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Screening of Herbicides for Rice Seedling Safety and *Echinochloa colona* Management under Australian Conditions

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Abstract: Different herbicides are currently required for sustainable weed management in aerobic rice. Three pot experiments were conducted using different herbicides to evaluate rice safety and for the control of *Echinochloa colona*, a major weed of aerobic rice. Among the pre-emergence (PRE) herbicides, it was found that pendimethalin (594 g ai ha^{-1}) and flumioxazin (60 g ai ha^{-1}) were relatively safe herbicides for rice and provided 100% control of *E. colona*. All other PRE herbicides, such as atrazine, cinmethylin, clomazone, dimethenamid-P, isoxaflutole, metribuzin, prosulfocarb + *S*-metolachlor, pyroxasulfone, trifluralin, and *S*-metolachlor reduced the biomass of rice compared with the non-treated control. Dose-response studies revealed that flumioxazin and pendimethalin even at low doses (30 g ai ha^{-1} for flumioxazin and 294 g ai ha^{-1} for pendimethalin) provided excellent control (>95%) of *E. colona*. Post-emergence (POST) application of paraquat (360 g ai ha^{-1}) at the time of rice emergence caused toxicity in the crop, but also provided excellent control of *E. colona*. When applied just after crop emergence (11 days after sowing), Pendimethalin was found to be safe for rice (2% mortality) and reduced the biomass of *E. colona* by 88% compared with the non-treated control. It is quite possible that the rice variety Reiziq used in this study may have a tolerance to flumioxazin, which needs further investigation involving more rice varieties. This study suggests that flumioxazin can be used as an alternative to pendimethalin for the sustainable management of *E. colona* in aerobic rice.

Keywords: flumioxazin; pendimethalin; paraquat; rice safety; weed control

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1. Introduction

Australia is known for rice (*Oryza sativa* L.) cultivation globally due to its possession of the world's highest average yield and level of high water-use efficiency [1,2]. Rice is grown only in a few pockets of Australia due to the strict water and land use required for its production. The main cultivation of rice occurs in the Riverina region, New South Wales. In the Queensland region, rice is grown in some parts of the Burdekin River [3]. Australian growers have placed a great deal of emphasis on the use of aerobic rice as growers have prioritized the efficient use of water resources, land use, and environmental protections for sustained production of rice [3–5].

Weeds are the main constraint on the production of a high yield in aerobic rice [6]. The effective use of rice herbicides has benefitted the Australian rice industry by providing effective weed control. However, the continuous use of herbicides with similar modes of action in rice production has contributed to herbicide resistance in many weeds such as *Oryza sativa* (red rice or weedy rice), *Echinochloa* spp., *Cyperus difformis*, *C. esculentus*, *C. iria*, *Fimbristylis miliacea*, and *Sagittaria montevidensis*. [4,7]. For example, ALS inhibiting herbicide (bensulfuron, fenoxaprop, and pyrazosulfuron-ethyl) resistance has been documented in many rice weeds [2,7]. The over-reliance on a single herbicide can lead to the evolution of resistant weed biotypes with a high selection pressure that may cause herbicide resistance against multiple groups of herbicides [8]. For example, *Lolium rigidum* Gaud. has evolved resistance to acetolactate synthase (ALS) and acetyl CoA carboxylase (ACCase)-inhibiting herbicides [8].

Echinochloa colona (junglerice) is a problematic weed in aerobic rice [9]. It negatively affects crop production by creating a competition for soil, water, and nutrients. In Australia, information on rice yield losses due to *E. colona* is very limited but losses are likely to be substantial as evident from other studies conducted overseas. In the Philippines, the *E. colona* density of 280 plants m^{-2} resulted in a 76% yield loss in rice [10]. Similarly, significant yield losses in aerobic rice were observed due to a high infestation of *E. colona* when rice was planted at a wide row spacing (30 cm row to row) in the Philippines [11]. In Arkansas, a high density of *E. crus-galli* (100–200 tillers m^{-2}) reduced the grain yield of rice by >40% [12].

