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Noxious Weed Species *Monochoria vaginalis* Resistant to Synthetic Auxin and Acetolactate Synthase Inhibitor Herbicides

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Abstract

Monochoria vaginalis (Burm. f.) C. Presl is a dominant weed in paddy rice cultivation, significantly threatening productivity and farming sustainability. In Subang, Indonesia, synthetic auxin and acetolactate synthase (ALS) inhibitors are commonly used to control *M. vaginalis*. However, farmers have currently reported declining efficacy of these herbicides. Therefore, this study aimed to confirm the resistance of *M. vaginalis* to the early post-emergence application of synthetic auxin and ALS inhibitors. Plant bioassays were conducted using the pot test method to determine resistance level. Herbicides were applied 2 weeks after planting *M. vaginalis* at 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 times the recommended dose, and untreated plants as control. Herbicides tested were 2,4-D, bensulfuron-methyl, bispyribac sodium, penoxulam, and sulfentrazone. The results obtained based on resistance index values (RI) showed that *M. vaginalis* populations from Kediri and Gandasari, Subang, had moderately to high resistance to 2,4-D (RI: 8.15 and 13.0) and bensulfuron-methyl (RI: 205.61 and 21.80). Weed was moderately resistant to bispyribac sodium (RI of Kediri biotype: 8.79) and had low to moderate resistance to penoxulam herbicides (RI: 8.94 and 3.56). In contrast, *M. vaginalis*-resistant biotypes remained susceptible to sulfentrazone (protoporphyrinogen oxidase/PPO inhibitors) herbicide. A significant increase in GR50 was observed between 28 and 56 days after herbicide application, signifying enhanced regrowth. The resistance of *M. vaginalis* to synthetic auxin and ALS inhibitors presents the need for farmers to consider alternative herbicides, such as PPO inhibitors, to prevent the development of resistant weeds.

Keywords: acetolactate synthase inhibitor; herbicide resistance; *Monochoria vaginalis*; sustainable agriculture; synthetic auxin

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INTRODUCTION

Monochoria vaginalis is a noxious aquatic weed that negatively impacts farmland productivity, aquatic biodiversity, and natural resource use. If noxious species develop, persist, and spread widely, they can inhibit the establishment, survival and spread of native plants, occupy agricultural land, and disrupt the biodiversity of native plant communities (Arun et al., 2022). Sustainable agriculture aims to protect the earth's soil and natural resources, promote agroecology, safeguard biodiversity, and improve the quality of life and health of farmers, farm workers, and communities (Muhie, 2022). However, noxious weeds have both direct and indirect negative impacts on these objectives. In farmland management and environmental sustainability, herbicide resistance remains a crucial issue. As an aquatic weed with a high reproductive and dispersal rate, *M. vaginalis* can grow aggressively and harm rice plants (Arun

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et al., 2022). This condition is increased by the tendency of species to develop resistance to herbicides, which has been the primary control method (Widianto et al., 2022).

Herbicides represent the primary tool for controlling weeds. In Indonesia, farmers have adopted acetolactate synthase (ALS) and synthetic auxin to control weeds in rice cultivation. However, the susceptibility of *M. vaginalis* to these herbicides has decreased over time. Resistance to ALS inhibitors was first reported in Sapto Mulyo Village, Kota Gajah Sub-district, Central Lampung Regency, Lampung Province; Ramadewa Village, Seputi Raman Sub-district, Central Lampung Regency, Lampung Province; Sarijaya Village, Majalaya Sub-district, Karawang Regency, West Java Province; and Kalentambo Village, Pusakanegara Sub-district, Subang Regency, West Java Province (Kurniadie et al., 2021a). The auxin herbicide 2,4-D has been used for over 7 decades (Peterson et al., 2016). Meanwhile, resistance to 2,4-D, has not been confirmed (Heap, 2024). It is currently the third-most widely applied herbicide worldwide, following ALS and 5-enolpyruvylshikimic acid-3-phosphate (EPSP) synthase-inhibitor (Busi et al., 2018). The ALS herbicides are widely recognized for their high efficiency, low toxicity to animals, and broad-spectrum activity (Powles and Yu, 2010).

The results of interviews with local farmers showed that the most commonly used herbicides for the control of broadleaf weeds in rice cultivation are ALS inhibitors and synthetic auxin. The resistance of *M. vaginalis* to these herbicides directly impacts productivity, presenting a significant challenge in the agricultural context. The failure of herbicides to control weeds leads to high-density growth, which exerts a more significant inhibitory effect on plant height, number of tillers per plant, and rice yield (Zhou et al., 2021). As a fast-growing aquatic plant, *M. vaginalis* can cause significant yield reductions when not managed effectively (Yokota et al., 2014). Therefore, rice plants will be increasingly vulnerable to competition with uncontrolled weeds. The crops will face competitive stress, which may negatively affect growth, development, and crop yields (Hazrati et al., 2023).

Observation of herbicide resistance is essential to prevent the spread of weeds to a broader area. The spread of resistant weeds poses a significant threat, as it can occur through seed mobility through irrigation canals, agricultural tools and machinery, livestock, human activity, and pollen movement (Duary, 2014; Jhala et al., 2021) Therefore, prompt assessment needs to be conducted to prevent potential agricultural impact.

The increasing incidence of weed resistance to synthetic auxin and ALS inhibitors poses a significant challenge in rice cultivation. There are 87 and 708 cases of resistance to synthetic auxin and ALS inhibitors, respectively. Instances of multiple resistance to these herbicides have been reported, including *Limnophila erecta* resistance to bensulfuron-methyl, cinosulfuron, pyrazosulfuron-ethyl, 2,4-D, and mesosulfuron-methyl as well as *Raphanus raphanistrum* resistance to metsulfuron-methyl, dicamba, 2,4-D, mesotrione, pyrasulfotole, and topramezone (Azmi and Baki, 2003; Owen et al., 2015; Heap, 2024). Despite global reports confirming *M. vaginalis* resistance to ALS inhibitors, no evidence has been discovered regarding synthetic auxins (Heap, 2024).

The primary objective of this study is to scientifically confirm the resistance of *M. vaginalis* to 2,4-D, bensulfuron-methyl, penoxsulam, bispyribac sodium, and sulfentrazone. The extent of resistance within weed species was determined, providing critical information on the efficacy of herbicides. Additionally, the study aims to identify alternatives to control resistant *M. vaginalis*, thereby contributing to more sustainable management practices in rice cultivation. Therefore, a specific current problem was addressed as a starting point for a deeper understanding of the weed resistance mechanism.

