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Original Article

Resistance of *Monochoria vaginalis* to Bensulfuron-methyl in Central Java and East Java Indonesia

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Abstract

Monochoria vaginalis is one of the dominant weeds in rice crop fields of Central Java and East Java Indonesia and it failed to be controlled by herbicides with the active ingredient bensulfuron-methyl. The research was carried out using split plot-design with the dosage of bensulfuron-methyl as a main plot (0, 80, 160, 320, 640 and 1280 g a.i. ha^{-1}) and *Monochoria vaginalis* from 5 areas as a subplot. The research was done from January until August 2019. Resistance ratio test of *Monochoria vaginalis* was performed using the whole plant pot test method. Results showed that *Monochoria vaginalis* originating from Kedaton, Tambakrejo, and Plumpang villages Indonesia was confirmed to have developed into weeds resistant to the methyl bensulfuron herbicide.

Keywords: weeds, *Monochoria vaginalis*, herbicide resistance, ALS

1. Introduction

The use of herbicides in Central Java and East Java Indonesia has been carried out for more than ten years. Rice productivity both in Central Java and East Java was about 9,51 and 10,53 million tons, respectively, in 2018. According to this result, Central Java and East Java become the rice production centers with the highest productivity in Indonesia (Subdirectorate of Food Crops Statistics, 2018). High yields of rice in Central Java and East Java were due to intensive application of herbicides. One of the herbicide that is often used is the mode of action of ALS (Acetolactate synthase) enzyme inhibitors. Bensulfuron-methyl belongs to the sulfonylurea herbicide group that inhibits the activity of ALS enzyme bensulfuron-methyl, a main enzyme in biosynthesis of branched chain amino acids such as valine, leucine, and isoleucine. Zimdahl (2018) reported that ALS enzyme inhibiting herbicides are the most widely used to control many species of weeds in crop fields. More than 90 herbicides of ALS enzyme inhibitor group have been widely used in Indonesia, such as bensulfuron, methyl metsulfuron, ethyl pyrazosulfuron, triaculfuron, and sodium bispiribak (Directorate of Fertilizers and Pesticides, 2019).

M. vaginalis is a broadleaf weed that is often found in the lowland rice fields (*Oryza sativa* L.) in Indonesia and has a history of resistance to ALS enzyme inhibiting herbicides. *M. vaginalis* was identified to be resistant to bensulfuron-methyl in a Japanese rice cultivation area (Hamamura *et al*., 2003). Kuk *et al*. (2003) reported that *M. vaginalis* is resistant to bensulfuron-methyl, cyclosulfamuron and pyrazosulfuron ethyl in rice fields that intensively use herbicides in Chonnam Korea. ALS enzyme inhibiting herbicides have been used for at least more than ten years to control weed of *M. vaginalis*. The use of herbicides with the

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same mode of action without alternate use of herbicides can trigger the occurrence of *M. vaginalis* resistance to ALS enzyme inhibiting herbicides (Nandula, 2016). The nature of weed resistance to herbicides is a trait that can be derived, genes that express resistance can be distributed through pollen so that similar weeds around it can produce seeds that carry resistant properties (Jugulam & Godar, 2013).

Weed identification to confirm that the weeds are truly resistance to herbicide is required before determining steps should be taken to control weed resistance, so the control management can be carried out effectively and on target. Management of weed resistance is not carried out properly can lead to wider weed resistance distribution and the emergence of weeds with higher resistance can even cause weeds to become multiple resistance (CropLife, 2017). In addition, identification of resistant weeds must be done immediately to prevent the spread of resistant weeds to a wider area. Duary (2014) reported that mobility of weed seeds is very high; the risk of spreading of weed seeds is higher in irrigation-based agricultural areas, and weed seeds carried by irrigation water flow will cause the distribution of resistant weeds to continue to expand. Some species of weeds resistant to glyphosate that occur in New South Wales Australia are not caused by the frequency of herbicide use, but due to mobility of seeds and pollen (Stanton *et al*., 2004).

