

ORIGINAL ARTICLE

Confirmation of resistance *Monochoria vaginalis* (Burm. f.) C. Presl from West Java and Lampung Indonesia to bensulfuron-methyl herbicide

Denny Kurniadie^{1*}, Ryan Widiyanto³, Dedi Widayat¹, Uum Umiyati¹, Ceppy Nasahi²

¹ Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, Bandung, Indonesia

² Department of Pest and Diseases, Faculty of Agriculture, Universitas Padjadjaran, Bandung, Indonesia

³ Graduate student, Faculty of Agriculture, Universitas Padjadjaran, Bandung, Indonesia

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*Corresponding address:
denny.kurniadie@unpad.ac.id

Abstract

Several national rice centers in Indonesia have used acetolactate synthase herbicide inhibitors for years, especially in several regions of Lampung and West Java provinces. This practice has led to the failure of the application of bensulfuron-methyl herbicide to control *Monochoria vaginalis* (Burm. f.) C. Presl. The purposes of this study were to confirm that the failure of herbicide application in several areas of the provinces of Lampung and West Java was caused by weed resistance, and to determine the level of resistance. A resistance test of *M. vaginalis* was performed using the whole plant pot test method and split plot design with three replications. *Monochoria vaginalis* which indicated resistance was sampled from several regions, namely Supto Mulyo, Ramadewa, Sarijaya, and Kalentambo. The susceptible samples of *M. vaginalis* as a control were taken from Cibodas and Sumberagung. The six levels of doses of herbicide bensulfuron-methyl used were: 0, 80, 160, 320, 640 and 1,280 g · ha⁻¹. The experimental results show that *M. vaginalis* from Supto Mulyo, Ramadewa, Kalentambo and Sarijaya was confirmed to have developed into weeds resistant to bensulfuron-methyl herbicide. *Monochoria vaginalis* from Suptomulyo, Kalentambo and Sarijaya were included in the high resistance category with a resistance ratio of more than 12, while *M. vaginalis* from Ramadewa was included in the moderate resistance category with a resistance ratio of 9.39.

Keywords: resistance ratio, rice, weed

Introduction

Weeds are still an important problem in agricultural production. On agricultural land, the presence of weeds is considered to be a threat because it can reduce the productivity of the land (Zimdahl 2018). In rice plants, yield losses caused by weeds may reach 50–60% in puddled transplanted rice and 70–80% in direct seed rice (Dass *et al.* 2017). Crop yield losses can be reduced by weed control. According to Tu *et al.* (2001), weed control techniques can be carried out by prevention, physically, biologically and chemically. Chemical weed control using herbicides is still a mainstay of farmers,

especially in Indonesia, because herbicides have a high level of efficiency and effectiveness (Sigalingging *et al.* 2014). Herbicides are considered to be more profitable than other control techniques because they can suppress weed growth more quickly, need less labor, are relatively inexpensive, and do not directly damage the physical structure of the soil (Clout and Williams 2009).

The use of herbicides with the same active ingredients over a long period of time will cause resistance. Herbicide resistance occurs because of the selection pressure that starts from random mutations in plants. Mutations cause changes in the composition of genes so that weeds that are initially susceptible to herbicides

turn resistant. Susceptible weeds die more easily when exposed to herbicides, while resistant weeds will be more adaptive to herbicides so they can survive and dominate a population (Qasem 2013). According to Beckie *et al.* (2006), the main factor driving the formation of resistant weed populations is the continuous use of herbicides with the same active ingredient on a land, causing the resistant weeds to dominate. The problem of weed resistance has become a problem of the agricultural sector in each country. In addition to the continuous and intensive use of herbicides with the same active ingredients, farmers' dependence on one control technique, the use of improper doses, and the same planting system also promote quick selection of resistant weeds (Menne and Köcher 2008).

Herbicide bensulfuron-methyl is a selective post-emergence herbicide. This herbicide has an enzyme acetolactate synthase (ALS) inhibitor which can be applied through leaves and soil/roots. The target weeds of this herbicide are broad leaf weeds and sedges in direct rice planting and transplanted rice planting systems (Lee *et al.* 2004). The ALS herbicide that is commonly used in paddy fields in Indonesia is bensulfuron-methyl. One of the broad leaf weeds that can be controlled by this herbicide is *M. vaginalis*. It was found to be resistant to the sulfonylurea herbicide in two different paddy fields in Akita and Ibaraki, Japan (Koarai and Morita 2002). In Indonesia, *M. vaginalis* is still not officially registered as a resistant weed in paddy fields. Some rice fields in rice production centers in Indonesia have been using the herbicide bensulfuron-methyl for more than 10 years, which allows *M. vaginalis* to be resistant to the bensulfuron-methyl herbicide.

