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Allelopathic Effect of Oryzalexine A on the Germination and Growth of Several Weeds

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ABSTRACT: Oryzalexine A, a potent growth inhibitor against several weeds such as *Digitaria sanguinalis* (L) Scop., and *Amaranthus lividus* L. was purified by conventional solvent partitioning and column chromatographies. This substance showed strong inhibitory activity on several weeds : Germination of seeds of *Poa annua* L. was inhibited by 36.5% at 1.0 mM and *Amaranthus lividus* L. by 56.1% at 2.5 mM. Growth of root and shoot of *Digitaria sanguinalis* (L.) Scop. by 10.2% and 22.4% at 2.5mM, respectively. These suggest that Oryzalexine A in rice straw might affect the germination and growth of susceptible weeds and other plants.

Keywords : Oryzalexine A, allelopathic effect, *Poa annua* L., *Amaranthus lividus* L., *Digitaria sanguinalis* (L.) Scop.

Rice straws are usually left in the paddy fields after harvest. Allelochemicals from the straws due to soil moisture, rain, and microorganisms are released into the soil by leaching and decomposition.

There has been a number of reports about allelopathic effect of rice plants (*Oryza sativa* L.). Lee *et al.* (1991) had shown that when rice straw was mulched with 700 g/m^2 in the upland field, dry weight of *Alopecurus aequalis* L. was decreased more than 35%. The inhibitory activity was different depending on varieties and extraction methods (Lee *et al.* 1998), and the contents or composition of allelochemicals in rice were diserved (Chou *et al.*, 1977). Allelopathic compounds in rice straw were identified mainly as phenolic compounds (Chandrasekaran and Yosida 1973; Olofsdotter *et al.*, 1995). Since the phenolic compounds had weak inhibitory activity, they were utilized as a nutrient source or converted into non-toxic forms by microorganisms in the soil (Shindo and Kuwatsuka 1978). Several research groups isolated momilactones A and B and oryzalexines A and C from rice husk as major growth inhibitors against several weed species (Cartwright *et al.*, 1981; Kato *et al.*, 1973; Kodama *et al.*, 1988a; William *et al.*, 1981). They inhibit the root growth of rice and the germination of lettuce (Kato *et al.*, 1973, 1977). Howerever, there has been no report on the inhibitory effect of oryzalexine A against the growth of weeds. This report described the isolation and identification of oryzalexine A from rice straw and its inhibitory activity against several weeds.

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MATERIALS AND METHODS

Plant material and isolation

Plant material

Rice (*Oryza sativa* L. cv. Haresugata) straws were obtained from paddy fields after harvest. These were dried in air for 2 weeks and cut into small pieces.

Isolation

The straw (20 kg dry weight) were extracted with 200 liters of 80% aqueous methanol (MeOH) within one day. After filtration, the MeOH solution was evaporated at 40°C under reduced pressure. The residues (1,467 g) were dissolved in distilled water, and were further extracted with successively equal volumes of ethyl acetate (EtOAc) and butanol (BuOH). The EtOAc fraction showing the germination inhibitory activity against *Digitaria sanguinalis* (L.) Scop. was separated by silica gel column chromatography. The column (80 cm×5 cm) was eluted with a stepwise increase of EtOAc in hexane.

The active fractions (30%-70% EtOAc in hexane) were combined and loaded on a silica gel column (100 cm×3 cm; EtOAc in chloroform). The strong activity was detected in the 20% EtOAc in chloroform fraction, and was further purified by ODS column chromatography (50~100% acetonitrile, stepwise). The active fractions (50~60% acetonitrile) was combined and purified by ODS column chromatography (50% acetonitrile) dividing out 12.1 mg of almost pure crystals.

Bioassay

One hundred μl acetone solution of the crude or purified fraction was placed in a glass vial (15 mm diameter, 45 mm

high) containing a filter paper. The solvent was allowed to evaporate before 100 μl of distilled water was added. Then, 50 seeds of *Digitaria sanguinalis* (L.) Scop. were placed on each filter paper and kept in a growth chamber maintained at 25°C for 12 hrs photoperiod. Germination percentage of seeds was determined after 6 days. All the experiments were replicated three times. Results were expressed as percent of the control.

Instrumental analyses

HPLC

In order to check the purity of the isolated compound, HPLC analysis was conducted. The condition and instrument were as follows; HPLC (Hitachi, Japan) equipped with LiChrosorp RP18 was used for analysis. Analysis was carried out at absorbance mode of 0.2 AU, absorbance scale of 0.200 AU, spectral bandwith of 4 nm, spectial interval of 400 msec and wave length of 245 nm. And solvent eluted from 30% to 100% MeOH stepwisely.

GC/MS

In order to check the molecular weight of the isolated compound GC equiped DB-1 capillary column (15 m×0.25 mm) was used. The temperature program was from 100°C to 280°C at 45°C/min. Flow rates of carrier gas were 3.0 m/min for He. The injector temperature was 260°C and detector temperature was 280°C. MS (Varian 3300 gas chromatography) was equipped with a split injector.

