *E. prostrata* L., *Lindernia procumbens* (Krock.) Philcox, *M. vaginalis* (Burm. f.) Presl ex Kunth, and *P. lapathifolium* (Table 2). The total aboveground dry weight of these weeds was 17.236 g in the CK pots, while it reduced to 7.146 g and 2.873 g in the TR1 and TR2, respectively. The inhibitory rates of paddy weeds were 58.54% for TR1 and 83.33% for TR2, respectively, compared to the control. The differences in inhibitory rate (IR3) between IR2 and IR1 was 24.79%. The inhibitory rate of paddy weeds (IR4) was 59.80% for TR2, when TRI was used as control.

In contrast to the suppression on paddy weeds, the terpenoid mixture treatment in TR2 enhanced the growth of rice seedlings in both experiments. The aboveground dry weights of rice plants significantly increased from 0.931 g (TR1) to 1.021 g (TR2) in the 2007 experiment with the hybrid cultivar Shan-You 63, and from 1.032 g (TR1) to 1.135 g

Table 1. Effects of terpenoids mixture on shoot dry weight (g) of paddy weeds in 2007

Weed spp	Shoot dry weight (g)			Inhibitory rate (IR, %)			
	Control	TR1	TR2	IR1	IR2	IR3	IR4
Echinochloa crus-galli (L.) Beauv.	0.686Aa	0.025 Bb	0 Bc	96.36	100.00	3.64	100.00
Echinochloa crus-galli (L.) Beauv. Var. *zelayensis* (H.B.K.) Hithc.	2.36 Aa	0.97 Bb	0.13 Cc	58.90	94.49	35.59	86.60
Leptochloa chinensis (L.) Nees.	2.304 Aa	0.781 Bb	0.233 Cc	66.10	89.89	23.78	70.17
Leptochloa panicea(Retz.) Ohwi.	0.652 Aa	0.091 Bb	0.071 Bb	86.04	89.11	3.07	21.98
Cyperus difformis L.	4.919 Aa	2.846 Bb	0.71 Cc	42.14	85.57	43.42	75.05
Cyperus rotundus L.	0.347 Aa	0.028 Bb	0 Bb	91.93	100.00	8.07	100.00
Eclipta prostrata L.	0.158 Aa	0.071 Bb	0 Cc	55.06	100.00	44.94	100.00
Mazus japonicus (Thunb.) O. Kuntze.	0.312 Aa	0.107 Bb	0.01 Cc	65.71	96.79	31.09	90.65
Monochoria vaginalis (Burm. f.) Presl ex Kunth	0.128 Aa	0.052 Bb	0.012 Bb	59.38	90.63	31.25	76.92
Lemna minor L.	0.034 Aa	0.045 Aa	0.053 Aa	-32.35	-55.88	-23.53	-17.78
Polygonum lapathifolium L.	0.397 Aa	0.066 Bb	0 Cc	83.38	100.00	16.62	100.00
Unknown	0.981 Aa	0.552 Bb	0.113 Cc	43.73	88.48	44.75	79.53
All weeds	13.278 Aa	5.634 Bb	1.332 Cc	57.57	89.97	32.40	76.36

TR1: Rice plants without terpenoid mixture, TR2: Rice plants with terpenoid mixture, CK: paddy soil without rice plants and without terpenoid mixture. Different uppercase letters and lowercase showed significant differences in shoot dry weight of paddy weeds over CK at $P < 0.05$ and $P < 0.01$; *Significant differences in inhibitory rate from IR1 at $P < 0.05$; **Very Significant differences in inhibitory rate from IR1 at $P < 0.01$.

Table 2. Effects of terpenoids mixture treatment on shoot dry weight (g) of paddy weeds in 2009

Weed spp	Shoot dry weight (g)			Inhibitory rate (IR, %)			
	Control	TR1	TR2	IR1	IR2	IR3	IR4
Echinochloa crus-galli (L.) Beauv.	1.994Aa	0.745Bb	0.041Cc	62.64	97.94**	35.31	94.50
Cyperus difformis L.	4.653Aa	1.984Bb	0.868Cc	57.36	81.35**	23.98	56.25
Eclipta prostrata L.	2.162Aa	1.647Bb	0.841Cc	23.82	61.10**	37.28	48.94
Lindernia procumbens (Krock.) Philcox	5.844Aa	1.685Bb	0.648Cc	71.17	88.91**	17.74	61.54
Monochoria vaginalis (Burm. f.) Presl ex Kunth	0.599Aa	0.379Bb	0.261Cc	36.73	56.43**	19.70	31.13
Polygonum lapathifolium L.	0.877Aa	0.241Bb	0.076Cc	72.52	91.33**	18.81	68.46
Unknown	1.107Aa	0.465Bb	0.138Cc	57.99	87.53**	29.54	70.32
All weeds	17.236Aa	7.146Bb	2.873Cc	58.54	83.33**	24.79	59.80