The provinces of Lampung and West Java are among the top five provinces that have the largest paddy fields

and are the national centers of rice in Indonesia (Sub-directorate of Food Crops Statistics 2018) The use of herbicides has become part of rice cultivation activities, especially the continuous use of ALS herbicides during the last 10 years. Based on reports from farmers from Sapto Mulyo village, Kota Gajah District, Central Lampung Regency, Ramadewa village, Seputi Raman District, Central Lampung Regency, Lampung Province, Sarijaya village, Majalaya District, Karawang Regency and Kalentambo village, Pusakanegara District, Subang Regency, West Java Province, broad leaf weed *M. vaginalis* can no longer be controlled with the bensulfuron-methyl herbicide.

The purposes of this study were to see the extent of resistance of *M. vaginalis* (Burm. f.) C. Presl weeds from several regions in Lampung and West Java provinces against the bensulfuron-methyl herbicide and to determine the level of resistance of *M. vaginalis*.

Materials and Methods

The material used was *M. vaginalis* weed from low-land rice plantations with high frequency of herbicide use (Fig. 1). The weed *M. vaginalis* originated from Sapto Mulyo village, Kota Gajah District, Central Lampung Regency, Lampung Province (5°02'19.9"S, 105°18'38.9"E), Ramadewa village, Seputi Raman District, Central Lampung Regency, Lampung Province (4°55'41.5"S, 105°22'35.8"E), Sarijaya village Majalaya District, Karawang Regency, West Java province (6°16'34.0"S, 107°22'03.7"E), and Kalentambo village, District of Pusakegara, Subang Regency, West Java Province (6°15'32.8"S, 107°53'34.6"E). The susceptible



Fig. 1. Map of sample locations of *Monochoria vaginalis*

biotype of *M. vaginalis* from lowland rice plantations that do not use herbicides were taken from Cibodas village, Selokan Jeruk District, Bandung, West Java (7°00'15.3"S, 107°45'34.3"E), and the susceptible biotype of *M. vaginalis* from lowland rice plantations with low frequency of herbicide use were taken from Sumberagung village, Godong District, Grobogan Regency, Central Java Province (7°04'25.7"S, 110°47'41.8"E).

The research consisted of two stages, namely: (1) preliminary research in the form of a field survey and seed collection, and (2) a weed resistance level test to determine the level of weed resistance to bensulfuron-methyl herbicide. Seed collection was conducted by moving the weed samples to a greenhouse and keeping them until they produced seeds. Then the seeds were collected. Seed collection was conducted in the greenhouse of the Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, Sumedang Regency, West Java from January to May 2019, whereas further research to determine the weed resistance level was conducted at the Syngenta Research and Development Station, village of Jatisari, Sarijaya Regency, West Java from July to August 2019.

The resistance level test was performed by the Whole Plant Pot Test method (Burgos 2015). The experimental design used was a split plot design consisting of two factors with three replications for each experiment. The first factor was the origin of weeds as the main plot consisting of six locations of weeds, namely: G_0 (*M. vaginalis* from Cibodas), G_1 (*M. vaginalis* from Sumberagung), G_2 (*M. vaginalis* from Sapto Mulyo), G_3 (*M. vaginalis* from Ramadewa), G_4 (*M. vaginalis* from Sarijaya) and G_5 (*M. vaginalis* from Kalentambo). The second factor was the dose of bensulfuron-methyl 20% as a subplot consisting of six dosage levels, namely: $0 \text{ g} \cdot \text{ha}^{-1}$; $80 \text{ g} \cdot \text{ha}^{-1}$; $160 \text{ g} \cdot \text{ha}^{-1}$; $320 \text{ g} \cdot \text{ha}^{-1}$; $640 \text{ g} \cdot \text{ha}^{-1}$, and $1,280 \text{ g} \cdot \text{ha}^{-1}$. Weed seeds (50 seeds) both exposed and not exposed to herbicides from each location were germinated in pots containing paddy soil. The weeds were then thinned, leaving 5 samples of weeds per pot. In order to break the dormancy, weed seeds of *M. vaginalis* needed to be stored in a refrigerator at 4°C for 1 month. After weed seeds germinated and 2.5 leaves appeared, weeds were treated with herbicides. Spraying was done by using a T-boom sprayer with a pressure of 138 kPa using a flat fan nozzle on a plot ($2 \times 2 \text{ m}$). Weeds were arranged randomly and then sprayed evenly according to the treatment dose. Then they were arranged in a greenhouse and dry weight was observed 20 days after herbicide application.

