# Sulfonylurea Herbicide-Resistant Study on Broadleaf Weeds in The Lowland Rice Production Center in West Java, Indonesia

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## Abstract

Most rice growers in Indonesia use herbicides for weed control. However, intensive use of herbicides can lead to the weeds to become resistant to the chemicals. The objective of this study was to determine the resistance status of weeds Ludwigia octovalvis, Sphenoclea zeylanica, Monochoria vaginalis in lowland rice in West Java, Indonesia. The study was started by planting three species of weeds, L. octovalvis, S. zeylanica, M. vaginalis, which were then treated with metsulfuron-methyl and penoxsulam herbicides. The study was conducted from January to May 2020 at Cikabayan Experimental Greenhouse, IPB University. The experiment was arranged in a randomized complete block design with four replications. The first factor was the origin of weeds, exposed weeds (Karawang and Subang), and not exposed to herbicides (Bogor); the second factor was the rates of the herbicide, i.e., 0, 2.5, 5, 10, 20, 40, 80 and 160 g. ha<sup>-1</sup> for metsulfuron- methyl, and 0, 50, 100, 200, 400, 800, 1600, and 3200 ml.ha<sup>-1</sup> for penoxsulam. The results showed that based on the resistance ratio, S. zeylanica and M. vaginalis from Karawang and Subang, and L. octovalvis from Subang indicated a low resistance to metsulfuron-methyl, L. octovalvis from Karawang was still sensitive to metsulfuronmethyl. L. octovalvis, S. zeylanica, and M. vaginalis from Karawang and Subang were still sensitive to penoxsulam herbicides. This information would be useful to develop a strategy of weed management for important food crops.

Keywords: effective dose, methyl metsulfuron, mortality, penoxulam

### Introduction

Weeds are undesirable plants that are out of place, not intentionally grown, that reduce the production and quality of the cultivated crops (Hussain et al., 2021). Crop yields can still be maintained by controlling the growth of the weeds (Zarwazi et al., 2016). Herbicides are the chemicals used for weed control because they are considered effective and efficient (Travlos et al., 2020). However, the use of herbicides for weed control are currently facing a big challenge, the emergence of resistant weeds.

The presence of herbicide-resistant weeds is an example of a very rapid evolutionary form of how weeds responded to the chemicals (Powles and Yu, 2010). Currently, 262 weed species have been reported to have herbicide resistance; most cases of weed resistance occurred in herbicides of the ALS inhibitor class (Heap, 2018). Every ten years, new weed species are found to be resistant, from 400 biotypes 30% are resistant to acetolactate inhibitors (ALS), 20% are resistant to acetyl-CoA carboxylase (ACCase) inhibitors (Jhala and Knezevic, 2017). Weeds are said to be resistant when the herbicide inhibitors no longer control the target weeds.

Weed resistance is a severe problem in herbicide use that affects farmers, industry, and eventually, the society. Once herbicide resistance occurs, weeds become more challenging to control so that they can cause yield losses (Baucom, 2019). There are a number of herbicide products with various active ingredients in Indonesia, but few had reported the resistant weeds, especially rice weeds. Two weed species in rice had demonstrated herbicide resistance in the Philippines, they are *S. zeylanica* 

which is resistant to 2.4-D, and *E. cruss-galli* which is resistant to butachlor and propanil (Baltazar, 2017). Several broadleaf rice weeds have been reported to be resistant to ALS inhibitors and synthetic auxin herbicides in Malaysia, namely *S. zeylanica, L. flava, S. guayanensis,* and *B. rotundifolia* (Dilipkumar et al., 2020), whereas *C. difformis* and *S. mucronatus* were cross-resistant to several ALS inhibitor herbicides, and this has been reported in California, Italy, and Spain (Kraehmer et al., 2017).

Rice cultivation in West Java primarily uses herbicides to control weeds, so that it is possible that the weeds have become herbicide-resistant. Razo et al. (2019) stated that herbicide-resistant weeds can survive after the application of herbicides, and these traits can be inherited. Directorate General of Agricultural Infrastructure and Facilities (2019) reported 172 types of systemic herbicides in rice plants with various active ingredients. The most active ingredient used in herbicide products in rice is metsulfuronmethyl. In addition to metsulfuron-methyl, there is a relatively recently used sulfonylurea herbicide, namely penoxulam. Metsulfuron-methyl is sold under 75 trademarks, whereas penoxulam is sold under three different trademarks. However, from the many herbicide trademarks currently marketed, there is no information regarding the case of broadleaf weed resistance in lowland rice in Indonesia. Appropriate control measures can prevent more significant losses due to resistant weeds with accurate information about the resistance. This study aims to determine the resistance of the species of broadleaf weeds in lowland rice in West Java to sulfonylurea herbicides.

