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Herbicidal Selectivity and Mode of Action of EK–2612 in Rice and Barnyardgrass

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EK–2612 [5–(2–3 dihydro–2,2,4,6,7–pentamethyl benzofuran–5–yl)–2–[1–(allyoxyimino) butyryl]–3 hydroxy cyclohex–2–en–1–one] is a new herbicide being developed for the selective post–emergence control of a wide range of grass weeds. It is a member of the class of cyclohexane–1,3–diones which inhibits chloroplastic acetyl–CoA carboxylase (ACCase, EC 6.4.1.2) and block fatty acid biosynthesis. A study was conducted to evaluate the herbicidal performances of EK–2612 especially on barnyardgrass and rice. The compound EK–2612 performed excellent post–emergence activity against barnyardgrass exhibiting the growth inhibition of barnyardgrass by 74 and 84% at 60 and 120 g a.i. ha^{-1}, respectively. With increasing EK–2612 concentration, acetyl–CoA carboxylase activity was more inhibited in barnyardgrass than in rice. Chlorophyll content in barnyardgrass was reduced greatly with the increase of EK–2612 concentrations, whereas chlorophyll content in rice was not affected by EK–2612. No phytotoxic symptoms were observed on rice due to EK–2612, having a good rice safety up to the level of 120 g a.i. ha^{-1}. These results suggest that EK–2612 has an excellent pre–emergence herbicidal activity against barnyardgrass without hampering the growth of rice.

Key words: Herbicide selectivity, mode of action, ACCase

INTRODUCTION

Weeds have always been recognized as one of the major constraints on yield and quality of rice and have been proved a significant pest problem in temperate rice culture (Ioannis and Kico, 2005). The barnyardgrass (*Echinochloa crus–galli L.*) is a highly prolific annual grass weed that is widely distributed in the tropics as well in most of the rice growing regions in the world believed to control it for maximizing rice production.

EK–2612, a new synthesized herbicide commonly known as grass killers has been developed for the selective post emergence control of a wide range of grass weeds including barnyardgrass. This compound belongs to cyclohexanediones (CHDs) family, which was developed in the late 1970's and has been used to control grass species in dicotyledonous crops (Iwataki and Hirono 1978; Swisher and Corbin 1982). Two classes of grass killer herbicide i.e., the aryloxyphenoxy propionates (AOPPs) and the CHDs have been reported as acetyl–CoA carboxylase (ACCase) inhibitors. In plants, two forms of ACCase have been identified those are located in the chloroplast, the primary site of plant fatty acid biosynthesis, and in the cytosol (Sasaki *et al.*, 1995; Konishi *et al.*, 1996). Chloroplastic ACCase is a vital point of plant metabolism (Ohlrogge and Jaworski 1997). The CHD herbicides act on the plastidic enzyme ACCase. ACCase is a key enzyme involved in the biosynthesis of fatty acids, particularly is active in meristematic tissues, and its inhibition results in cessation of growth followed by the chlorosis of the young leaves and the eventual necrosis of the whole plant (Harwood 1989; Gronwald 1991). This enzyme acts by catalyzing the formation of malonyl–CoA from the ATP–dependent carboxylation of acetyl–CoA (Powles & Yu, 2010).

The objective of this study was to determine the herbicidal activity of the compound EK–2612 and to determine the mechanism of selectivity between rice and barnyardgrass.

MATERIALS AND METHODS

Herbicidal activity of EK–2612

Ek–2612, [5–(2–3 dihydro–2,2,4,6,7–pentamethyl benzofuran–5–yl)–2–[1–(allyoxyimino) butyryl]–3 hydroxy cyclohex–2–en–1–one] was synthesized by Korea Research Institute of Chemical Technology, Daejeon, Korea. Rice cv. Chuchung (*Oryza sativa* L.) and barnyardgrass (*Echinochloa crus–galli* Beauv.) were grown in an environmental control room (light; 12 hr, 30C, dark; 12 hr, 25C). Ten pre–germinated rice seeds and 20 barnyardgrass seeds (dormancy was broken by 30 days submerged in cold water) were sown in 6 cm diameter round shape pot including a commercial potting mix. Rice and barnyardgrass seedlings at 2–2.5 leaf were treated with EK–2612 at 15, 30, 60 and 120 g a.i. ha^{-1} using a hand sprayer. Plant height and dry weight of aboveground biomass were measured at 12 days after herbicide treat-

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ment. Percent of rice growth inhibition and barnyardgrass control were calculated as compared with untreated control. The experiment was in a completely randomized design with three replications and was repeated.