The purpose of this research was to determine the weed resistance of *M. vaginalis* in Central Java and East Java Indonesia and to find out the level of weed resistance. The information is very important to measure the current status of *M. vaginalis* resistance from ALS enzyme inhibiting herbicide. The results can be used to develop potential weed management strategies for controlling weed resistance.

2. Materials and Methods

2.1 Collection of seed samples

The locations of survey and seed samples are collected based on information from farmers who reported that *M. vaginalis* could not be controlled by the bensulfuron-methyl herbicide, while sensitive *M. vaginalis* was taken from rice fields that never used herbicides. There were five locations were focused to collected seed samples, including three predicted resistant *M. vaginalis* sampling locations, namely: R_1 (Kedaton Village, Kapas District, Bojonegoro Regency, East Java, 7°11'52.9″S, 111°55'37.2″E), R_2 (Tambakrejo Village, Pemalang District, Pemalang Regency, Central Java, 6°53'44.4" S, 109°21'12.2' 'E), and R_3 (Plumpang Village, Plumpang District, Tuban Regency, East Java, 7°02'41.5″ S, 112°05'41.5″ E) (Figure 1). Sensitive *M. vaginalis* was taken from two locations, the first location is rice fields that have never used herbicides (S_0) and the second location is rice fields that exposed herbicides (S_1). Rice fields that have never used herbicides are located in Cibodas Village, Solokan Jeruk District, Bandung Regency, West Java (7°00'15.3" S, 107°45'34.3″ E), and rice fields that exposed herbicides located in Sumberagung Village, Godong District, Grobogan Regency, Central Java (7°04'25.7″ S, 110°47'41.8″ E) (Figure 1).

At all locations a survey was carried out to obtain information about *M. vaginalis* weeds which were predicted to be resistant to ALS inhibiting herbicides in the rice fields.

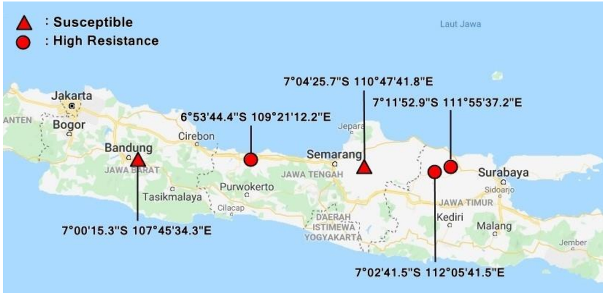

Figure 1. Map of the spread of *M. vaginalis* resistance to bensulfuron-methyl herbicide in Central Java and East Java

This stage included interviewing farmers to obtain the history of land use data and taking sample of *M. vaginalis* propagule. The history of land use included a history of herbicide use, land area and GPS (global positioning system) coordinates for sampling locations.

Weed seeds were collected by taking weed propagules which still survived after the application of bensulfuron-methyl herbicide, were then planted in a greenhouse to produce seeds. This was carried out in order to control the maturity of *M. vaginalis* seeds, Collected seeds were stored at the refrigerator with temperature of 4 °C for one month to break seed dormancy (Kuk *et al*., 2003).

2.2 Investigation of resistance

The study was conducted at the greenhouse owned by Research and Development PT Syngenta Indonesia Cikampek, Karawang West Java, from June until August 2019. The statistical design used was split-plot design with three replications. The ANOVA test was performed using IBM SPSS Statistics version 19.0 software (IBM Corp, 2010) to determine the significance level. Resistance tests were carried out using the whole plant pot test method (Burgos, 2015). The soil for this experiment was sterilized by autoclave at 120 °C and 15 psi for two hours; this is done to sterilize the soil from other weed seeds. *M. vaginalis* seeds were planted in plastic pots with a diameter of 20 cm, the germinated of *M. vaginalis* was restricted to five samples per pot ten days after planting. *M. vaginalis* was given an herbicide application at 20 days after planting. The herbicide used was the bensulfuron-methyl herbicide 20 WP (wettable powders) with a recommended dosage of 80 g a.i. ha^{-1} with a spray volume of 300 L ha^{-1}. The herbicide application was carried out using a semi-automatic knapsack sprayer using a flat fan nozzle with a pressure of 138 kPa. Herbicides were applied at six dosage levels based on recommended doses (0, 80, 160, 320, 640, and 1280 g a.i. ha^{-1}). At 30 days after treatments dry weight samples of *M. vaginalis* were observed in the greenhouse.