TR1: Rice plants without terpenoids mixture, TR2: Rice plants with terpenoids mixture, CK: paddy soil without rice plants and without terpenoids mixture. Different uppercase letters and lowercase showed significant differences in shoot dry weight of paddy weeds over CK at $P < 0.05$ and $P < 0.01$; *Significant differences in inhibitory rate from IR1 at $P < 0.05$; **Very Significant differences in inhibitory rate from IR1 at $P < 0.01$.

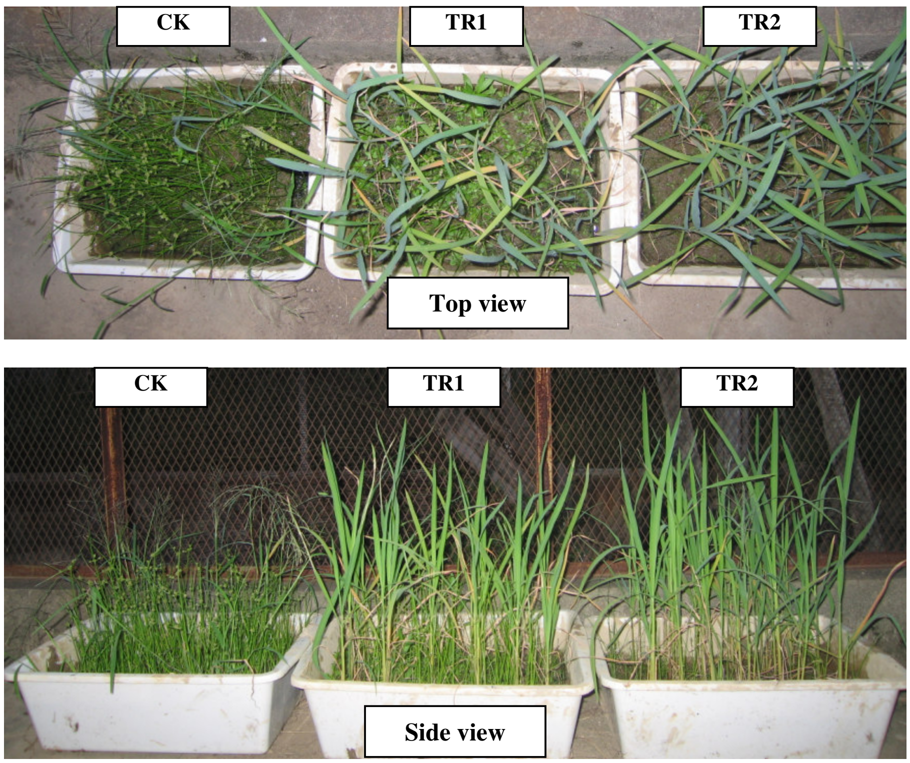

Figure 1. The herbicidal effects of terpenoids mixture in paddy soil on hybrid cultivar Shan-You 63 in 2007. CK: paddy soil only without rice plants and without the terpenoids mixture treatment. TR1: with rice plants and without the terpenoids mixture treatment. TR2: with rice plants with terpenoids mixture treatment.

(TR2) in the 2009 experiment with the hybrid cultivar Te-You-Hang II (Table 3). Similarly, the terpenoid mixture also increased the rice plant heights from 35.26 cm (TR1) to 38.13 cm (TR2) in the 2007 experiment (Shan-You 63) and from 37.14 cm (TR1) to 39.05 cm (TR2) in the 2009 experiment (Te-You-Hang II).

Table 3 Effects of terpenoid mixture treatment on seedlings growth of two hybrid rice cultivars (data ± SE)

Rice cultivar (year)	Shoot dry weight (g)		Plant height (cm)	
	TR1	TR2	TR1	TR2
Shan-You 63 (2007)	0.931±0.028	1.021±0.037*	35.26±1.38	38.13±1.08**
Te-You-Hang II (2009)	1.032±0.042	1.135±0.019*	37.14±1.42	39.05±1.35ns

TR1: Rice plants without terpenoids mixture, TR2: Rice plants with terpenoids mixture. *Significant differences over TR1 at $P < 0.05$; **Very Significant differences over TR1 at $P < 0.01$; ns: No significant differences.