Chlorophyll contents assay

Rice and barnyardgrass were grown as mentioned above. To evaluate the effects of EK–2612 on chlorophyll contents of rice and barnyardgrass, EK–2612 was treated at 3rd leaf stage of plants and new coming 4th leaf was harvested five days after herbicide treatment. The leaf sample (0.2–0.4 g fresh weight) was homogenized with 10 ml 95% methanol and centrifuged at 10,000 g for 10 min. The supernatant was analyzed by spectrophotometer at 470.0, 652.4, 665.2 nm wavelength. Total chlorophyll contents were calculated as μg chlorophyll per g fresh weight following the procedure of Lichtenthaler (1987).

Cellular damage

To examine the peroxidation of cell membrane, cellular leakage was determined in rice and barnyardgrass. Eighty leaf tissue discs (4 mm diameter) were placed in a 6–cm diameter Petri dishes containing 7 ml of 1% sucrose, 1 mM 2–(N–morpholino) ethanesulfonic acid pH 6.5 with or without EK–2612 compound dissolved in acetone. The tissues were incubated in a growth chamber at 25°C in darkness for 5 days. Electrolyte leakage into the bathing medium was detected by the conductivity meter daily.

Acetyl–CoA carboxylase assay

Introduction of $NaH^{14}CO_3$ into acetyl–CoA to form malonyl–CoA via acety–CoA carboxylase was assayed using 10–day–old rice and barnyardgrass leaves by modified methods of Stoltenberg *et al*. (1989). Fresh leaf tissue was homogenized in a medium of 100 mM tricine–KOH pH 8.3, 10% glycerol, 10 mM β–mercaptoethanol, 1 mM Na_2 EDTA and 1 mM phenylmethyl sulfonyl fluoride. The homogenate was filtered through 4 layers of cheese cloth and centrifuged at 25,000 g for 30 minutes at 4C. Supernatant was saturated with 10.6% $(NH_4)_2SO_4$ for 20 minutes and centrifuged at 25,000 g and supernatant was saturated with 11.3% $(NH_4)_2SO_4$ again, and centrifuged at 25,000 g for 30 minutes. The precipitate was resuspended in 10 mM tricine–KOH pH 7.8, 10% glycerol and desalted on a Sephadex G–25 column. Acetyl–CoA carboxylase activity was assayed at 35C for 10 minutes in a volume containing 20 mM DTT, 50 mM $MgCl_2$, 20 mM ATP, 150 mM NaH^{14} CO_3 (0.34 μCi/μmol) and 0.1 ml enzyme preparation. Reactions were initiated by addition 0f 20 ml 30 mM acetyl–CoA and stopped by addition of 50 μl 12N HCl and radioactivity quantified by liquid scintillation spectrometer (Packard Tricarb 2300TR).

Acetate incorporation

To investigate the influence of EK–2612 on lipid synthesis in rice and barnyardgrass leaves, 30 leaf discs (4 mm diameter) taken from 10–day–old rice and barnyardgrass were added to 10 ml ^{14}C–acetate (0.1 μCi/ml) + 0.1 M potassium phosphate buffer pH 7.5 solution and 0–200 μM EK–2612 were added to the medium and incubated for 24 hours in growth chamber (25C). After being rinsed with distilled water, the leaf discs were boiled with water–saturated butanol containing 0.005% butylated hydroxyl–toluene and then homogenized and centrifuged at 15,000 g for 10 minutes. The supernatant was dried under nitrogen at 40C water bath. This fraction was then dissolved in 0.5 ml of chloroform–methanol–water (86:14:10 v/v). A 0.2 ml aliquot of the fraction was transferred to a scintillation vial and 4 ml scintillation cocktail solution was added and radioactivity was determined using a scintillation spectrometer.