2.3 Dry weight of weed

Weed dry weights were obtained by weed destruction for each unit of experiment and were carried out 30 days after herbicide application. *M. vaginalis* was dried in an oven at 80 °C for 48 hours until the dry weight was constant and then weighed.

2.4 Percentage of growth reduction

Weed dry weight value was converted to growth reduction by comparing the dry weight value of herbicide treatments (T) with controls (C) using the following equation (Juraimi *et al*., 2012):

$$\text{Growth reduction (\%)} = 100-((T/C)*100) \quad (1)$$

with T is the dry weed weight value by herbicide treatment, and C is the dry weed weight value without herbicide treatment

2.5 Resistance index

The resistance index is obtained from the comparison of GR_{50} of sensitive weeds with GR_{50} of resistant weeds. Sensitive weeds used as a comparison are sensitive weeds originating from exposed herbicides rice field. This was carried out because sensitive populations from unexposed herbicides rice field were likely to have very high sensitivity to herbicides, and would result in an artificially high resistance index value. In such cases, it is possible that the sample will record a high resistance index value, but it can still be controlled at the full recommended dose (Burgos, 2015). Resistance levels are divided into several categories, including: High resistance: R/S >12, medium resistance: R/S = 6-12, low resistance: R/S = 2-6, and sensitive: R/S <2 (Ahmad-Hamdani, Owen, Yu, & Powles, 2012).

2.6 Determination of GR_{50} value

Growth reduction (GR_{50}) is a dose of herbicide required to reduce weeds growth with a probability of 50%. The GR_{50} value obtained from the simple linear regression equation, namely $Y = a + bx$. The Y value is the probit value obtained from the probit table based on the percent weed growth reduction value, and x is the log of the herbicide dose. After the x value is obtained, GR_{50} can be known by calculating the antilog of the x value (Finney & Stevens, 1948; Vincent, 2008). Regression equations are obtained using Minitab17 software (Minitab Inc, 2010).

3. Results and Discussion

3.1 Dry weight of *M. vaginalis*

Table 1 shows that application of herbicide bensulfuron-methyl at the lowest dose (80 g a.i. ha^{-1}) was able to control the dry weight accumulation in sensitive biotype of *M. vaginalis* from Cibodas (S_0) and Sumberagung (S_1), and was significantly different compared to the control (0 g a.i. ha^{-1}). *M. vaginalis* derived from Plumpang (R_3) had the highest average of dry weight at control (0 g a.i. ha^{-1}) but was not significantly different compared to the treatment of bensulfuron-methyl 80-1,280 g a.i. ha^{-1}. Application of herbicide bensulfuron-methyl 80-640 g a.i. ha^{-1} showed that the dry weight average of *M. vaginalis* derived from Tambakrejo (R_2) and Kedaton (R_1) was not significantly different compared to the control (0 g a.i. ha^{-1}).

Dry weed weight illustrates the success of herbicides in controlling weed of *M. vaginalis*. High value of weed dry weight after herbicide application and equivalence to weed dry weight without herbicide application indicate that the herbicide has failed to control the weed. The failure of herbicides to control weeds target at a dose ten times the recommended dose can be the basis for confirming the occurrence of resistance (Sereda, Erasmus, & Coetzer, 1996). In addition, Ahmad-Hamdani (2012) states that the failure of herbicides to control weed targets more than two times the recommended dose describes the weed as being resistant.