Weed dry weights were obtained by weed destruction for each unit of experiment. *Monochoria vaginalis* was dried in an oven at 80°C for 48 h until the dry weight was constant, and was then weighed. Weed dry weight data obtained was converted to a percentage of

growth reduction (GR) by comparing the dry weight value of *M. vaginalis* applied by herbicide (P) with *M. vaginalis* without the application of herbicide (K) in each region of origin using the following equation:

$$GR = [1 - (P/K)] \times 100\%.$$

The difference response due to the treatment average was tested by *F* test, and if the difference in treatment mean had a significant effect, then the test was continued with Duncan's Multiple Range test at the 5% level.

The GR_{50} value (growth reduction with a probability of 50%) can be calculated by using dose response log-logistic non-linear regression (Seefeldt *et al.* 1995):

$$y = C + \frac{D - C}{1 + \left(\frac{x}{GR_{50}}\right)^b},$$

where: *C* – lower limit; *D* – upper limit; *b* – slope; GR_{50} – dose required to give 50% effect.

Resistance ratio was used to determine the level of resistance of a weed. Calculating resistance ratios was done by comparing GR_{50} *M. vaginalis* originating from areas exposed to high frequency of herbicide use (G_2, G_3, G_4 and G_5) with GR_{50} of *M. vaginalis* of susceptible biotype (G_0 and G_1). If the resistance ratio is known, then the level of resistance of each weed that comes from areas exposed to herbicides can be calculated. According to Ahmad-Hamdani *et al.* (2012) the level of resistance is classified into several classes based on the value of the resistance ratio (*R/S*), namely: high resistance (*R/S* >12), moderate resistance (*R/S* = 6–12), low resistance (*R/S* = 2–6), and susceptible (*R/S* <2).

Results

Growth reduction percentage

Table 1 shows that *M. vaginalis* G_0 and G_1 had the highest average of growth reduction percentage (100%) with the application of $80 \text{ g} \cdot \text{ha}^{-1}$ herbicide bensulfuron-methyl, whereas *M. vaginalis* G_5 showed the lowest average of growth reduction percentage. The growth reduction of G_5 ranged from 17.12% at the lowest dose ($80 \text{ g} \cdot \text{ha}^{-1}$) to 45.54% at the highest dose ($1,280 \text{ g} \cdot \text{ha}^{-1}$). This shows that G_5 could not be controlled despite using the highest dose (Table 1). The growth reduction of G_2, G_3, G_4 and G_5 had a low percentage, less than 50%, of growth reduction with a treatment dose of $80 \text{ g} \cdot \text{ha}^{-1}$. This means that *M. vaginalis* G_2, G_3, G_4 and G_5 could not be controlled with bensulfuron-methyl herbicide at recommended doses.

Table 1. The effect of origin of *Monochoria vaginalis* weed and doses of bensulfuron-methyl herbicide on the average growth reduction percentage of *M. vaginalis* (%)

<i>Monochoria vaginalis</i>	Dose of herbicide [g · ha ⁻¹]					
	0	80	160	320	640	1,280
G ₀	0.00 a B	100.00 c A	100.00 c A	100.00 c A	100.00 c A	100.00 c A
G ₁	0.00 a B	81.48 c A	85.00 c A	100.00 c A	100.00 c A	100.00 c A
G ₂	0.00 a A	17.68 b AB	22.59 a AB	32.95 ab B	65.08 bc C	81.34 bc C
G ₃	0.00 a A	23.37 b B	48.28 b C	60.90 bc C	62.33 b C	85.73 bc D
G ₄	0.00 a A	6.57 a AB	19.97 c B	20.54 a B	25.43 a B	64.51 ab C
G ₅	0.00 a A	17.12 b AB	32.23 ab BC	38.94 ab BC	40.98 b C	45.54 a C

Numbers followed by the same letter do not differ significantly according to the Duncan test at 5% level. Lowercase letters are read vertically (columns) and uppercase letters are read horizontally (lines)