RESULTS AND DISCUSSION

EK–2612 showed excellent herbicidal activity on barnyardgrass showing almost no inhibition in rice plant in this study. Growth of barnyardgrass was significantly inhibited by the application of EK–2612 in all the concentration used in this study (Table 1). The level of inhibition was 84 and 100%, when EK–2612 was applied at 60 and 120 g a.i. /ha, respectively. Even at the lowest concentration (15 g a.i. /ha) of this compound inhibited growth of barnyard grass more than 50%. Whereas, no significant inhibition was observed in case of rice even at the highest rate, 120 g a.i./ha of EK–2612 based on plant height and aboveground dry weight (Table 1). From this

Table 1. Effect of EK–2612 foliar application on plant height and aboveground dry weight of rice and barnyardgrass 12 days after treatment

EK–2612 (g a.i./ha)	Rice			Barnyardgrass		
	Plant height (cm)	Dry weight (g/pot)	Inhibition (%)	Plant height (cm)	Dry weight (g/pot)	Control (%)
0	28.4a	0.233a	–	21.7a	0.192a	–
15	26.4a	0.229a	1.9a	12.7b	0.106b	53.0c
30	25.4a	0.228a	2.1a	5.3c	0.048c	76.8b
60	26.6a	0.229a	1.9a	4.5c	0.034c	83.5b
120	25.4a	0.221a	5.5a	0	0	100a

The symbols with the different letters in the same column denote a significant difference at the 5% level by Tukey's Honestly Significant Difference test.

study we can easily say that rice is quite safe to EK–2612 even at the highest doses where barnyardgrass was completely inhibited. Similar type of finding was observed by Kim *et al.*, 2004 where they reported that the compound EK–2612 demonstrated a strong post–emergent herbicidal activity on grass weeds under upland conditions in a greenhouse keeping rice plant in safe.

Like growth inhibition in barnyardgrass, chlorophyll content was also reduced greatly with the increase of EK–2612 concentrations in barnyardgrass, whereas chlorophyll content in rice was not affected using EK–2612 (Fig. 2). The reason of reduced chlorophyll by the application of EK–2612 could be the damage of chloroplast and photo–oxidation. Our findings are agreed with the findings of Gealy and Slife (1983) where they reported that chlorophyll contents in corn were significantly reduced by BAS 9052, a cyclohexanedione herbicide and that this effect was due to the damage of chloroplast and photo–oxidation. Cellular leakage did not occur until three days of incubation both in rice and barnyardgrass

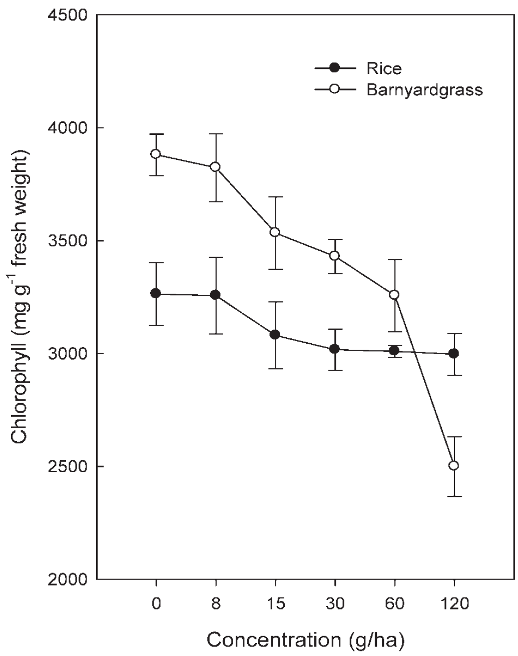

Fig. 1. Effect of EK–2612 on chlorophyll content in rice and barnyardgrass leaves 5 days after treatment.

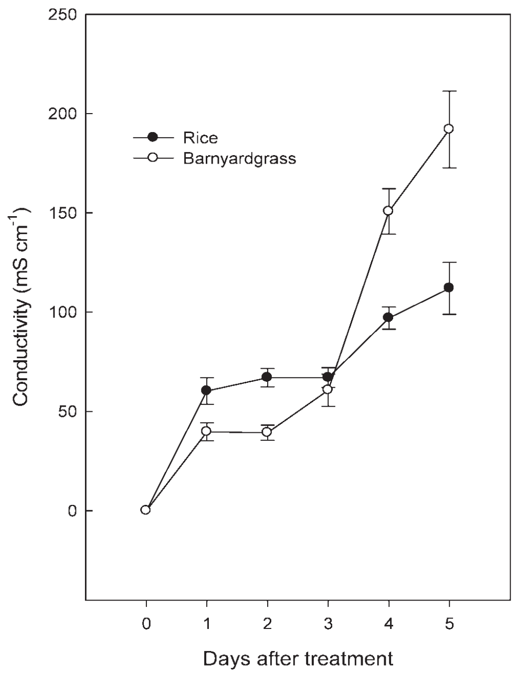

Fig. 2. Effect of EK–2612 on electrolyte leakage from rice and barnyardgrass.