3.2 Growth Reduction of *M. vaginalis*

Table 2 shows that *M. vaginalis* derived from Cibodas (S_0) and from Sumberagung (S_1) had the highest average of growth reduction percentage on the treatment of bensulfuron-methyl 80-1,280 g a.i. ha^{-1} and significantly different compared to the control (0 g a.i. ha^{-1}). *M. vaginalis* derived from Kedaton (R_1) with 80-320 g a.i. ha^{-1} bensulfuron-methyl application had a lower growth reduction average and was not significantly different compared to the control, while *M. vaginalis* derived from Tambakrejo (R_2) with 80-640 g a.i. ha^{-1} application had a growth reduction average that is not significantly different compared to the control. *M. vaginalis* derived from Plumpang (R_3) had the lowest average of growth reduction at the treatment of bensulfuron-methyl 80-1280 g a.i ha^{-1}, and not significantly different compared to control (Table 2, Figure 2). This indicates that the resistance level of *M. vaginalis* to bensulfuron-methyl herbicide from high to low was *M. vaginalis* derived from Plumpang, Tambakrejo and Kedaton. The growth reduction that occurs can be observed in Figure 3, resistant biotype (R) can survive from the application of bensulfuron-methyl and has a lower growth reduction value than the sensitive biotype (S).

Table 1. Effect of different dosage of bensulfuron-methyl herbicide on the average of dry weight (g) of *M. Vaginalis*

Dosage (g a.i. ha^{-1})	*Monochoria vaginalis*				
	S_0 (Cibodas)	S_1 (Sumberagung)	R_1 (Kedaton)	R_2 (Tambakrejo)	R_3 (Plumpang)
0	0.70 ± 0.09^{a}BC	0.38 ± 0.04^{a}C	2.45 ± 0.28^{a}A	1.32 ± 0.23^{a}B	0.94 ± 0.25^{a}BC
80	0.00 ± 0.00^{b}C	0.08 ± 0.09^{b}C	2.42 ± 0.37^{a}A	1.05 ± 0.06^{a}B	0.85 ± 0.14^{a}B
160	0.00 ± 0.00^{b}C	0.06 ± 0.03^{b}C	2.42 ± 0.37^{a}A	0.94 ± 0.07^{ab}B	0.83 ± 0.15^{a}B
320	0.00 ± 0.00^{b}C	0.00 ± 0.00^{b}C	2.17 ± 0.47^{a}A	0.94 ± 0.15^{ab}B	0.81 ± 0.16^{a}B
640	0.00 ± 0.00^{b}C	0.00 ± 0.00^{b}C	1.58 ± 0.25^{ab}A	0.93 ± 0.05^{ab}B	0.77 ± 0.13^{a}B
1280	0.00 ± 0.00^{b}C	0.00 ± 0.00^{b}C	0.29 ± 0.11^{b}B	0.47 ± 0.04^{b}A	0.47 ± 0.11^{a}A

Note: The average values marked with lowercase letters in the same column (vertical) and capital letters in the same row (horizontal) show values that are not significantly different based on Duncan's test at 5% level. ($\bar{x}$ ± SD).

Table 2. Effect of different dosages of bensulfuron-methyl herbicide on the average of weeds growth reduction (%) of *M. Vaginalis*

Dosage (g a.i. ha^{-1})	*Monochoria vaginalis*				
	S_0 (Cibodas)	S_1 (Sumberagung)	R_1 (Kedaton)	R_2 (Tambakrejo)	R_3 (Plumpang)
0	0.00 ± 0.00^{b}A	0.00 ± 0.00^{b}A	0.00 ± 0.00^{c}A	0.00 ± 0.00^{b}A	0.00 ± 0.00^{b}A
80	100.0 ± 0.00^{a}A	81.48 ± 1.91^{a}A	9.65 ± 2.40^{bc}B	18.77 ± 3.94^{ab}B	12.06 ± 3.08^{ab}B
160	100.0 ± 0.00^{a}A	85.00 ± 0.70^{a}A	18.81 ± 3.95^{bc}B	23.46 ± 3.12^{ab}B	12.28 ± 2.12^{ab}B
320	100.0 ± 0.00^{a}A	100.0 ± 0.00^{a}A	24.42 ± 3.12^{bc}B	26.86 ± 3.29^{ab}B	17.85 ± 2.71^{ab}B
640	100.0 ± 0.00^{a}A	100.0 ± 0.00^{a}A	35.03 ± 1.65^{ab}B	26.89 ± 3.45^{ab}B	25.00 ± 3.22^{ab}B
1280	100.0 ± 0.00^{a}A	100.0 ± 0.00^{a}A	88.60 ± 0.30^{a}A	59.05 ± 1.37^{a}AB	40.12 ± 2.95^{a}B