MATERIALS AND METHOD

Dose-response experiments

The study was conducted from July to October 2023 in the greenhouse of the Faculty of Agriculture, Universitas Padjadjaran. Herbicides used included synthetic auxin from the phenoxy-carboxylate class (a.i.: 2,4-D; recommended dose: 432.5 g a.i. ha^{-1}), ALS inhibitors from the sulfonylurea (a.i. bensulfuron-methyl; recommended dose: 4 g a.i. ha^{-1}), pyrimidinyl-thiobenzoate (a.i. bispyribac sodium; recommended dose: 20 g a.i. ha^{-1}), and triazolopyrimidines chemical classes (a.i. penoxulam; recommended dose: 10 g a.i. ha^{-1}), as well as protoporphyrinogen oxidase (PPO) inhibitors from the N-Phenyl-triazolinones chemical class (a.i. sulfentrazone; recommended dose: 48 g a.i. ha^{-1}). The soil used was inceptisols obtained from paddy fields in the experimental area of Universitas Padjadjaran, Sumedang Regency, West Java. It was adopted as a planting medium after being sterilized at 120 °C for 6 hours to prevent other weed seeds from growing. Additionally, 20 to 30 *M. vaginalis* seeds were planted in 20 cm diameter pots with a height of 25 cm. The seeds were scattered on the soil surface without being covered but could sink (anaerobic) with a water level 1 to 2 cm above the surface. After 10 days, weeds were removed and replanted, leaving 10 samples, and then herbicide was applied 14 days later (1 to 2 leaf stage). The application was conducted at 400 l ha^{-1} using a semiautomatic knapsack sprayer with a flat fan nozzle and 138 kPa pressure. The *M. vaginalis* was collected 28 days later, and 5 plants from each treatment were used to measure the dry weight by cutting surviving weed propagules at the base of the stem or ground level. Subsequently, the weed was dried in an oven at 80 °C for 48 hours and weighed. In survival, a maximum of 2 individuals were left in each treatment to be observed again at 56 days after treatment (DAT).

Plant material

Samples of *M. vaginalis* were collected in February 2023 from rice paddy fields where synthetic auxins and ALS inhibitors herbicides were extensively used. Susceptible samples were obtained from Kertajaya Village, Tambakdahan Sub-district, Subang Regency, West Java (107°48'07.8" E and 6°21'39.9" S) (S). The susceptible weeds were collected from rice fields using herbicides with appropriate dosing and rotation practice. This includes the alternating use of ALS inhibitors, synthetic auxins (from different chemical groups), and mixed herbicides across seasons. Conversely, resistant samples were collected from Kediri Village, Binong Sub-district, Subang Regency, West Java (107°48'02.4" E and 6°22'46.7" S) (R1), and Gandasari Village, Cikaum Sub-district, Subang Regency, West Java (107°44'45.4" E and 6°23'44.2" S) (R2). Resistant biotypes were sourced from rice fields subjected to continuous and intensive application of the same herbicides. Farmers often replace the chemicals for weed control when deemed ineffective. However, the change was conducted based on the commercial name. This method can result in selecting a product with the same active ingredient or mode of action as the previous solution. The samples were chosen after preliminary tests using the recommended doses of herbicides, and the classification of resistant and susceptible biotypes was confirmed. The study was conducted using *M. vaginalis* seeds to ensure excellent uniformity. Seed collection includes harvesting weed species from rice fields and replanting in a greenhouse to produce seeds. Subsequently, the collected seeds were dried under direct sunlight for a week to increase maturity and reduce moisture content.

Statistical analysis

The experiment was arranged in a split-plot design of 2 factors with 4 replications. The first factor was the biotype of *M. vaginalis*, which originated from 3 locations and served as the main plot. The second factor was the 7 levels of application dose, namely 0.0, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 times the recommended, respectively (Tampubolon et al., 2019; Kurniadie et al., 2023). Furthermore, the dry weight of weed data was subjected to a square root transformation (square root of data + 0.5) before being analyzed with ANOVA. The relationship between pesticide doses and weed populations was analyzed based on a *p*-value of less than 0.05. The Turkey test was adopted to determine significant differences in the data set in the event of an interaction.

The growth reduction percentage data was calculated by comparing the dry weight of weeds treated with herbicide (T) to untreated control (C) using Equation 1 by Widayat and Sumekar (2019).

$$\text{Growth reduction (\%)} = \left(1 - \frac{T}{C}\right) \text{ x } 100\% \quad (1)$$

The GR50 herbicide dosages were determined by nonlinear regression analysis using the log-logistic dose-response equation (Equation 2) (Seefeldt et al., 1995).

$$Y = \frac{c + (d\text{-}c)}{1 + \left(\frac{X}{GR50}\right) b} \quad (2)$$

Where c and d represent the lower and upper limits, and b is the slope of the response curve. Defined as the dose required to achieve a 50% growth reduction, GR50 was derived from the percentage of damage calculated from the dry weight of the weed. The doses included the entire spectrum of reactions, ranging from no observable effects to total mortality. Furthermore, dose-response analysis was conducted using OriginPro 9.0 software.

The resistance level was determined by comparing the GR50 resistant (R) and susceptible (S) biotypes (R/S). The classification of resistance levels includes R/S > 12 (high resistance), R/S = 6 to 12 (moderate resistance), R/S = 2 to 6 (low resistance), and R/S < 2 (susceptible).

RESULTS AND DISCUSSION

The experimental results signified an increase in dry weight at different observation periods. *M. vaginalis*, which showed growth inhibition due to 2,4-D at 28 days after application, experienced a recovery in the later period. As presented in Table 1, 28 DAT with 2,4-D applied at 865 g a.i. ha^{-1}, the dry weight of the R1 biotype was 0.03 g. However, biotype R1 grew at 56 DAT and had a high dry weight of 11.24 g, which was not significantly different from the application of 0.5 and 1.0 times the recommended dose of 216.25 and 432.5 g a.i. ha^{-1}, respectively. A similar pattern was observed in the population of the R2 biotype, where the dry weight at 28 DAT was 0.02 g at an application of 1,730 g a.i. ha^{-1} (4.0 times the recommended dose), which then increased to 8.53 g at 56 DAT, as shown in Table 1 and Figure 1.