### **Material and Methods**

The research was conducted at the Cikabayan Experimental Greenhouse, IPB Dramaga, Bogor Regency (6°33'01.6"S 106°42'51.4"E), from January to June 2020. The materials used in this study were seeds of *L. octovalvis, M. vaginalis,* and *S. zeylanica*, which were assumed to be resistant, weed seeds of *L. octovalvis, M. vaginalis,* and *S. zeylanica* which are susceptible, metsulfuron-methyl herbicide, penoxulam herbicide, topsoil.

This study was structured using a factorial design in the form of a complete randomized group design with two factors, namely the area of origin of the weeds, namely weeds exposed to herbicides (Karawang and Subang), and not exposed to herbicides (Bogor). The second factor was the herbicide dose consisting of 8 doses: 0,  $\frac{1}{8}X$ ,  $\frac{1}{4}X$ ,  $\frac{1}{2}X$ , X, 2X, 4X, where X is the recommended dose of herbicide. The doses of metsulfuron-methyl were 0, 2.5, 5, 10, 20, 40, 80,

and 160 g.ha<sup>-1</sup>, and penoxulam were 0, 50, 100, 200, 400, 800, 1600, and 3200 ml.ha<sup>-1</sup>, respectively. Each treatment consisted of four replications.

Weed resistance testing stages refer to the method used in Burgos (2015). The planting medium used was topsoil that has been filtered using wire mesh. Seeds were evenly distributed in trays, with each tray containing 40 weed seeds. Thinning was conducted when the plant has two true leaves until each tray contains 20 plants. Plant watering was conducted every day so that the plants remain in a state of watersufficient. Herbicide application was through foliar application, conducted when the weather was clear. Herbicide application was carried out using a manual knapsack sprayer with a yellow fan nozzle (polijet nozzle). Calibration of the sprayer was carried out to determine the spray volume. Herbicide application was carried out during sunny weather by spraying the entire weed canopy with a spray volume of 250 L.ha-<sup>1</sup>, when weeds have three true leaves. After applying herbicides, the plants were watered every day, and if any weeds grow other than the weeds planted, the weeds would be cleaned. Observations started three days after application by recording the number of surviving plants, the level of damage, effective dose, and weed shoot weight. Measurements of the percentage of mortality were carried out once every two days. The recorded weight was the dry weight of the whole plants of the weeds. Parameters measured were the percentage of mortality, plant dry weight, effective dose (ED<sub>50</sub>) of the herbicide, and resistance ratio.

Dry weight was measured two weeks after treatments by removing weeds from the soil surface. Each weed in each treatment was put into a paper envelope and labeled, then placed in the 80°C oven until the dry weight, then and weighed to calculate their dry weight.

Weed mortality percentage is calculated according to the following formula: Weed mortality percentage (%) =  $\frac{Number of dead weeds}{Number of plant weeds} \times 100\%$ 

Damage percentage (Guntoro and Fitri, 2013):

Damage percentage (%) = (1-(P/K))\*100%

where :

P = value of the dry weight of weeds with herbicide treatment K = dry weight value of control weeds

The resistance ratio (RR) is the value of the  $ED_{50}$  ratio of resistant weeds to susceptible weeds. Based on the resistance ratio, weed resistance levels were classified in the tested species. According to Ahmad-Hamdani et al., (2012), Weeds are classified as high resistance if the RR value > 12, moderate resistance if the RR value > 6-12, low resistance if the RR value

is 2-6, and classified as sensitive if the RR value <2. The value of the effective dose was determined based on the percentage of damage, which were transformed into the probit value with the help of the probit table. The tested dose levels were converted into log form. Based on the probit percent mortality value (Y) and the dose log (X), a simple regression equation was determined Y = aX + b (Guntoro and Fitri, 2013). Data were analyzed by analysis of variance at the level of = 5%. Analysis of variance, which showed significantly different was continued with the DMRT at 95% confidence interval. All calculations were carried out using Microsoft Excel and SAS 9.1.