Note: The average values marked with lowercase letters in the same column (vertical) and capital letters in the same row (horizontal) show values that are not significantly different based on Duncan's test at 5% level. ($\bar{x}$ ± SD).

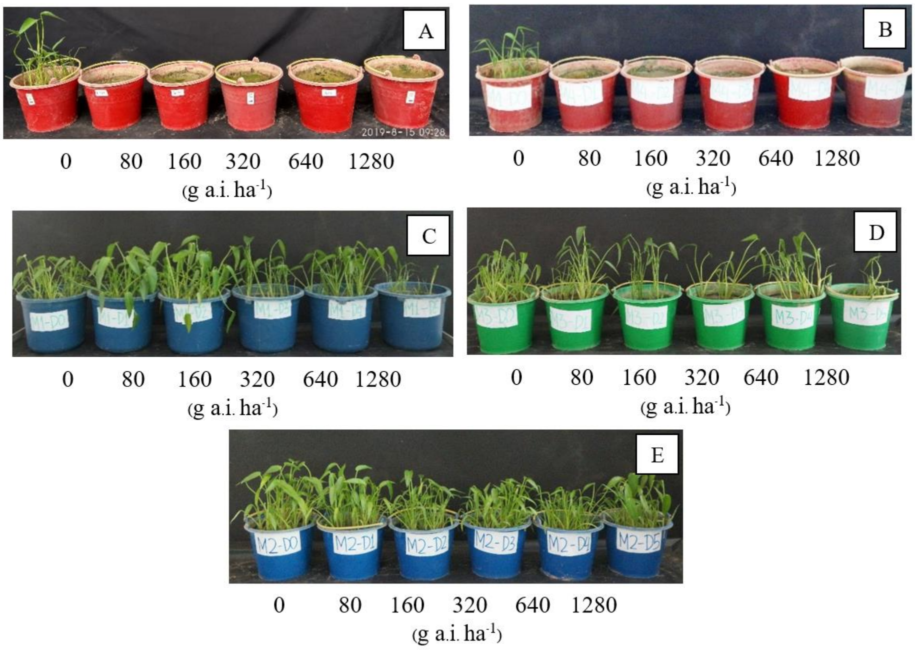

Figure 2. Effect of different dosage of bensulfuron-methyl herbicide to *M. vaginalis* A: Cibodas, B: Sumberagung, C: Kedaton, D: Tambakrejo, E: Plumpang

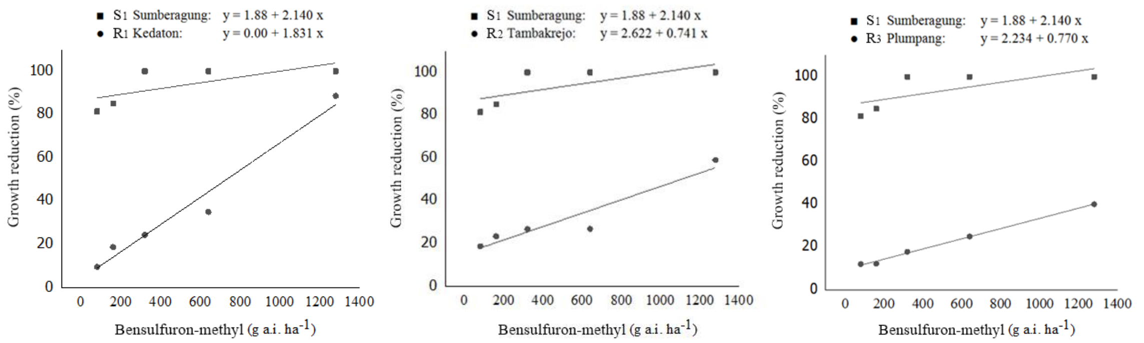

Figure 3. Growth reduction of susceptible and resistant population of *M. vaginalis* to besulfuron-methyl 30 days after herbicide application