GR₅₀ and resistance ratio

GR₅₀ is a parameter that can provide information about the herbicide dosage which can control weed *M. vaginalis* to achieve 50% growth reduction. Dose response analysis was carried out globally with a lower limit (C) of 6.57 and an upper limit (D) of 100. Table 2 shows that *M. vaginalis* G₁ required a dose of 28.7 g · ha⁻¹, G₂ required a dose of 363.1 g · ha⁻¹, G₃ required a dose of 489.8 g · ha⁻¹, G₄ required a dose of 1,078.9 g · ha⁻¹, and G₅ required a dose of 1399.6 g · ha⁻¹ to obtain 50% growth reduction. GR₅₀ of *M. vaginalis* G₁ could not be determined because the percentage of growth reduction at the lowest dose was 100%. The resistance ratio was then obtained by comparing the GR₅₀ of *M. vaginalis* G₂, G₃, G₄ and G₅ with GR₅₀ of *M. vaginalis* G₁.

Based on the resistance ratio obtained from the comparison of GR₅₀ of resistant to susceptible *M. vaginalis*, it is known that *M. vaginalis* G₂, G₃, G₄ and G₅ had resistance ratios of 12.6; 17.07; 37.59 and 48.76, respectively, so they are categorized as weeds with a high level of resistance (Ahmad-Hamdani *et al.* 2012). Figure 2 shows that the growth percentage reduction

from resistant *M. vaginalis* was lower than susceptible *M. vaginalis*. This illustrates that after the application of bensulfuron-methyl herbicide, resistant *M. vaginalis* could maintain its growth rate.

Discussion

Growth reduction percentage illustrates how much the bensulfuron-methyl herbicide can reduce the growth rate of *M. vaginalis* from each regional origin. Weeds from areas that are accustomed to using herbicides are more difficult to control than weeds from areas that do not or rarely use herbicides. Weed growth reduction percentage is different in each location and is influenced by several factors such as history of land use, farmers' practices in using herbicides, herbicide application frequency, and cropping patterns applied in each location of weed origin. *M. vaginalis* of G₄ and G₅ had the smallest growth reduction percentage compared to *M. vaginalis* originating from other locations. This might

Table 2. Analysis of weed resistance level of *Monochoria vaginalis* from various locations

<i>Monochoria vaginalis</i>	B	r ²	C	D	GR ₅₀ [g · ha ⁻¹]	Resistance ratio	Level of resistance (Ahmad-Hamdani <i>et al.</i> 2012)
G ₀	–	–	–	–	–	–	susceptible
G ₁	1.26	0.79	6.57	100	28.77	–	susceptible
G ₂	1.47	0.97	6.57	100	498.29	17.32	high
G ₃	0.92	0.89	6.57	100	270.10	9.39	moderate
G ₄	1.72	0.86	6.57	100	1,073.13	37.30	high
G ₅	0.46	0.96	6.57	100	2,066.66	71.83	high

C – lower limit; D – upper limit; GR₅₀ – growth reduction with probability of 50%
The value of GR₅₀ tested using non-linear regression log-logistic dose response (Seefeldt 1995)

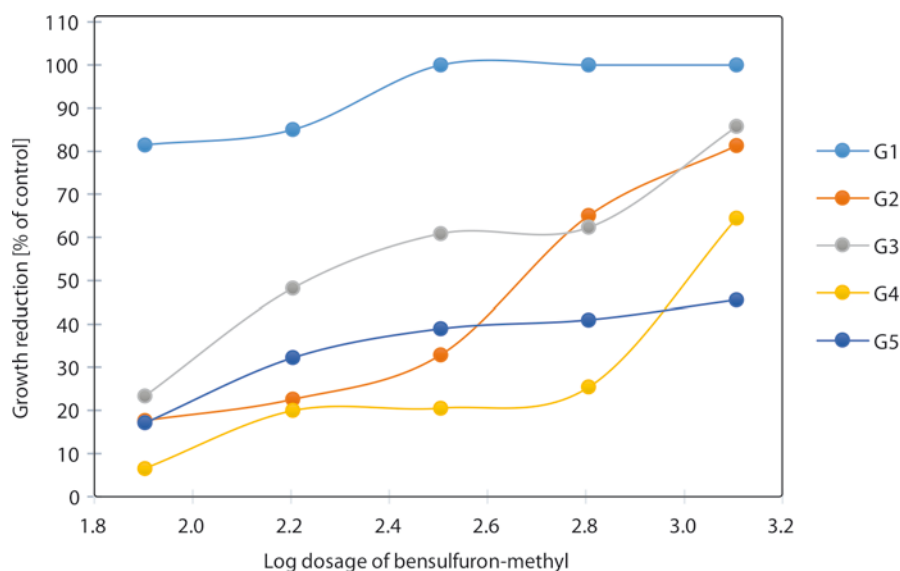


Fig. 2. Growth reduction percentage of susceptible and resistant *Monochoria vaginalis* (G1–G5) to bensulfuron-methyl herbicide 20 days after application