#### **Result and Discussion**

#### Mortality percentage

The percentage of mortality of each weed species increased with increasing doses of the herbicide metsulfuron-methyl and the herbicide penoxulam (Figure 1). Weeds that were treated with penoxulam demonstrated responses that coincided with each other compared to metsulfuron-methyl. This is because these plants have similar of sensitivity to the herbicide penoxulam, and there is no indication that weeds have shown resistance.

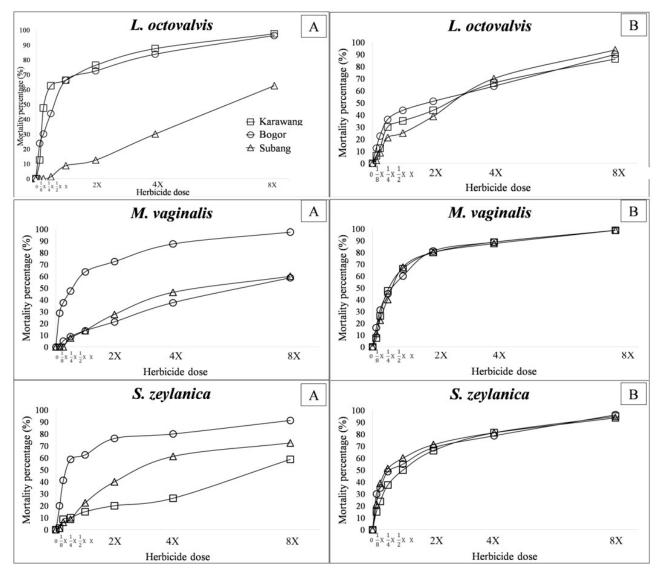


Figure 1. Mortality percentage of *Ludwigia octovalvis, Sphenoclea zeylanica, Monochoria vaginalis vaginalis* to various doses of the metsulfuron-methyl and penoxulam herbicides. X = recommended dose of herbicide; A = metsulfuron-methyl; B = penoxulam

There was a difference in the mortality percentage in each weed species treated with metsulfuron-methyl. *Ludwigia octovalvis* from Karawang, *S. zeylanica*, and *M. vaginalis* from Bogor had the highest percentage of deaths of 97.5%, 97.5%, and 91.25%, respectively. The mortality percentage of *L. octovalvis* from Karawang was higher (97.5%) than *L. octovalvis* from Bogor, indicating that *L. octovalvis* from Karawang has not yet demonstrated resistance symptoms when sprayed with metsulfuron-methyl and penoxulam at the recommended herbicide dosage. According to Heap (2020) there has been no reports of *L. octovalvis* resistance to metsulfuron-methyl and penoxulam.

The percentage of weed mortality can indicate the intensity of herbicide selection carried out in the field (Hada et al., 2020). The highest mortality percentage of *S. zeylanica* and *M. vaginalis* applied to metsulfuron-methyl was from Bogor, a susceptible weed. The highest dose of metsulfuron-methyl could not suppress the growth of *S. zeylanica* and *M. vaginalis* from Karawang and Subang, as opposed to *S. zeylanica* and *M. vaginalis* from Bogor. These results indicate the of resistance symptoms in *S. zeylanica* and *M. vaginalis* from Karawang and Subang to metsulfuron-methyl. The low percentage of weed mortality after being applied with the same herbicide indicated the occurrence of resistance in these weeds (Lubis et al., 2012).

ED50 Value

 $ED_{50}$  value is the effective dose that causes 50% death in the weed population after the herbicide is applied. The higher the  $ED_{50}$  values, the higher the

doses required to cause 50% death. The results of variance analysis showed that the application of metsulfuron-methyl to *L. octovalvis, S. zeylanica*, and *M. vaginalis* from all origins had a significant effect on the ED<sub>50</sub> of metsulfuron-methyl values. In contrast, the application of penoxulam to *L. octovalvis, S. zeylanica*, and *M. vaginalis* from all origins did not significantly affect the ED<sub>50</sub> of penoxulam values (Table 1 and 2).

The results showed that the  $ED_{50}$  value of the herbicide metsulfuron-methyl was higher in some areas of weed origin compared to weeds from Bogor. The origin of weeds that had a higher  $ED_{50}$  value of the herbicide metsulfuron-methyl than Bogor indicated that the weeds demonstrated resistance to the herbicide metsulfuron-methyl. Weeds that indicated resistance to the herbicide metsulfuron-methyl were *L. octovalvis* from Subang, *S. zeylanica*, and *M. vaginalis* from Karawang and Subang intensively in each season by farmers in the area.