Pendimethalin is quite popular for the control of *E. colona* in rice in South Asia [13,14]. In an Australian pot study, atrazine, isoxaflutole, dimethenamid-P, isoxaflutole, prosulfocarb + *S*-metolachlor, and *S*-metolachlor were found to effectively control *E. colona*; however, the selectivity of these herbicides towards rice was not evaluated in that study [15,16]. In another study on rice, it was found that pyroxasulfone (170 g ai ha^{-1}) and trifluralin (1680 g ai ha^{-1}) injured rice seedlings by 28–36%, while clomazone (840 g ai ha^{-1}) did not cause any injury to rice [17]. Metribuzin caused injury to rice seedlings by 3–6%, but that injury did not translate into the yield [18]. Clomazone could effectively control *E. colona* in rice [19]. Cinmethylin effectively controlled *E. colona* in rice when applied at a rate of 25–100 g ha^{-1} [20]. Isoxazolidinone is a new herbicide for residual weed control in cereals [21]. Flumioxazin is registered for broad-leaf and some annual weeds in soybeans, sorghum, maize, and beans [22]. Paraquat is labeled as a PRE or preplant for use in rice [23,24]. Rice growers in Australia use paraquat combined with other herbicides representing multiple modes of action prior to crop emergence [25].

The frequency of herbicide resistance in *E. colona* is quite high, as *Echinochloa* species have evolved resistance to at least seven herbicidal modes of action (synthetic auxins (i.e., quinclorac), acetolactate synthase (ALS) inhibitors, acetyl coenzyme A carboxylase (ACCase) inhibitors, photosystem II inhibitors, microtubule inhibitors, long-chain fatty-acid inhibitors, and lipid synthesis inhibitors) globally [26]. In Australia, herbicide resistance in *E. colona* is still restricted to glyphosate [27]; however, its high potential for evolving resistance could lead to major implications for the management of this weed in the aerobic rice system. Many herbicides are available for the management of *E. colona* in aerobic rice in Australia. However, due to the threat of herbicide resistance within the sole use of particular herbicides, there is a need for additional and alternative herbicide programs to complement the sustainable chemical weed control programs in aerobic rice systems. The objective of this study was to evaluate different options for herbicides, and their application times for rice safety and *E. colona* control.

2. Materials and Methods

Three pot experiments were conducted at the weed science research facility at the Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Gatton, Australia (2020–2021). Experiments 1 and 2 were conducted in an automatic temperature-controlled glasshouse bay maintained at a day/night temperature of 30/20 °C (12 h/12 h). Experiment 3 was conducted in a screenhouse under natural conditions. The population of *E. colona* used in this study was resistant to glyphosate (data not shown). The list of herbicides used in this study, their trade names, and manufacturers have been provided in Table A1.

2.1. General Protocol

PRE herbicides were sprayed immediately after sowing of rice. Herbicides were sprayed using a research track sprayer equipped with Teejet XR 110015 flat fan nozzles calibrated to an output spray volume of 108 L ha^{-1}. Plants were allowed to grow for 28 days after treatment (DAT) of herbicide application to determine herbicide efficacy. Plants were assumed dead if they did not have at least one new leaf at 28 DAT. Plants were harvested from the base and dried in an oven at 70 °C for 72 h and then weighed for biomass.

2.2. Experiment 1. Performance of PRE Herbicides

This experiment was conducted with 12 PRE herbicides (atrazine, cinmethylin, clomazone, dimethenamid-P, flumioxazin, isoxaflutole, metribuzin, pendimethalin, prosulfocarb + *S*-metolachlor, pyroxasulfone, trifluralin, and *S*-metolachlor) and an onenon-treated control (Table 1). All treatments were tested in a randomized block design (RBD) with three replicates. For the weed study, pots (20 cm diameter) were filled with field soil (51% sand, 17.6% silt, and 31.2% clay) that was sieved through 0.5 cm mesh. About 20 viable seeds of *E. colona* were sown in each pot at a depth of 0.5 cm. Similarly, for the study on rice, 20 seeds of the rice variety Reiziq were sown in each pot at a depth of 2 cm. Sowing was performed on 23 June 2021. PRE herbicides were sprayed immediately after sowing using a research track sprayer as mentioned in the general protocol. Pots were kept dry until 24 h after spray and thereafter were watered with a sprinkler system. To estimate the mortality and biomass reduction percentage, surviving plants and shoot biomass data of each pot at 28 d after herbicide application was converted into a survival percentage or a percent reduction of shoot biomass compared with the nontreated control: [(survived plants or shoot biomass of nontreated pot -survived plants or shoot biomass of treated pot)/survived plants or shoot biomass of nontreated pot] × 100.