The application of sulfentrazone at the recommended dose showed effective control of *M. vaginalis* biotypes S, R1, and R2 at 28 and 56 DAT. This was evidenced by the dry weight of these biotypes, which was recorded as 0.00 g at the recommended dose of 48 g a.i. ha^{-1}, signifying complete eradication of weed, as shown in Table 2 and Figure 1. Sulfentrazone is rarely used by rice farmers in Subang, which is probably why *M. vaginalis* R1 and R2 biotypes became susceptible.

The occurrence of 2,4-D resistance has been documented globally. This study showed that *M. vaginalis* belongs to a group of species with high resistance to herbicides, a phenomenon not previously reported. Currently, there are 87 cases of weed resistance to synthetic auxins, and 47 are related to 2,4-D (Heap, 2024). In paddy rice, 7 cases have been recorded in Southeast Asia, affecting species such as *Limnocharis flava* (Indonesia, Malaysia), *Fimbristylis miliacea* (Malaysia), *L. erecta* (Malaysia), and *Sphenoclea zeylanica* (Malaysia, Philippines, Thailand) (Heap, 2024). Meanwhile, resistance to synthetic auxin herbicides can be caused by several factors, including 2,4-D metabolism, mutations, and inhibition in auxin transporters (Auxin transporting ATP-binding cassette (ABC) transporters of the B subclass) (Goggin et al., 2016; Schulz and Segobye, 2016). Additionally, resistance to 2,4-D has been observed in several other species, such as *Amaranthus tuberculatus* (United States), *Papaver rhoeas* (Spain), *Amaranthus hybridus* (Argentina), *Conyza canadensis* (Hungary), *Conyza sumatrensis* (France), *Hirschfeldia incana* (Argentina), and *Parthenium hysterophorus* (Dominican Republic). The resistance mechanism in these species primarily includes the ability to metabolize 2,4-D herbicides (Torra et al., 2017; Figueiredo et al., 2018; Palma-Bautista et al., 2020).

Table 1. Dry weight of *M. vaginalis* due to 2,4-D herbicide application

DAT	Biotype	2,4-D herbicide dosages (g a.i. ha^{-1})						
		0	108.12	216.25	432.5	865	1,730	3,460
28	S	3.97^{c}	0.82^{c}	0.66^{c}	0.00^{b}	0.00^{b}	0.00^{a}	0.00^{a}
		A	B	C	D	D	D	D
	R1	4.87^{b}	2.87^{b}	1.25^{b}	1.25^{a}	0.03^{b}	0.00^{a}	0.00^{a}
		A	B	C	C	D	D	D
	R2	5.42^{a}	3.60^{a}	3.04^{a}	1.60^{a}	0.87^{a}	0.02^{a}	0.00^{a}
		A	B	C	D	E	F	F
56	S	12.44^{a}	6.28^{b}	5.11^{b}	0.00^{c}	0.00^{c}	0.00^{b}	0.00^{a}
		A	B	B	C	C	C	C
	R1	13.15^{a}	12.33^{a}	11.89^{a}	11.78^{a}	11.24^{a}	0.00^{b}	0.00^{a}
		A	A	B	B	B	C	C
	R2	12.17^{a}	11.88^{a}	10.17^{a}	9.88^{b}	8.62^{b}	8.53^{a}	0.00^{a}
		A	A	B	B	C	C	D

Note: S = Susceptible; R = Resistant. Values in each column followed by the same lowercase (vertical direction) and uppercase letters (horizontal direction) were not significantly different at $p < 0.05$ according to the Tukey Test for each herbicide. Significance followed by letters at 28 and 56 DAT are presented separately

Table 2. Dry weight of *M. vaginalis* due to sulfentrazone herbicide application

DAT	Biotype	Sulfentrazone herbicide dosages (g a.i. ha^{-1})						
		0	12	24	48	96	192	384
28	S	3.90^{a}	1.71^{a}	0.54^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
		A	B	C	D	D	D	D
	R1	3.78^{a}	0.73^{b}	0.24^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
		A	B	C	C	C	C	C
	R2	3.91^{a}	2.69^{a}	0.58^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
		A	B	C	D	D	D	D
56	S	9.31^{b}	8.83^{b}	8.09^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
		A	A	A	B	B	B	B
	R1	10.79^{a}	3.05^{c}	1.53^{c}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
		A	B	C	D	D	D	D
	R2	11.04^{a}	10.25^{a}	9.63^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
		A	A	A	B	B	B	B

Note: S = Susceptible; R = Resistant. Values in each column followed by the same lowercase (vertical direction) and uppercase letters (horizontal direction) were not significantly different at $p < 0.05$ according to the Tukey Test for each herbicide. Significance represented by letters at 28 and 56 DAT were presented separately

Table 3. Dry weight of *M. vaginalis* due to bensulfuron-methyl herbicide application

DAT	Biotype	Bensulfuron-methyl herbicide dosages (g a.i. ha^{-1})						
		0	1	2	4	8	16	32
28	S	4.11^{a}	2.53^{b}	1.87^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{c}
		A	B	C	D	D	D	D
	R1	4.36^{a}	4.25^{a}	4.21^{a}	3.03^{b}	2.90^{a}	2.40^{a}	2.33^{a}
		A	A	A	B	B	C	C
	R2	4.25^{a}	4.09^{a}	4.03^{a}	3.96^{a}	2.98^{a}	2.93^{a}	1.90^{b}
		A	A	A	A	B	B	C
56	S	11.68^{b}	10.42^{b}	7.12^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}
		A	B	C	D	D	D	D
	R1	13.25^{a}	12.41^{a}	12.39^{a}	12.14^{a}	11.89^{a}	11.83^{a}	10.17^{a}
		A	B	B	B	B	B	C
	R2	12.18^{b}	10.25^{b}	9.76^{b}	9.65^{b}	7.42^{b}	7.22^{b}	7.03^{b}
		A	B	B	B	C	C	C

Note: S = Susceptible; R = Resistant. Values in each column followed by the same lowercase (vertical direction) and uppercase letters (horizontal direction) were not significantly different at $p < 0.05$ according to the Tukey Test for each herbicide. Significance represented by letters at 28 and 56 DAT were presented separately

An increase in dry weight also occurred with the application of ALS inhibitors such as bensulfuron-methyl, bispyribac sodium, and penoxulam herbicides (Table 3, 4, 5). The dry weight values of *M. vaginalis* biotypes R1 and R2 were 2.33 and 1.90 g, respectively, at 28 DAT, with bensulfuron-methyl at 32 g a.i. ha^{-1}. This signified that 8 times the recommended dose of this treatment was insufficient to control the biotypes. The significance value of weed dry weight also changed. At 28 DAT, the dry weight of R1 with a dose of 16 g a.i. ha^{-1} showed a considerable difference compared to lower doses. However, at 56 DAT, it increased and was not significantly different compared to lower doses up to 0.5, which is the recommended dosage at 1 g a.i. ha^{-1}. A similar phenomenon was observed in R2, which featured an increase in dry weight following the application of bensulfuron-methyl herbicide at 32 g a.i. ha^{-1}. This was different from the dose of 8 g a.i. ha^{-1} at 28 DAT, but not indifferent at 56 DAT, as shown in Table 3 and Figure 2.