be due to the higher intensity of herbicide application in both areas. According to interviews with farmers, the paddy fields at those two locations used herbicides twice during each planting season. Annually there are three rice crops, so the frequency of herbicide application at these locations was higher than at other locations.

Areas that intensively use herbicides for a long time do not kill 100% of weeds, and there will be a small portion of weeds that can survive and evolve into resistant weed biotypes (Manik 2019). Nandula (2016) stated that the repeated use of herbicides having the same mode of action with no rotation can trigger the occurrence of *M. vaginalis* resistance to ALS (acetolactate synthase) enzyme inhibiting herbicides. In addition, Baucom (2019) reported that natural selection processes where genetic mutations occur naturally in plants due to continuous use of similar herbicides will stimulate weed resistance. Weeds that survive will persistently reproduce, whereas weeds that are susceptible to herbicides will die in the presence of herbicides (Vrbničanin *et al.* 2017). In general, based on the dry weight of weeds, weeds that are exposed to herbicides have a lower average of weed growth reduction than weeds that are not exposed to herbicides. The higher the dose of herbicide, the higher the inhibitory effect on weed growth causing, a decrease in dry weight (Majd *et al.* 2019). According to Costa and Rizzarda (2014), the dry weights of resistant weed *Raphanus raphanistrum* are much higher than susceptible ones. The decrease in weed dry weight is related to the ability of bensulfuron-methyl to inhibit the formation of the ALS enzyme. ALS enzymes play an important role in the formation of amino acids leucine, valine, and isoleucine. If the work of the ALS enzyme is inhibited, the metabolic process in *M. vaginalis* weeds is disrupted,

causing the weeds to have decreased growth due to poisoning and even death. Inhibition of the formation of ALS enzymes can also cause decreased biomass and decreased seed production in *Lactuca serriola* (Alcocer-Ruthling *et al.* 1992) and *Amaranthus powellii* (Tardif *et al.* 2006).

In the long run weed resistance levels will continue to increase along with the use of herbicides having the same mode of action (Vrbničanin *et al.* 2017). It has been reported that *M. vaginalis* has become resistant to bensulfuron-methyl in China, Japan, and South Korea (Heap 2020). Kuk *et al.* (2003) reported that *M. vaginalis* originating from monoculture rice cultivation in Chonnam, South Korea proved to be resistant after being tested in vitro on all four types of ALS enzyme inhibiting herbicides with a resistance ratio of 183 for imazosulfuron herbicide, 130 for cyclosulfamuron herbicide, 35 for bensulfuron-methyl herbicide, 183 for imazosulfuron herbicide, 130 for cyclosulfamuron herbicide, 35 for bensulfuron-methyl herbicide, and 31 for ethyl pyrazosulfuron herbicide. ZongZhi *et al.* (2009) stated that *Monochoria korsakowii* is resistant to bensulfuron-methyl in two different locations, namely in Dehui and Liuhei, China, with resistance ratios of 6 and 13.6 respectively. The resistant type of *M. vaginalis* showed high levels of cross-resistance to pyrazosulfuron-ethyl, bensulfuron-methyl, cyclosulfamuron, and flumetsulam, but can controlled by imazaquin (Hwang *et al.* 2001). Determination of resistance levels is important, because it will provide an overview of the mechanism of resistance that occurs, so that appropriate strategies can be formulated to control weed resistance (Burgos 2015). Rotating the use of herbicides with different modes of action can also prevent the occurrence of herbicide resistance. According to

Hwang *et al.* (2001), *M. vaginalis* that is resistant to ALS enzyme inhibiting herbicides can be controlled by rotating herbicides which have different modes of action, such as 2,4-D, simazine, and oxadiazon.

Conclusions

Monochoria vaginalis originating from Sapto Mulyo, Ramadewa, Sarijaya, and Kalentambo areas were classified as weeds with a high level of resistance, whereas *M. vaginalis* originating from the villages of Cibodas and Sumberagung were included in the weed category which is susceptible to the herbicide bensulfuron-methyl.

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