Table 1. Effects of pre-emergent herbicides on rice and *Echinochloa colona* biomass.

Treatment	Dose (g ai ha^{-1})	Biomass Reduction of Rice over Control (%)	Biomass Reduction of *Echinochloa colona* over Control (%)
Non-treated control	-	0	0
Atrazine	2250	41.6	41.9
Cinmethylin	37	100	100
Clomazone	270	79.2	100
Dimethenamid-P	720	100	100
Flumioxazin	60	14.3	100
Isoxaflutole	75	100	100
Isoxazolidinone	500	56.8	100
Metribuzin	144	73.9	100
Pendimethalin	594	9.4	100
Prosulfocarb + *S*-Metolachlor	2000	100	100
Pyroxasulfone	94	100	100
S-Metoalchlor	1400	100	100
Trifluralin	600	37.1	100
LSD (0.05)	-	16.2	18.1

LSD: Least significant differences at 5% level of significance.

2.3. Experiment 2. Optimizing Herbicide-Dose

In this experiment, five doses each of pendimethalin (0, 149, 298, 596, and 1192 g ai ha^{-1}) and trifluralin (0, 150, 300, 600, and 1200 g ai ha^{-1}); six doses each of metribuzin (0, 18, 36, 72, 144, and 288 g ai ha^{-1}) and atrazine (0, 282, 564, 1128, 2256, and 4512 g ai ha^{-1}); and four doses of flumioxazin (0, 15, 30, 60, and 120 g ai ha^{-1}) were tested against rice and *E. colona* in an RBD design in three replicates. The purpose of this experiment was to optimize the dose of these selected herbicides for rice safety and reasonable weed control. We hypothesized that herbicides such as trifluralin, metribuzin, and flumioxazin could be safer to rice at lower doses and thus effective for weed control. Sowing of rice and *E. colona* was conducted on 21 July 2021 by following a similar procedure as in Experiment 1. PRE spray was conducted immediately after sowing using the procedure described in the general protocol.

2.4. Experiment 3. Efficacy of Paraquat in Rice

In this experiment, treatments comprised early POST application of pendimethalin, trifluralin, atrazine, flumioxazin, and metribuzin, and were compared with paraquat alone and as a tank mixture with paraquat. There was a total of 12 treatments including a non-treated control that was tested in an RBD in three replicates. For *E. colona* pots, 10 seeds per pot were sown on 20 August 2021, and again 10 seeds per pot were added (sown) in the same pot on 2 September 2021. The purpose was to evaluate the herbicide's efficacy on emerged plants (i.e., sown on 20 August 2021) as well as on seeds (i.e., sown on 2 September 2021). For rice pots, sowing was conducted on 2 September 2021 by using 20 seeds per pot and by following a similar procedure as described in Experiment 1. The pot size of 20 cm diameter was used, and each pot was filled with field soil (sieved through 0.5 cm mesh). POST spray was conducted on 13 September 2021 (11 days after sowing, rice plants were 2 cm tall at that time) using the method described above.

2.5. Statistical Analyses

In Experiments 1, 2, and 3, data were subjected to the analysis of variance (ANOVA) test using the statistical software CPCS1-Punjab at the Agricultural University, Ludhiana, India. Where the ANOVA found significant treatment effects, means were separated at $p \leq 0.05$ using Fisher's protected LSD test (Tables A2, A3a–d and A4). Data were also validated to meet the assumptions of normality and variance before analysis using Levene's and Shapiro-Wilk's tests.

3. Results and Discussion

3.1. Experiment 1. Performance of PRE Herbicides

All tested herbicides exhibited excellent control (100%) of *E. colona* except atrazine (Table 1). Atrazine reduced the biomass of *E. colona* by only 42% compared with the non-treated control. Cinmethylin, dimethenamid-P, isoxaflutole, prosulfocarb + *S*-metolachlor, pyroxasulfone, and S- metolachlor caused 100% mortality of rice plants; therefore, they were not found to be safe for rice. The percent biomass reduction of the rice over the control was the lowest with pendimethalin and it was similar with the flumioxazin treatment. The percent biomass reduction of rice over the non-treated control with clomazone, isoxaflutole, metribuzin, and trifluralin was 79, 57, 74, and 37%, respectively. This study revealed that pendimethalin and flumioxazin are relatively safe herbicides for rice and provide excellent control of *E. colona*. No signs of toxicity in the rice plants with pendimethalin and flumioxazin treatments were observed (visual observations). A slight biomass reduction with the pendimethalin and flumioxazin compared with the non-treated control was due to a slight suppression of the rice plants at an early stage, which was recovered at a later stage as there was no sign of toxicity in the plants.