RESULTS AND DISCUSSIONS

The straw were extracted with methanol. After extraction and evaporation, 1,467 g of solid was obtained. *Poa annua L.* germinated to 0% at 30,000 μl/ml. After fractionating with BuOH, EtOAc and aqueous, bioassay was carried out at 5,000 ppm. EtoAc fraction among them showed the highest activity to decrease the germination to 0%. EtoAc fraction was purified by silica gel column chromatography that eluted with stepwise increase of EtOAc in hexane. After bioassay the actives were 30~70% EtOAc fractions, which showed 54.1% germination at 2,000 ppm. These EtOAc fractions (20 g) were combined and loaded on a silica gel column which eluted with stepwise increase of EtOAc in chloroform. The strong activity was detected in the 20% EtOAc fraction, which was germinated to 41.9% at 1,000 μl/ml and obtained 8.4 g of solid. This solid further purified by ODS column chromatography eluted with stepwise of 50 to 100% acetonitrile. The strongest activity was showed at 50% acetonitrile fraction (980 mg), which germinated 37.1% of *Poa annua* L. at 500 ppm. This solid further purified by ODS column chromatography (100% acetonitrile) and get 140 mg crystals (the 17th of 19 fractions). These crystals were crystallized with Hexane and EtOAc and got 68.9 mg crystals. These crystals were purified by silica gel column chromatography that eluted with 10 chloroform in 1 EtOAc, and finally got 12.1 mg crystals (Table 1).

Isolated crystals was determined for the purity and molecular weight with HPLC and GC/MS, respectively, and were finally confirmed the structure by PMR and CMR. By considering these spectra data and the literatures of oryzalexine A, the crystal isolated was identified oryzalexine A, that had molecular formula $C_{20}H_{30}O_2$, molecular weight 302.2246.

Although oryzalexine A was originally isolated from rice husks as growth and germination inhibitors (Kono *et al.*, 1984), it has been known to participate in the plant defense systems against pathogens along with phytoalexins (Cartwright *et al.*, 1981; Matsuyama, 1983). The endogenous levels of phytoalexins are different by both biotic and abiotic stresses (Kodama 1988b; Tamogami *et al.*, 1995), and growth stage (Lee *et al.*, 1999b). In this experiment, oryzalexine A isolated from rice straw exhibited the pronounced inhibition on both germination and early growth of weeds. The germination of *Amaranthus lividus* L. was inhibited by

Table 1. Germination inhibition of *Poa annua* L. in each separation step.

Purification method	Fraction	Purified Solid	Bioassay concentration (ppm)	Germination rate (% of control)
Rice straw		20 kg		
80% MeOH, distilled Water	MeOH	1,467 g	30,000	0 ± 0
EtOAc, BuOH, Aqueous	EtOAc	170 g	5,000	0 ± 0
EtOAc in hexane, Stepwise	30-70%	20.0 g	2,000	54.1 ± 2.8
EtOAc in chloroform, Stepwise	20%	8.4 g	1,000	41.9 ± 3.7
50-100% acetonitrile, Stepwise	5060%	980 mg	500	37.1 ± 1.0
100% acetonitrile	Rf 5.5	140 mg	100	38.7 ± 4.5
Recrystallize with Hexane/EtOAc	17th	68.9 mg	2.2	47.3 ± 8.6
Chloroform 10 : EtOAc 1	3rd	12.1 mg	-	-

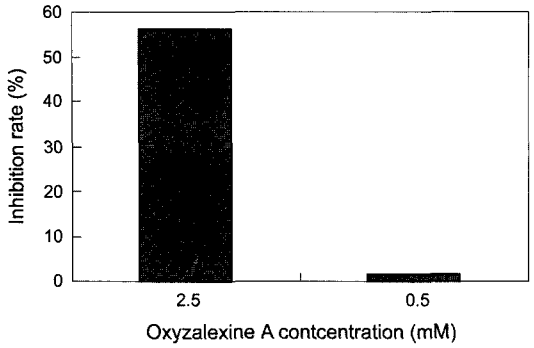

Fig. 1. Influence of oryzalexine A on the germination of *Amaranthus lividus* L.

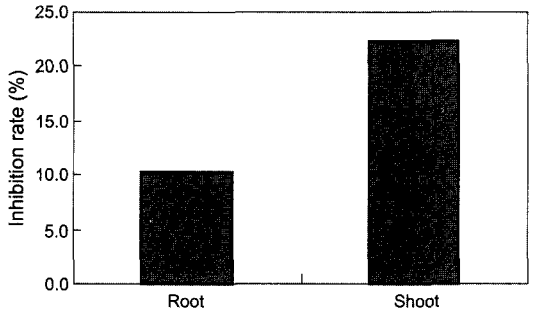

Fig. 2. Influence of 2.5 mM oryzalexine A on the growth of *Digitaria sanguinalis* (L.) Scop.

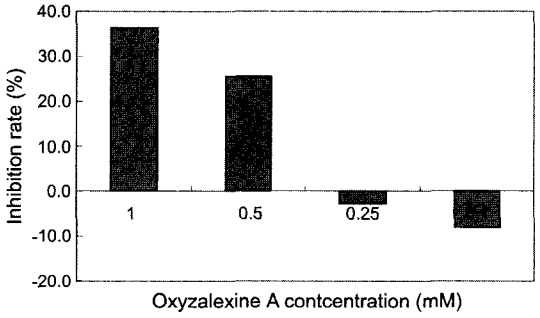

Fig. 3. Influence of oryzalexine A on the germination of *Poa annua* L.

56.1% and the growth of root and shoot of *Digitaria sanguinalis* (L.) Scop. was inhibited by 10.2% and 22.4% at 2.5 mM, respectively, The germination of *Poa annua* L. was inhibited by 36.5% at 1.0 mM (Fig. 1, 2, 3).

Among allelochemicals such as momilactone A, B, oryzalexine A, C and ineketon, from rice plant, Momilactone B showed the most strong inhibition against crops (Kato *et al.*, 1973; Lee *et al.*, 1999a). Lee *et al.* (1999b) reported that concentrations of momilactone A and B in rice plants increased with growth, became maximal levels at the heading stage and then decreased gradually. This experiment indicated that oryzalexine A in rice straws may cause some inhibitory effects on the germination and growth of susceptible weed species when straw is left in the field after harvesting.

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