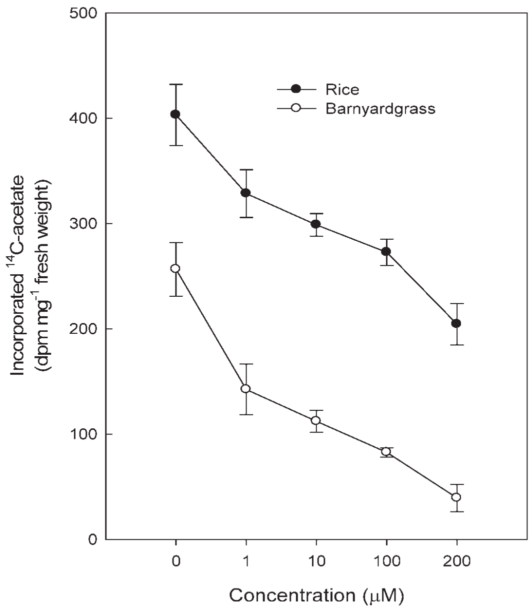

Fig. 3. Effect of EK–2612 on ^{14}C–acetate incorporation into lipid in rice and barnyardgrass leaf.

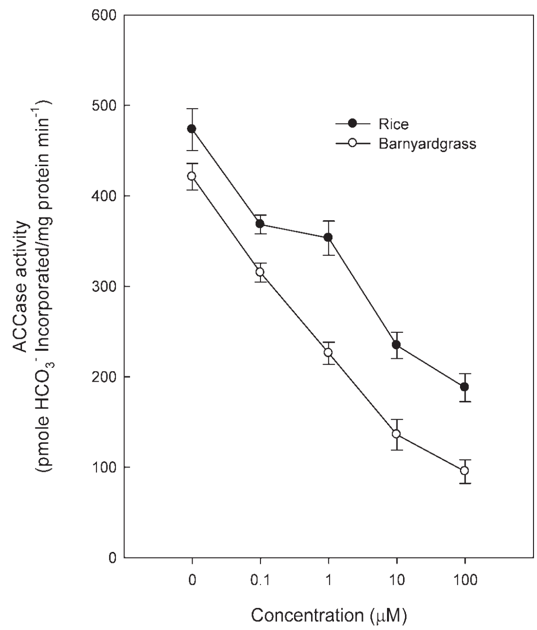

Fig. 4. Effect of EK–2612 on acetyl–CoA carboxylase activity in rice and barnyardgrass.

treated with EK–2612 (Fig. 3). However, cellular leakage started from both species three days after incubation showing more rapid changes in barnyardgrass than in rice. Total lipid synthesis, as measured by incorporation of ^{14}C–acetate, was reduced by 70% in barnyardgrass and 50% in rice with 200 μM EK–2612 treatment (Fig. 4). Similar findings were observed from the results of haloxyfop and diclofop–methyl effects on lipid synthesis in corn (Cho *et al*., 1986; Hoppe, 1981).

Detail studies about Acetyl–coenzyme A carboxylase has been reported as the target for cyclohexane–1,3–dione herbicides (Herbert *et al*., 1997;Rendina and Felts, 1988; Secor and Cseke, 1988). It has also been reported that selectivity and resistance mechanisms of grass killers are mainly attributable to the differential susceptibility of this enzyme (Burton *et al*., 1989; Martinez–Ghersa *et al*., 1997; Matthews, 2000). Here in this study, acetyl–CoA carboxylase activity on rice and barnyardgrass was inhibited by EK–2612 and the response to inhibition was linear with increasing EK–2612 concentration (Fig. 4). However, acetyl–CoA carboxylase was more sensitive to EK–2612 in barnyard grass than in rice.

These results indicate that EK–2612 showed selectivity between rice and barnyardgrass and mode of action of EK–2612 may be primarily attributed to the inhibition of acetyl–CoA carboxylase and lipid synthesis, and also the damage of membrane by peroxidation. EK–2612 is targeting ACCase enzyme both in rice and barnyardgrass. However, the selectivity of rice and barnyardgrass was not based on sensitivity of the enzyme but probably metabolism or translocation of EK–2612. Finally it may be concluded that the compound EK–2612 is a potential herbicide candidate for rice cultivation having a good barnyardgrass control efficacy.

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