Different weed species were encountered during the experiments in 2007 and 2009 (Tables 1 and 2). This may be because of the different experimental periods (month and year) and climate conditions such as temperature, humidity, the characteristics of the two rice cultivars tested, and the growth characteristics of paddy weeds at different experimental time.

The results obtained in the present study indicate that the optimum terpenoid combination can inhibit the growth of most paddy weeds, including *E. crus-galli*; the inhibitory rates of total paddy weeds were about 90% in 2007 and 83% in 2009. However, the terpenoid mixture promoted the growth of rice plants (Figure 1, Table 3), although it is not clear if the promotion of rice growth was due to the terpenoid mixture. Vergara (23) suggested that paddy weeds are harmful to rice seedlings at 30 days after planting. Our experimental results showed that the terpenoid mixture inhibited the growth of most paddy weeds during the 30 days after planting, thereby minimising weed competitive effect on rice plants. The results indicate that the exploitation of the joint effect of natural terpenoids may replace, or at least reduce, the use of synthetic herbicides in rice production. The use of natural terpenoids could serve as a novel, sustainable approach for weed management.

Herbicidal activity of terpenoids and its potential use as bioherbicides has been widely studied. Vaughn and Spencer (21) reported that several oxygenated monoterpenes decreased germination of *Abutilon theophrasti*, *Lolium multiflorum*, *Amaranthus retroflexus* and *Digitaria sanguinalis*, and found that species with larger seed, such as *Zea mays*, *Glycine max*, and *Cucumis sativus,* exhibited greater tolerance to the terpenoids than smaller seeded species, such as *Digitaria sanguinalis*, *Amaranthus retroflexus and Lolium multiflorum.* Volatile oil from *Eucalyptus citriodora* Hook. significantly reduced seed germination of *Triticum aestivum, Zea mays, Raphamus sativus, Cassia occidentalis, Amaranthus viridis*, and *E. crus-galli* in laboratory bioassay (2). Under field conditions, application of 5% eucalypt oil caused nearly 80% and 40% of growth reduction in *C. occidentalis* and *E. crus-galli,* respectively (2). Essential oil of *Artemisia scoparia* significantly reduced germination, seedling growth and dry matter accumulation of *A. fatua, Cyperus rotundus*, and *Phalaris mino*r) (19). Romagni *et al.* (15) reported that 1,4- and 1,8-cineole inhibited germination and radicle elongation of *E. crus-galli* and *Cassia obtusifolia*.

Although the mechanism of terpenoids for inhibitory activity is largely unknown, some evidences have been reported. Batish *et al.* (2) suggested that eucalypt oil adversely affected on the photosynthetic machinery and the energy metabolism of *C. occidentalis* and *E. crus-galli*. Singh *et al.* (19) concluded that *Artemisia* oil inhibited plant root growth through generation of reactive oxygen species (ROS) -induced oxidative damage. Savelev *et al*. (16) suggested that the inhibitory activity of *Salvia lavandulaefolia* essential oil resulted from a complex interaction between its constituents, including both synergistic and antagonistic activities. Nishida *et al*. (13) reported the eucalyptol, pinene, camphene and camphor could inhibit cell proliferation in the roots of *Brassica campestris* by disturbing their organelle and nuclear DNA synthesis within the meristem cells. Our previous results confirmed that this terpenoid mixture significantly reduced the activity of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in roots and leaves of E. *crus-galli*, thereby inhibiting the growth of the weed (10).

The competitive ability of the two rice cultivars on weed growth was also noted in our experiments. Without the terpenoid mixture treatment, the presence of rice seedlings alone significantly inhibited weed growth by approximately 58% (IR1) with both hybrid cultivars Shan-You 63 and Te-You-Hang II, compared to their control (Tables 1 and 2). The addition of the terpenoid mixture treatment further improved the inhibitory rates (IR2) to approximately 90% with Shan-You 63 in the 2007 experiment and to approximately 83% with Te-You-Hang II in the 2009 experiment, respectively. By aid of the terpenoid mixture, the net inhibitory rates (IR3) have been raised by approximately 32% and 25% in 2007 and 2009, respectively (Table 1 and Table 2). These results demonstrated that with the help of the optimum terpenoid mixture, some rice cultivars can suppress the growth of almost all paddy weeds without the need of applying synthetic herbicides at rice seedlings stage. The results of this study highlight the possibility of using natural compounds as an alternative approach for weed management in rice production. However, additional work is required to assess the reliability, practical aspects and application technology of the terpenoid mixture under field conditions. Its persistence in the soil and the long-term impacts on aquatic organisms and on the soil biological, physical and chemical properties would also need to be addressed in further research prior to extensive application of natural products for weed management in rice production systems.

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