The same phenomenon was subsequently observed in R1 following the application of bispyribac sodium and in R1 and R2 after being administered penoxulam, as shown in Table 4 and 5. The results showed that R1 had significant regrowth after receiving bispyribac sodium and penoxulam herbicides at doses exceeding the

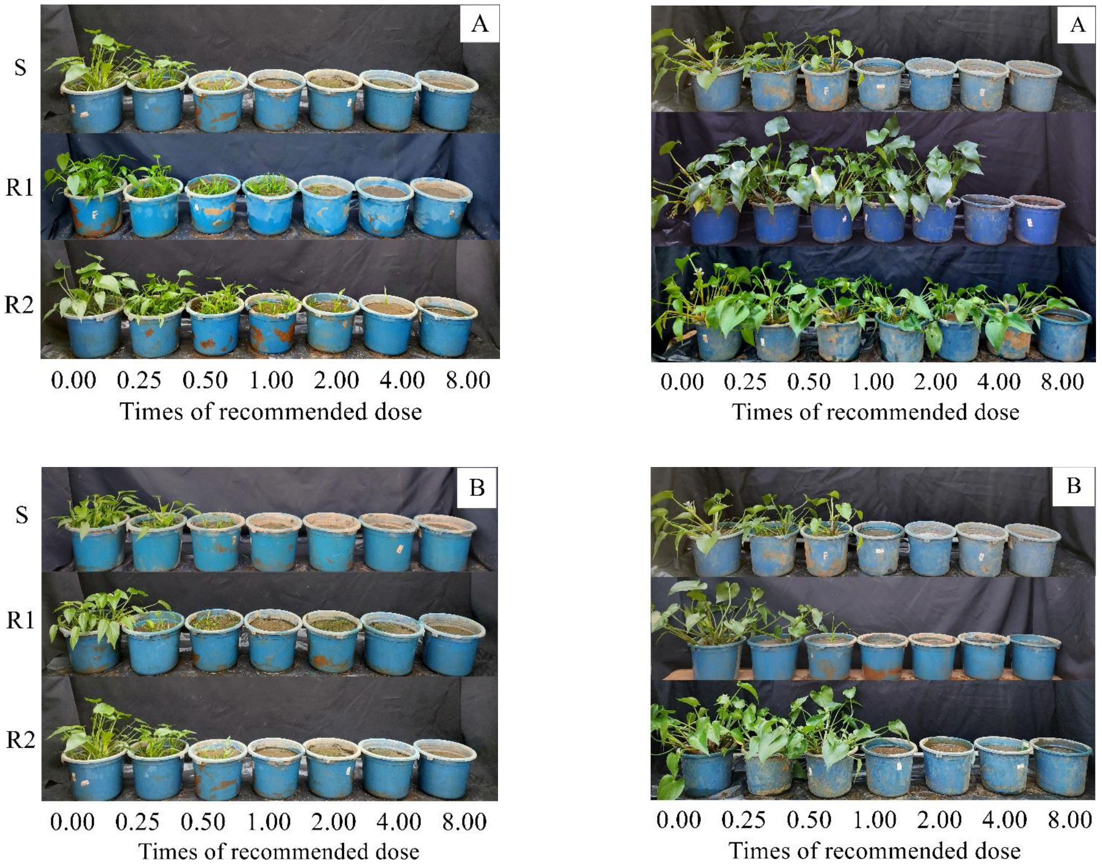


Figure 1. Effect of A) 2,4-D (0.00; 108.12; 216.25; 432.50; 865; 1,730; 3,460 g a.i. ha^{-1}) and B) Sulfentrazone (0; 12; 24; 48; 96; 192; 384 g a.i. ha^{-1}) herbicides application on resistant *M. vaginalis* weeds at 28 (left) and 56 (right) DAT
Note: S = Susceptible; R = Resistant

recommended levels. In contrast, biotype R2 did not regrow after bispyribac sodium herbicide was applied at doses above the recommended levels, as presented in Figure 2. The dry weight values of R1 were 0.42 and 0.28 after this herbicide was applied at doses of 40 and 80 g a.i. ha^{-1}, respectively, and significantly differed in lower doses at 28 DAT. However, at 56 DAT, the weights were not significantly different from biotype R1 at 0.5 of the recommended dose (10 g a.i. ha^{-1}).

Significant differences in weed dry weight were observed following penoxulam application at 28 and 56 DAT, with only biotype R1 showing substantial changes. However, both R1 and R2 regrowed following herbicide application. The dry weight of biotype R1 at 28 DAT presented very low values of 0.52 and 0.64 g at 384 and 96 g a.i. ha^{-1}, respectively. Meanwhile, significant regrowth occurred at 56 days after application with dry weight values of 2.72 and 3.19 g. This showed that penoxulam herbicide could suppress the growth of the *M. vaginalis*-resistant biotype at the early stages of the development cycle. Resistant biotype weeds could recover, resume growth, and proceed to flowering, as shown in Figure 2.

Weed that survives after treatment application at or above the recommended dose, while the same dose effectively controls wild types, signifies herbicide resistance. This phenomenon was observed in the application of ALS inhibitors bensulfuron-methyl, bispyribac sodium, and penoxulam, which showed efficacy in controlling *M. vaginalis* biotype S weeds but were ineffective against R1 and R2.