Applying one type of herbicide to the weed population that gives satisfactory control does not rule out the possibility that one individual has genes that are resistant to the applied herbicide (Yulivi et al., 2014). Rotation or combining herbicides with different active ingredients and modes of action (MoA) is one of the effective efforts to overcome resistant weeds (Norsworthy et al., 2012).

The application of the herbicide penoxulam to *L. octovalvis, S. zeylanica* and *M. vaginalis* from Karawang and Subang showed that the  $ED_{50}$  value of penoxulam was slightly higher than that of *L.* 

Table 1. ED50 value of metsulfuron-methyl h	herbicide to the weed origin of L. octovalvis, S. zeylanica, and	d <i>M.</i>
vaginalis		

Weed origin	Metsulfuron-methyl				
	L. octovalvis	S. zeylanica	M. vaginalis		
Karawang	8.68b	28.86a	26.30a		
Subang	33.25a	28.28a	18.39ab		
Bogor	8.90b	8.30b	8.61b		

Note: Values accompanied by different letters in the same column show significant differences according to DMRT at  $\alpha$ =5%.

Table 2. EDEDSphenoclea zeylanica,Monochoria vaginalis.

Weed origin	Penoxulam				
	L. octovalvis	S. zeylanica	M. vaginalis		
Karawang	206.33	236.49	280.61		
Subang	298.82	246.46	204.52		
Bogor	258.31	209.74	196.16		

*octovalvis, S. zeylanica,* and *M. vaginalis* from Bogor. However, these weeds could still be controlled by using the recommended herbicide dosage.

#### Resistance Ratio

The results showed that S. zeylanica and M. vaginalis from Karawang and Subang and L. octovalvis from Karawang, which were applied to the herbicide metsulfuron-methyl, had low resistance (Table 3). Based on Table 3, the administration of the herbicide metsulfuron-methyl at the recommended dose in Karawang and Subang has not been able to control S. zeylanica and M. vaginalis. The ineffectiveness of the herbicide metsulfuron-methyl in suppressing the growth of S. zeylanica and M. vaginalis indicated that the weed demonstrated resistance to this chemical. Beckie and Tardif (2012) suggested that a dependence on one type of herbicide and continuous use of herbicides with the same mode of action (MoA) can cause weeds to become resistant. ALS inhibitor herbicide resistance occurred after several years of intensive herbicide uses (Knezevic et al., 2017). Yang et al. (2021) added that the widespread population of multiple herbicide-resistant E. crus-galli in Ningxia Province, China, had shown resistance to several ALS and ACCase inhibitors. Non-target site-based mechanisms are most likely involved in the weed resistance to herbicides.

The weed resistance symptoms of *L. octovalvis*, *S. zeylanica*, and *M. vaginalis* is affected by rice cultivation techniques carried out by the growers. Based on our survey of the rice growers in the area, farmers in Karawang and Subang have been using

metsulfuron-methyl herbicide for approximately 15 to 20 years continuously without rotation with other herbicides, because this chemical has been effective to control weeds in their rice fields. The use of inappropriate herbicide doses by farmers is also one the causes of weed resistance in Karawang and Subang. Farmers tend to use doses that are higher than the recommended herbicide doses to control weeds.

Ludwigia octovalvis, S. zeylanica, and M. vaginalis from Karawang and Subang were still sensitive to the herbicide penoxulam (Table 4), so herbicide penoxulam at the recommended dose can still be used to control L. octovalvis, S. zeylanica, and M. vaginalis from Karawang and Subang. Umiyati et al. (2020) suggest that using herbicide with the active ingredient penoxulam 25 g.L<sup>-1</sup> at a dose of 10 g.ha<sup>-1</sup> was able to suppress the growth of broadleaf weeds such as *L. octovalvis* and *S. zeylanica*. Green and Owen (2011) added that controlling resistant weeds is more complicated than weeds that are not resistant. Therefore, rotation or alternation of selective herbicides with active ingredients and different modes of action (MoA) for weed control is essential to reduce the emergence of resistant weeds. Dong et al. (2021) added that the development of herbicide-resistant crops is also being developed to be an effective strategy for controlling resistant weeds because it can reduce plant phytotoxicity and broaden the herbicide spectrum. Biotypes of weed that had become resistant to ALS inhibitors will be very hard to control. Integrated control for resistance management need to be developed to control broadspectrum cross-resistant weeds (Fang et al., 2019).