In a previous study, PRE herbicides pyroxasulfone, *S*-metolachlor, and trifluralin caused injury to rice plants [17]. These authors also found that clomazone was safe for rice; however, we observed toxicity with the use of clomazone. Clomazone can cause significant damage to drilled rice if applied in loamy soils and rains occur soon after the sowing and the herbicide application [28]. In the present study, water was applied to plants every day through a sprinkler system. This practice could have been the reason for the clomazone toxicity in rice in our study; therefore, further investigation under field conditions is required. Rice plants showed toxicity to atrazine in the present study. A previous study suggests that the accumulation of atrazine in rice plants may cause toxicity due to the over-generation of hydrogen peroxide and superoxide anions [29].

Pendimethalin and flumioxazin exhibited excellent control of *E. colona* and did not cause injury to the rice plants in this study. Pendimethalin and flumioxazin were found to be effective (>80% control) against *Chloris virgata* Sw. (a summer grass weed) when these herbicides were evaluated in the mung bean crop [30]. In northwestern India, pendimethalin is a widely used herbicide for weed control in dry direct-seeded rice [19,20]. A recent study in the US found that some accessions of *Oryza* spp. (B20, B2, S11, B49, and S59) had reduced sensitivity to flumioxazin [31]. These authors further suggested that as rice and *Oryza* spp. (weedy rice) are closely related, flumioxazin could be effective for *Oryza* spp. control if flumioxazin tolerant rice varieties are used. The rice variety used in this study might have a natural tolerance to flumioxazin; however, further testing comparing this variety with other rice varieties for flumioxazin tolerance is needed for robust information.

3.2. Experiment 2. Optimizing Herbicide-Dose

Atrazine at the lowest dose (282 g ai ha^{-1}) caused a 58% reduction in the biomass of *E. colona* compared with the non-treated control, and this biomass reduction increased by 93% when atrazine was applied at 4512 g ai ha^{-1} (Table 2). Atrazine caused toxicity in the rice plants even at the lowest dose (282 g ai ha^{-1}). The biomass of rice was reduced by 24 and 49% compared with the non-treated control when atrazine was applied at 282 g ai ha^{-1} and 4512 g ai ha^{-1}, respectively.

Table 2. Effect of atrazine on percent biomass reduction of *Echinochloa colona* control and rice.

Atrazine Doses (g ai ha^{-1})	Biomass Reduction of *E. colona* over Vontrol (%)	Biomass Reduction of Rice over Vontrol (%)
0	0	0
282	58.3	23.7
564	67.8	24.8
1128	70.4	25.4
2256	77.7	26.0
4512	93.3	48.8
LSD (0.05)	32.4	17.8

LSD: Least significant differences at 5% level of significance.

Flumioxazin, even at the lowest dose of 15 g ai ha^{-1}, reduced the biomass of *E. colona* by 75% and demonstrated complete control of *E. colona* at 60 g ai ha^{-1} (Table 3, Figure 1). The biomass of the rice did not decline compared with the non-treated control when flumioxazin was applied at 30 g ai ha^{-1}, and the biomass of *E. colona* at this dose was reduced by 96% compared with the non-treated control (Figure 2).

Table 3. Effect of flumioxazin on percent biomass reduction of *Echinochloa colona* control and rice. LSD: Least significant differences at 5% level of significance.

Flumioxazin Doses (g ai ha^{-1})	Biomass Reduction of *E. colona* over Control (%)	Biomass Reduction of Rice over Control (%)
0	0	0
15	75.2	9.2
30	96.1	10.6
60	100	18.8
120	100	36.3
LSD (0.05)	20.6	13.9

Figure 1. Rice seedlings in response to flumioxazin doses (Left to Right: Flumioxazin doses are in increasing order: 0, 15, 30, 60, and 120 g ai ha^{-1}.

Figure 2. *Echinochloa colona* seedlings in response to flumioxazin doses (Left to Right: Flumioxazin doses are in increasing order: 0, 15, 30, 60, and 120 g ai ha^{-1}.