Dose-response studies of ALS enzyme-inhibiting herbicides were conducted. *M. vaginalis* from Kalentambo, Subang, Indonesia, was identified as cross-resistant to bensulfuron-methyl, penoxulam, and bispyribac sodium (Widianto et al., 2022). Resistant to ALS herbicides have been identified in several regions of the country, including Lampung, Central Java, and East Java (Kurniadie et al., 2021a; 2021b). In China, *Monochoria corsakowii* was reportedly resistant to bensulfuron-methyl in Dehui and Liuhei, at ratios of 6 and 13.6, respectively

Table 4. Dry weight of *M. vaginalis* due to bispyribac sodium herbicide application

DAT	Biotype	Bispyribac sodium herbicide dosages (g a.i. ha^{-1})						
		0	5	10	20	40	80	160
28	S	4.08^{a}	3.54^{a}	1.82^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{a}
		A	A	B	C	C	C	C
	R1	4.44^{a}	3.42^{a}	3.24^{a}	3.23^{a}	0.42^{a}	0.28^{a}	0.00^{a}
		A	B	B	B	C	C	D
	R2	3.92^{a}	0.92^{b}	0.16^{c}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{a}
		A	B	C	C	C	C	C
56	S	8.95^{b}	7.66^{b}	5.39^{c}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
		A	B	C	D	D	D	D
	R1	10.21^{a}	10.04^{a}	8.15^{a}	8.11^{a}	8.02^{a}	7.78^{a}	0.00^{a}
		A	A	B	B	B	B	C
	R2	10.04^{a}	9.35^{a}	7.23^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{a}
		A	A	B	C	C	C	C

Note: S = Susceptible; R = Resistant. Values in each column followed by the same lowercase (vertical direction) and uppercase letters (horizontal direction) were not significantly different at $p < 0.05$ according to the Tukey Test for each herbicide. Significances identified by letters at 28 and 56 DAT were presented separately

Table 5. Dry weight of *M. vaginalis* due to penoxulam herbicide application

DAT	Biotype	Penoxulam herbicide dosages (g a.i. ha^{-1})						
		0	2.5	5	10	20	40	80
28	S	4.88^{a}	3.19^{a}	2.58^{b}	0.00^{c}	0.00^{c}	0.00^{b}	0.00^{b}
		A	B	C	D	D	D	D
	R1	4.98^{a}	3.96^{a}	3.71^{a}	3.65^{a}	3.61^{a}	0.90^{a}	0.52^{a}
		A	B	B	B	B	C	C
	R2	4.22^{a}	3.46^{a}	3.34^{a}	1.31^{b}	0.64^{b}	0.03^{b}	0.00^{b}
		A	B	B	C	D	E	E
56	S	10.36^{b}	6.86^{b}	3.89^{b}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{b}
		A	B	C	D	D	D	D
	R1	10.14^{b}	9.66^{a}	9.57^{a}	8.17^{a}	6.89^{a}	3.77^{a}	2.72^{a}
		A	A	A	B	C	D	D
	R2	11.86^{a}	10.21^{a}	9.82^{a}	8.04^{a}	3.19^{b}	0.93^{b}	0.00^{b}
		A	B	B	C	D	E	E

Note: S = Susceptible; R = Resistant. Values in each column followed by the same lowercase (vertical direction) and uppercase letters (horizontal direction) were not significantly different at $p < 0.05$ according to the Tukey Test for each herbicide. Significances identified by letters at 28 and 56 DAT were presented separately

(ZongZhi et al., 2009). *M. vaginalis* from Chonnam rice plantation in Korea had a resistance ratio of 31 and 7 for bensulfuron-methyl and pyrazosulfuron ethyl herbicides (Kuk et al., 2003). *L. flava* showed resistance to bensulfuron-methyl, as reported by a ratio (RI) value exceeding 109. Additionally, species reported resistance to methyl metsulfuron (RI: 9), ethyl pyrazosulfuron (RI: 5.4), pyribenzoxim (RI: 4.6), and bispyribac sodium (RI: 6) (Zakaria et al., 2018).

Applying herbicides during the early post-emergence period to resistant weeds can lead to misconceptions among farmers. Herbicide may be wrongly presumed effective in controlling weeds when it only temporarily inhibits growth. The treatment with early-post-emergence herbicide applications such as ALS inhibitors and synthetic auxin shows significant growth inhibition before returning to normal and competing with rice plants. Significant growth was observed at 56 DAT, and when converted to the age of rice, the plants were in the vegetative phase, leading to inevitable competition. According to Kurniadie et al. (2021), weed *Ludwigia decurrens* experienced inhibition in early growth after being given penoxulam. However, the species grew almost similar to the