Table 3. The resistance ratio of *Ludwigia octovalvis, Sphenoclea zeylanica, Monochoria vaginalis* from different weed origins to metsulfuron-methyl herbicide.

Weed origin	L. 0	L. octovalvis S. zeylanica		M. vaginalis		
	RR	Response	RR	Response	RR	Response
Karawang	0.97	S	3.48	LR	3.05	LR
Subang	3.73	LR	3.41	LR	2.14	LR
Bogor	1		1		1	

Note: RR = Resistance ratio, S = sensitive, LR = low resistance

Table 4. The resistance ratio of *Ludwigia octovalvis, Sphenoclea zeylanica, Monochoria vaginalis* from different origin to penoxulam herbicide.

Weed origin	L. 0	L. octovalvis		S. zeylanica		M. vaginalis	
	RR	Response	RR	Response	RR	Response	
Karawang	0.80	S	1.13	S	1.43	S	
Subang	1.16	S	1.18	S	1.04	S	
Bogor	1		1		1		

Note: RR = Resistance ratio, S = sensitive

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Based on the survey conducted in the field, herbicide penoxulam is still rarely used by farmers because the level of confidence of farmers to metsulfuron-methyl is very high. Therefore, farmers are reluctant to replace the herbicides they have been using for a long time, so weeds *L. octovalvis, S. zeylanica,* and *M. vaginalis* are still sensitive to the herbicide penoxulam.

Metsulfuron-methyl and penoxulam herbicides are systemic herbicides with a mode of action inhibiting the formation of the enzyme acetolactate synthase (ALS) (Tang et al., 2021), which are readily absorbed by both roots and foliage and translocated in both the xylem and phloem to the site of action at the growing points (Anderson, 2002). Acetolactate is a key enzyme in the pathway of biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine, Plant deaths result from events in response to inhibition of branched-chain amino acids, but the actual sequence of phytotoxic processes is still unclear (Jugulam and Shyam, 2019). Systemic herbicides in plants are transported or translocated to the growing point, and changes in the target site usually triggerred the mechanism of weed resistance to ALS inhibitor herbicides (Pangestuning et al., 2017). Several point mutations within the gene encoding ALS can result in a varying degrees of herbicide-resistant ALS (Matzrafi et al., 2020).

The soil used in this study was from the Cikabayan Experimental Station, Darmaga, Bogor. Based on the report of Santana et al. (2020), the soil in the Cikabayan is classified as Latosol with clay content >60%, acidic with a pH value of 4.56, and has low organic matter content of 1.52%. Herbicides are usually more persistent in soils with low or alkaline organic matter and less in sandy soils.

The uses of herbicides can potentially harm the environment; the field study unveiled an acute decrease in biomass and organic matter soil degradation after prolonged uses of herbicide (Mada et al., 2013). The continuous application of herbicides inhibit the development of microbial populations and reduce the nutrients and organic materials of the soil (Hazanuzzaman, 2019). Herbicides can reach aquifers below ground from applications onto crop fields, and contaminate rivers and ponds through seepage of contaminated surface water (Mada et al., 2013). The soil in Karawang and Subang is acidic (Rachman et al., 2020); excessive accumulation of acid and base ions due to residues caused by herbicides can reduce the soil's buffering capacity, resulting the soil to become even very acidic.

The results of this study has provided a status of weed herbicide resistance in the rice farms in West Java,

and would be useful to design strategies to minimize the development of weed resistance.

# Conclusion

In the rice production center in West Java, we found indications of low resistance *L. octovalvis, M. vaginalis, S. zeylanica* of Subang, and *M. vaginalis, S. zeylanica* from Karawang, to the herbicide metsulfuron-methyl. There was no indication of the resistance of the examined weed species, both from Karawang and Subang, to the herbicide penoxulam. *Ludwigia octovalvis* from Karawang is still classified as sensitive to methyl metsulfuron and penoxulam indicated by their low resistance ratio. Strategic steps need to be taken by designing and socializing integrated weed management programs by combining various weed control methods that can be quickly adopted by the farmers.

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