Metribuzin at higher doses was found to be effective against *E. colona* (Table 4). Metribuzin at 72 g ai ha^{-1} and 144 g ai ha^{-1} reduced the biomass of *E. colona* by 70 and 100% compared to the non-treated control, respectively. The biomass of the rice was reduced by 35, 88, and 100% compared with the non-treated control when metribuzin was applied at 72, 144, and 288 g ai ha^{-1}, respectively.

Table 4. Effect of metribuzin on percent biomass reduction of *Echinochloa colona* control and rice.

Metribuzin Doses (g ai ha^{-1})	Biomass Reduction of *E. colona* over Control (%)	Biomass Reduction of Rice over Control (%)
0	0	0
18	15.9	10.9
36	24.4	27.3
72	69.6	35.4
144	100	88.5
288	100	100
LSD (0.05)	29.4	24.6

LSD: Least significant differences at 5% level of significance.

Pendimethalin at all tested doses provided 100% control of *E. colona* (Table 5, Figure 3). Rice biomass was reduced by 14 and 16% compared with the non-treated control when pendimethalin was applied at 298 and 1192 g ai ha^{-1}, respectively (Figure 4). Similarly, trifluralin at all tested doses demonstrated 100% control of *E. colona* (Table 6). Rice biomass was reduced by 24 and 83% compared with the non-treated control when trifluralin was applied at 300 and 1200 g ai ha^{-1}, respectively.

Table 5. Effect of pendimethalin on percent biomass reduction of *Echinochloa colona* control and rice.

Pendimethalin Doses (g ai ha^{-1})	Biomass Reduction of *E. colona* over Control (%)	Biomass Reduction of Rice over Control (%)
0	0	0
149	100	9.6
298	100	14.1
596	100	16.0
1192	100	16.5
LSD (0.05)	-	9.8

LSD: Least significant differences at 5% level of significance.

Figure 3. *Echinochloa colona* seedlings in response to pendimethalin doses (Left to Right: Pendimethalin doses are in increasing order: 0, 149, 298, 596, and 1192 g ai ha^{-1}.

Figure 4. Rice seedlings in response to pendimethalin doses (Left to Right: Pendimethalin doses are in increasing order: 0, 149, 298, 596, and 1192 g ai ha^{-1}.

Table 6. Effect of trifluralin on percent biomass reduction of *Echinochloa colona* control and rice.

Trifluralin Doses (g ai ha^{-1})	Biomass Reduction of *E. colona* over Control (%)	Biomass Reduction of Rice over Control (%)
0	0	0
150	100	16.1
300	100	24.1
600	100	26.7
1200	100	82.8
LSD (0.05)	-	11.2

LSD: Least significant differences at 5% level of significance.

This study suggests that pendimethalin and flumioxazin effectively controlled *E. colona* and were safe for use on the rice plants. Trifluralin also provided effective control of *E. colona*, but at the highest dose 1200 g ai ha^{-1} it caused toxicity in the rice. Metribuzin and atrazine at high doses demonstrated effective control of *E. colona*; however, at these doses, these herbicides caused toxicity to the rice crop and significantly reduced the biomass of the rice compared to the non-treated control.

Atrazine and metribuzin interfere with photosystem II of target plants and reduce the production of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH), and ultimately result in reducing the efficiency of the CO_2 fixation process [32,33]. Atrazine may also cause a rapid accumulation of reactive oxygen species (ROS) by limiting the electron transport system in chloroplasts that may result in membrane injury [34]. A previous study revealed that the shoot length of rice was reduced by 67% compared with the non-treated control when plants were exposed to atrazine at 0.40 mg L^{-1}.

In this study, pendimethalin was found to be safer for rice than trifluralin. Pendimethalin and trifluralin are mitotic poisons and may inhibit the target plants by restricting the polymerization of tubulin, inhibiting growth, and causing the death of the plants [35,36].

The role of pendimethalin and trifluralin in upland crops has been acknowledged by several researchers [14,37]. Trifluralin has a species-specific selectivity and is relatively more toxic to rice than pendimethalin [38]. Physical herbicide positioning and the movement of herbicides near the crop seeds with moisture may influence the toxicity of dintroaniline herbicides.