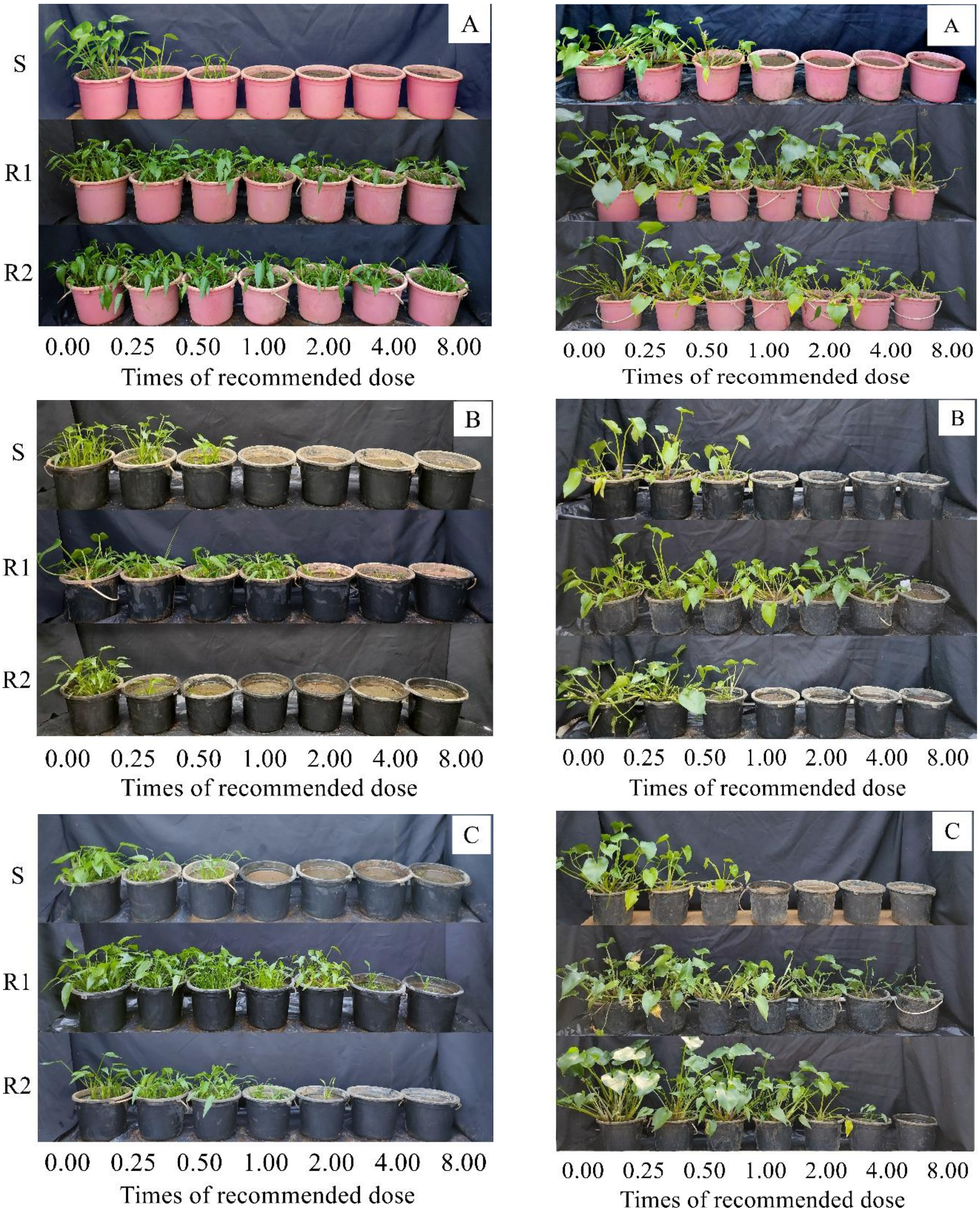


Figure 2. Effect of ALS inhibitor herbicides application on resistant *M. vaginalis* weeds at 28 (left) and 56 (right) DAT. A) Bensulfuron-methyl (0; 1; 2; 4; 8; 16; 32 g a.i. ha^{-1}), B) Bispyribac sodium (0; 5; 10; 20; 40; 80; 160 g a.i. ha^{-1}), C) Penoxulam (0; 2.50; 5; 10; 20; 40; 80 g a.i. ha^{-1})

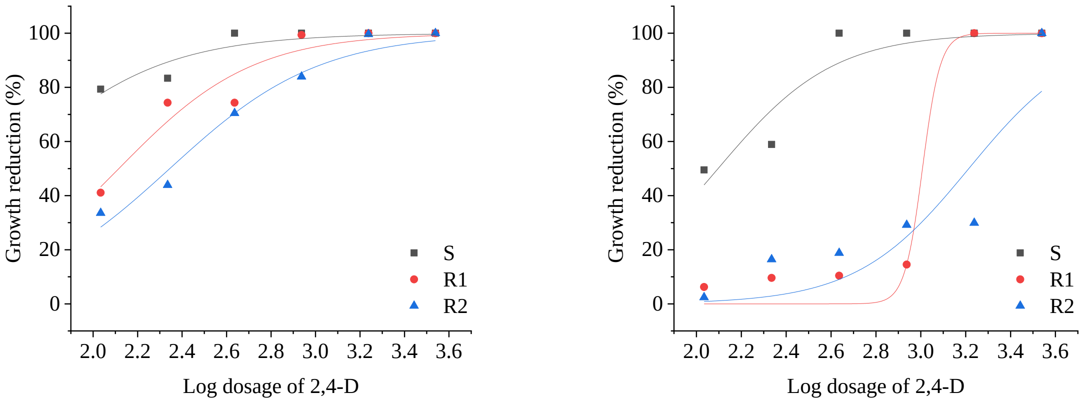
Note: S = Susceptible; R = Resistant

untreated control at 56 DAT. Regrowth occurred with post-emergence herbicide application, but the process was faster. Glyphosate-resistant *Ambrosia trifida* and *Conyza sumatrensis* weeds were subjected to rapid necrosis, and were proposed as Phoenix resistance. This was because "dead" plants could regrow a few days after herbicide application (Gressel, 2009; Brabham et al., 2011; De Queiroz et al., 2019).

The dose-response assay demonstrated that the *M. vaginalis* S biotype exhibited 100% mortality. However, the R biotype demonstrated a significantly reduced response to 2,4-D, bispyribac sodium, penoxulam, and bensulfuron-methyl herbicides, requiring higher doses than normal. The GR50 values for R plants were higher for S biotype (Figure 3 to 6; Table 6).

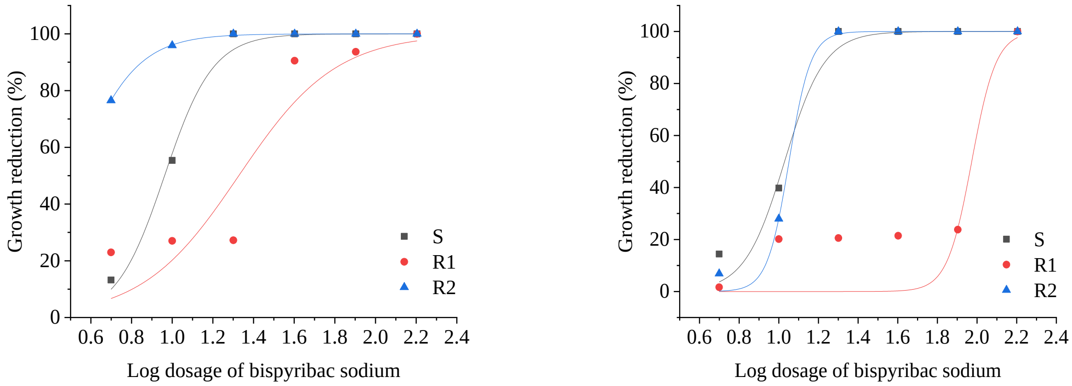
Application of 2,4-D herbicide at the recommended dose showed high growth inhibition of resistant *M. vaginalis* R1 and R2 at 28 DAT, but the weeds were able to recovery. These results had an impact on the GR50 evaluation where biotypes R1 and R2 were included in the low resistance category with GR50 values of 130.51 and 223.33, respectively. The weeds that did not experience death were able to regrow to the level of untreated plants at 56 DAT observation. In addition, biotype R2 weeds experienced good growth in the treatment of 4 times the recommended dose. This had an impact on a significant increase in the GR50 of biotypes R1 and R2 to 1,017.39 and 1,636.45, respectively. This had a highly significant impact on the resistance status of R1 and R2, resulting in an increase in resistance to 2,4-D from low resistance to medium and high resistance (Table 6).