Flumioxazin causes the inhibition of protoporphyrinogen oxidase (PPO) and leads to membrane lipid peroxidation [39]. A study conducted in Arkansas (US) revealed that rice cultivars CL163 and REX showed a high toxicity to flumioxazin (Shrestha et al., 2019). However, in the current study, flumioxazin was found to be safe for rice and provided excellent control of *E. colona*. The tested rice cultivar used in this study might have natural tolerance for flumioxazin that needs further investigation for more detail.

3.3. Experiment 3. Efficacy of Paraquat in Rice

In the experiment, all tested herbicides were applied as early POST when rice had just emerged. However, in Experiment 1, these herbicides were applied just after the rice was sown (as PRE). The sole application of atrazine caused toxicity to the rice crop alongside providing effective control (100%) of *E. colona* (Table 7). The sole application of paraquat, pendimethalin, trifluralin, flumioxazin, and metribuzin resulted in increases in the survival percentages of *E. colona* by 4, 32, 73, 25, and 43%, respectively, when compared with the non-treated control (Table 7).

Table 7. Efficacy of paraquat alone and its tank-mixed application with other pre-emergent herbicides against *Echinochloa colona* and rice.

Treatment	Dose (g ai ha^{-1})	Rice Mortality (%)	Rice Biomass Reduction Percentage over Control (%)	*E. colona* Survival Percentage	*E. colona* Biomass Reduction Percentage over Control (%)
Non-treated control	-	0	0	100	0
Paraquat	360	36.9	41.1	3.7	95.2
Pendimethalin	594	1.7	10.0	32.2	87.6
Paraquat + Pendimethalin	360 + 594	48.1	45.8	0	100
Trifluralin	300	9.8	34.6	73.2	65.5
Paraquat + Trifluralin	360 + 300	38.2	50.5	4.8	91.9
Atrazine	2250	67.5	87.3	0	100
Paraquat + Atrazine	360 + 2250	47.8	62.9	0	100
Flumioxazin	60	7.7	0.15	24.7	85.0
Paraquat + Flumioxazin	360 + 60	25.0	44.2	0	100
Metribuzin	36	13.3	11.4	42.6	72.7
Paraquat + Metribuzin	360 + 36	48.1	35.9	17.8	75.7
LSD (0.05)	-	19.5	17.8	22.8	17.6

LSD: Least significant differences at 5% level of significance.

The biomass reductions of *E. colona* over the non-treated control with the sole application of paraquat, pendimethalin, trifluralin, flumioxazin, and metribuzin were 95, 88, 65, 80, and 73%, respectively. The rice biomass reductions over the non-treated control with the sole application of paraquat, trifluralin, flumioxazin, atrazine, and metribuzin were 41, 10, 35, 0.1, 87, and 11%, respectively. Similarly, rice mortality percentages over the non-treated control with the sole application of paraquat, pendimethalin, trifluralin, flumioxazin, atrazine, and metribuzin were 37, 2, 10, 8, 67, and 13%, respectively.

Paraquat mixed with other herbicides was not found to be safe for rice. This study suggests that POST of paraquat causes toxicity to rice crops if sprayed after the rice emergence. Atrazine, although it provides excellent control of *E. colona*, causes toxicity to the rice crop. In Experiment 1, atrazine provided 42% control of *E. colona* compared with the non-treated control. This might be due to different environmental conditions as Experiment 1 was conducted under controlled conditions and atrazine was applied just after sowing. However, Experiment 3 was conducted under natural conditions, and atrazine was applied at the crop emergence stage and an enhanced accumulation of atrazine might have resulted in more toxicity [29].

Pendimethalin applied 594 g ai ha^{-1} and flumioxazin 60 g ai ha^{-1} were found to be safe for rice and reduced the biomass of *E. colona* by more than 80%. A lower dose of metribuzin (36 g ai ha^{-1}) was found to be safe for rice; however, this also reduced the biomass of *E. colona* by only 73% compared with the non-treated control.

We observed toxicity to the rice crop with paraquat as the spray was conducted at the crop emergence stage. Therefore, further investigations are needed to screen for rice varieties that have a tolerance to paraquat at the initial stages and to discover the proper time (crop stage) to apply it for rice safety and effective weed control. A recent study reported that early-season injury to rice following paraquat application had fewer effects on the yield [15]. Therefore, the use of paraquat with a crop-safener for improving rice safety and effective weed control needs to be evaluated in future pot and field studies.