A similar outcome was observed in the case of the bispyribac sodium and penoxulam herbicides application against biotype R1. This consequently increased the GR50 value and the resistance index. The GR50 value after applying bispyribac sodium was 3.34 (3.34 folds greater than the dose that is effective in controlling the susceptible biotype) at 28 DAT but increased to 8.15 at 56 DAT. This significantly impacted the resistance status, leading to an increase from low to moderate. In contrast, the GR50 value for biotype R2 was 0.58 and 1.05 at 28 and 56 DAT, respectively, signifying susceptibility. After penoxulam application, the GR50 values for the R1 and R2 biotypes were 99.44 and 36.73 at 28 DAT. This results in a change in the resistance status from low and susceptible to medium



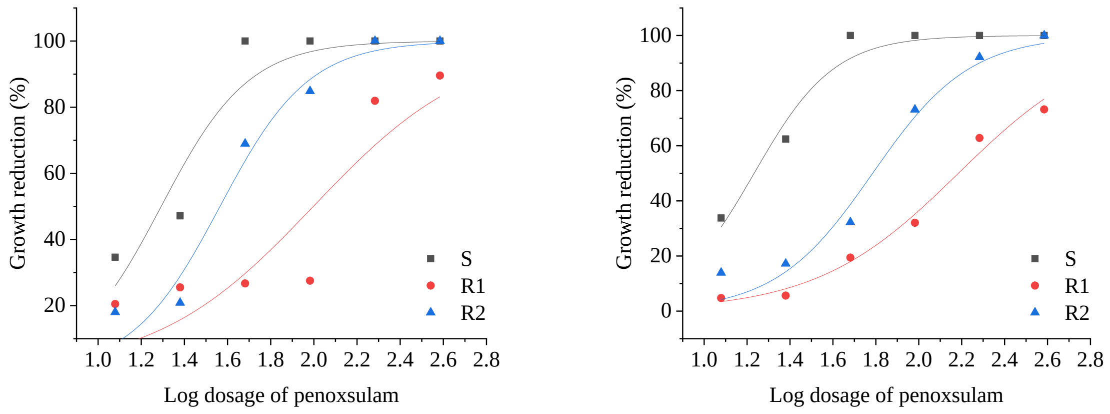
a. b.

Figure 3. Growth reduction observed by dose–response assay of herbicide resistant (R) and susceptible (S) *M. vaginalis* treated with 2,4-D at 28 (a) and 56 (b) DAT



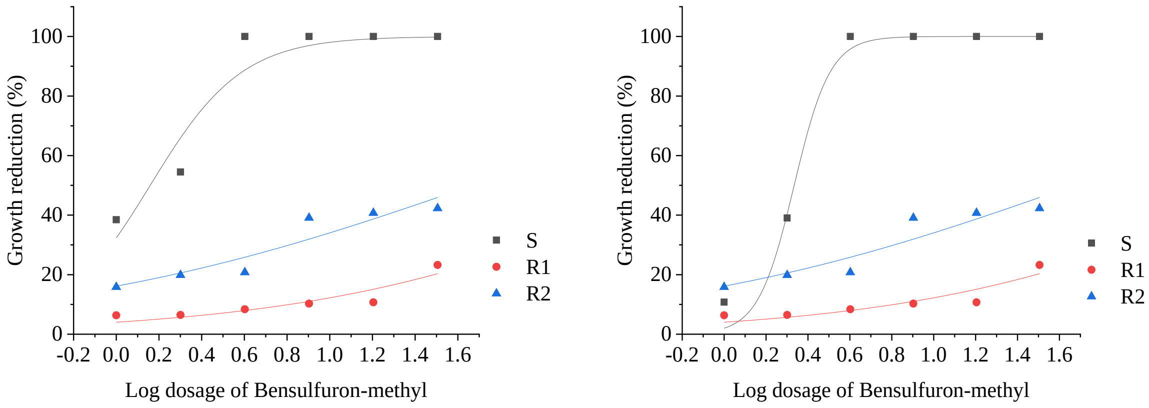
a. b.

Figure 4. Growth reduction observed by dose–response assay of herbicide resistant (R) and susceptible (S) *M. vaginalis* treated with bispirybac sodium at 28 (a) and 56 (b) DAT



a. b.

Figure 5. Growth reduction observed by dose–response assay of herbicide resistant (R) and susceptible (S) *M. vaginalis* treated with penoxulam at 28 (a) and 56 (b) DAT



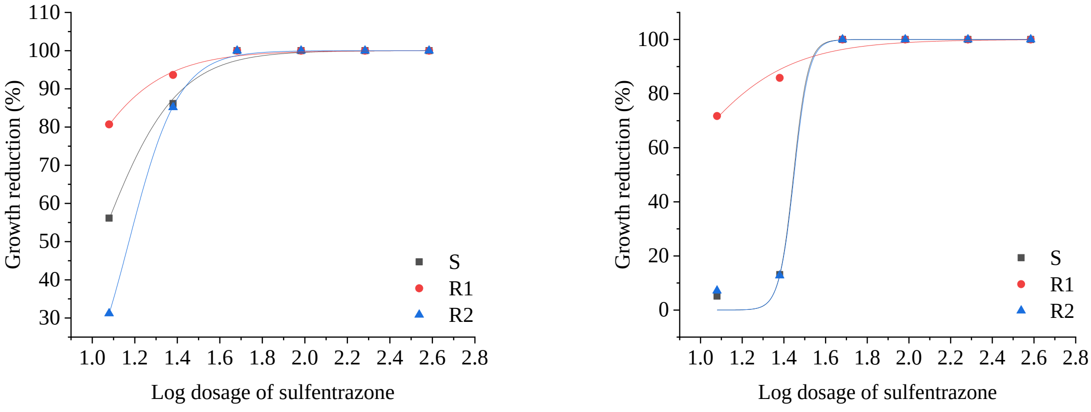
a. b.

Figure 6. Growth reduction observed by dose–response assay of herbicide resistant (R) and susceptible (S) *M. vaginalis* treated with bensulfuron-methyl at 28 (a) and 56 (b) DAT

resistance to penoxulam. Bensulfuron-methyl herbicide application could not suppress the growth of R1 and R2 biotypes, even at the highest dose at 28 DAT. Consequently, there was a significant increase in GR50 and resistance index values between 28 and 56 DAT observations. The R1 biotype had a GR50 value of 26.77 at 28 DAT, which increased to 442.07, while R2 increased from 27.65 to 46.86, as shown in Table 6.

Increased resistance levels were observed only in biotypes resistant to 2,4-D, bensulfuron-methyl, bispyribac sodium, and penoxulam. This phenomenon was not detected in the sulfentrazone application, as there was no evidence of an increase in the resistance index in either biotype. Based on the GR50 obtained, the application of these herbicides at the recommended dose controlled the R1 and R2 biotypes at 28 DAT, with no regrowth observed at 56 DAT. Therefore, sulfentrazone proved to be effective in controlling weed, with multiple resistance to 2,4-D, bensulfuron-methyl, bispyribac sodium, and penoxulam, as shown in Figure 7 and Table 6.

In West Java, Indonesia, an estimated one-and-a-half and a half million hectares of rice fields were potentially infested with noxious weeds such as *M. vaginalis*. This species evades typical paddy weed control measures due to late development, rapid growth under the rice canopy, flooding tolerance, and life cycle completion after rice harvest (Listyowati et al., 2022). The plant was a common weed in East Asian paddy fields (Yokota et al., 2014). The United States lists *M. vaginalis* as a regulated plant pest and



a. b.

Figure 7. Growth reduction observed by dose–response assay of herbicide resistant (R) and susceptible (S) *M. vaginalis* treated with sulfentrazone at 28 (a) and 56 (b) DAT

Table 6. Weed resistance level to each herbicide

Herbicides	Biotypes	28 DAT			56 DAT		
		GR50	Resistance index	Level of resistance	GR50	Resistance index	Level of resistance
2,4-D	S	40.99	-	Susceptible	124.88	-	Susceptible
	R1	130.51	3.18	Low	1,017.39	8.15	Moderate
	R2	223.33	5.45	Low	1,636.45	13.10	High
Bensulfuron-methyl	S	1.44	-	Susceptible	2.15	-	Susceptible
	R1	26.77	18.59	High	442.07	205.61	High
	R2	27.65	19.20	High	46.86	21.80	High
Bispyribac sodium	S	9.17	-	Susceptible	10.69	-	Susceptible
	R1	24.88	2.71	Low	93.99	8.79	Moderate
	R2	4.33	0.47	Susceptible	11.26	1.05	Susceptible
Penoxulam	S	19.61	-	Susceptible	17.09	-	Susceptible
	R1	99.44	5.07	Low	152.87	8.94	Moderate
	R2	36.73	1.87	Susceptible	60.95	3.56	Low
Sulfentrazone	S	10.90	-	Susceptible	27.93	-	Susceptible
	R1	5.98	0.55	Susceptible	7.12	0.26	Susceptible
	R2	14.88	1.37	Susceptible	28.12	1.01	Susceptible

Note: S = Susceptible; R = Resistant. R/S > 12 (High resistance), R/S = 6-12 (Moderate resistance), R/S = 2-6 (Low resistance), R/S < 2 (Susceptible). The resistance level is determined based on the classification according to Ahmad-Hamdani et al. (2012)

a federal noxious weed (USDA, 2010). It is also on the list of prohibited foreign plants in South Africa (Invasive Species South Africa, 2016). *M. vaginalis* can be controlled by synthetic auxin herbicides, including 2,4-D. In this study, a highly resistant population from Kediri and Gandasari was identified for the first time, with resistance index values of 8.15 and 13.10, respectively, as shown in Table 6. The *M. vaginalis* could flower and produce seeds 56 days after applying 2,4-D and ALS inhibitor herbicides.

Herbicide-resistant *M. vaginalis* had the potential to spread to larger areas and threaten the sustainability of Indonesian rice production. The R1 and R2 resistant biotypes originated from Kediri and Gandasari Villages, located in the sub-districts of Cikaum and Binong. These sub-districts were part of the Subang Regency, with a total rice area of approximately 84,570 ha (Rahadian et al., 2024). The data showed that the area potentially invaded by resistant *M. vaginalis* was extensive, from the village to the provincial level. The interference from *M. vaginalis* reduced rice grain yield by 32% (Hazrati et al., 2023). Furthermore, the spread of weeds, which was not confined to West Java Province, occurred through the movement of people, agricultural tools, vehicles, wind, irrigation systems, and contaminated rice seed (Chauhan and Johnson, 2008; Bajwa et al., 2018). This facilitated the spread of resistant weeds to farmland across Indonesia. In addition to seed mobility, the spread of resistant species can occur through pollen, as exemplified by the glyphosate-resistant *Amaranthus palmeri*, which spreads up to 300 m (Sosnoskie et al., 2012).

Pesticides represent the mainstay of crop protection in efforts to safeguard food production, with the potential elimination estimated to reduce yields by 20 to 40% (Lykogianni et al., 2021). The integrated use of herbicides at the appropriate dose and frequency, in combination with other control methods, ensured the continued viability of sustainable agriculture (Soteres et al., 2013). Furthermore, the development of new herbicides with innovative modes of action that offer high efficacy at low doses, minimal environmental impact, and enhanced selectivity is advancing (Umetsu and Shirai, 2020).

Sulfentrazone controlled weeds resistant to 2,4-D, bensulfuron-methyl, bispyribac sodium, and penoxulam. Alternative management strategies are essential to control weeds with multiple resistances. These may include the exclusion of herbicide application or the rotation of different herbicides that can control resistant biotypes, thereby inhibiting the development of higher resistance. Using mixed treatment and rotating different herbicide types in different growing seasons was an effective management strategy (Norsworthy et al., 2012). A wider range of options for rotation decreases the chances of weeds adapting to new products (HRAC, 2017). Based on the results, sulfentrazone can control resistant *M. vaginalis* biotypes as an option for rotational usage.

CONCLUSIONS

M. vaginalis from Kediri and Gandasari Villages, Subang, was identified as moderately and highly resistant to 2,4-D and bensulfuron-methyl. Resistant biotypes were controlled by the PPO inhibitor such as sulfentrazone. In addition to the PPO inhibitor, the ALS inhibitor remained a viable option for the management of *M. vaginalis* from Gandasari. Resistant biotypes showed growth inhibition due to herbicide pressure but were able to regrow to the level of untreated plants. A study on the resistance mechanism to 2,4-D, bispyribac sodium, penoxulam, and bensulfuron methyl was needed to learn more and provide a solid basis for future weed management options. Additionally, trials with different treatments, including mixtures of herbicides, were essential to increase the possibilities for controlling resistant weeds and ensuring proper implementation of the rotation program.

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