In conclusion, the present study found that flumioxazin can be used as an alternative to pendimethalin as a PRE herbicide for weed control in rice, especially when dominated with *E. colona*. This study was conducted in pots using a single rice variety. Therefore, paraquat application needs to be tested with more rice varieties to discover its safety for use on rice crops under field conditions. The application of paraquat at the time of rice emergence caused toxicity to the crop, suggesting the need to evaluate the performance of paraquat on rice at needlepoint under field conditions. The application of pendimethalin and flumioxazin may be safe to rice even if applied at the crop emergence stage and can provide effective control of *E. colona*. However, further evaluation is needed under field conditions using different varieties as there may be a variety-specific response to these herbicides.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. List of herbicides along with trade name and manufacturer used in the study.

Herbicide	Trade Name (g/L)	Registered for Rice	Manufacturer
Atrazine	Atrazine 900	No	FMC, Australia
Cinmethylin	Luximax® 750	No	BASF Crop Solution, Australia
Clomazone	Magister 480	Yes	FMC, Australia
Dimethenamid-P	Outlook 720	No	BASF Crop Solution, Australia
Flumioxazin	Terrain 500	No	NuFarm, Australia
Isoxaflutole	Balance 480	No	Bayer Crop Science, Australia
Isoxazolidinone	Overwatch 400	No	FMC, Australia
Metribuzin	Sencor 480	No	Bayer Crop Science, Australia
Pendimethalin	StompXtra 455	Yes	BASF Crop Solution, Australia
Prosulfocarb + *S*-Metolachlor	Boxer Gold 920	No	Syngenta Herbicide, Australia
Pyroxasulfone	Sakura 850	No	Bayer Crop Science, Australia
S-Metoalchlor	Bouncer 960	No	NuFarm, Australia
Trifluralin	Triflurx 480	No	FMC, Australia
Paraquat	Paraquat 360	Yes	Titan Ag Pvt Ltd., Australia

Table A2. Analysis of variance for different parameters in Experiment 1.

Source of Variation	**Degree of Freedom**	**Mean Sum of Square**	
		Biomass Reduction of Rice over Control (%)	**Biomass Reduction of *Echinochloa colona* over Control (%)**
Replication	2	327.8	125.5
Treatment	12	2830.1	778.6
Error	24	99.0	125.5

Table A3. Analysis of variance for different parameters for atrazine doses in Experiment 2.

Source of Variation	**Degree of Freedom**	**Mean Sum of Square**	
		Biomass Reduction of Rice over Control (%)	**Biomass Reduction of *Echinochloa colona* over Control (%)**
Replication	2	359.6	802.5
Treatment	5	716.3	313.3
Error	10	96.2	318.7

(**a**). Analysis of variance for different parameters for flumioxazin doses in Experiment 2.

Source of Variation	**Degree of Freedom**	**Mean Sum of Square**	
		Biomass Reduction of Rice over Control (%)	
Replication	2	78.6	93.9
Treatment	4	559.2	5488.6
Error	8	55.1	119.8

(**b**). Analysis of variance for different parameters for metribuzin doses in Experiment 2.

Source of Variation	**Degree of Freedom**	**Mean Sum of Square**	
		Biomass Reduction of Rice over Control (%)	
Replication	2	227.7	441.6
Treatment	5	5099.6	5820.8
Error	10	183.2	261.9

(**c**). Analysis of variance for different parameters for pendimethalin doses in Experiment 2.

Source of Variation	**Degree of Freedom**	**Mean Sum of Square**
		Biomass Reduction of *Echinochloa colona* over Control (%)
Replication	2	66.9
Treatment	4	141.3
Error	8	26.8

(**d**). Analysis of variance for different parameters for trifluralin doses in Experiment 2.

Source of Variation	**Degree of Freedom**	**Mean Sum of Square**
		Biomass Reduction of *Echinochloa colona* over Control (%)
Replication	2	100.8
Treatment	4	2945.0
Error	8	274.9

Table A4. Analysis of variance for different parameters for pendimethalin doses in Experiment 3.

Source of Variation	Degree of Freedom	Mean Sum of Square			
		Rice Mortality (%)	Rice Biomass Reduction Percentage over Control (%)	*E. colona* Survival Percentage	*E. colona* Biomass Reduction Percentage over Control (%)
Replication	2	43.0	1212.2	805.7	322.4
Treatment	12	1469.1	1609.5	1851.3	479.3
Error	24	133.4	11.0	186.4	118.6

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