3.3 Resistance index of *M. vaginalis*

Application of different dosage of herbicide to resistance levels of *M. vaginalis* shows the occurrence of resistance of *M. vaginalis* weeds from various locations. *M. vaginalis* derived from Kedaton, Tambakrejo, and Plumpang had a resistance index value of 18.71, 56.50, and 136.18, which means that *M. vaginalis* has a ratio of resistance more than 12 and belongs to the high resistance category. It illustrates that those *M. vaginalis* from Kedaton, Tambakrejo and Plumpang belonged to high resistant weed (Table 3).

Table 3. *M. vaginalis* weed resistance ratio to bensulfuron-methyl herbicide

M. vaginalis	GR_{50} (g a.i. ha^{-1})	r^2	Resistance index (R/S)	Resistance levels (Ahmad-Hamdani *et al*., 2012)
S_0	-	-	-	Sensitive
S_1	28.7	0.76	-	Sensitive
R_1	537.03	0.81	18.71	High Resistance
R_2	1621.81	0.65	56.50	High Resistance
R_3	3908.41	0.91	136.18	High Resistance

Many studies have been carried out to find out the weed resistance to ALS enzyme inhibiting herbicides. (Kuk *et al*., 2003) reported that *M. vaginalis* originating from Korean Chonnam rice plantations had a resistance index of 31 for the bensulfuron-methyl herbicide, and 7 for the pyrazosulfuron ethyl herbicide. ZongZhi *et al*. (2009) stated that *M. korsakowii* is resistant to bensulfuron-methyl in two locations: Dehui and Liuhei China. *M. korsakowii* from Dehui had a resistance index value of 6, while *M. korsakowii* from Liuhe had a resistance index of 13.6. According to Zakaria, Ahmad-hamdani, and Juraimi (2018) *Limnocharis flava* from Malaysia's Penang Island was resistant to several ALS enzyme inhibiting herbicides from the Sulfonylurea and Pyrimidinylthio-benzoates herbicide groups. *Limnocharis flava* was identified as resistant to bensulfuron-methyl with a ratio of resistance >109, resistant to methyl methsulfuron with a resistance index of 9, resistant to ethyl pyrazosulfuron with a ratio of resistance 5.4, resistant to pyribenzoim with a ratio of resistance 4.6, and resistant to sodium bispyribac with a ratio of resistance of 9.

4. Conclusions

M. vaginalis weed population originating from Kedaton, Tambakrejo and Plumpang Indonesia belongs to the category of weeds with high resistance to bensulfuron-methyl, while *M. vaginalis* population originating from Cibodas and Sumberagung belongs to the category of sensitive weeds to bensulfuron-methyl.

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References

Ahmad-Hamdani, M. S., Owen, M. J., Yu, Q., & Powles, S. B. (2012). ACCase-inhibiting herbicide-resistant *Avena spp*. populations from the Western Australian Grain Belt. *Weed Technology*, *26*(1), 130–136. doi:10.16 14/wt-d-11-00089.1

Burgos, N. R. (2015). Whole-plant and seed bioassays for resistance confirmation. *Weed Science*, *63*(1), 152–165. doi:10.1614/ws-d-14-00019.1

CropLife. (2017). *Herbicide resistance management guide*. CropLife Australia. Retrieved from https://www. croplife.org.au/wp-content/uploads/2016/08/2017-Herbicide-Resistance-Management-Strategies-.pdf

Directorate of Fertilizers and Pesticides. (2019). *Information system of Pesticides @PSP Kementan*. Jakarta, Indonesia: Ministry of Agriculture of the Republic of Indonesia. Retrieved from http://pestisida.id/ simpes_app/

Duary, B. (2014). Weed prevention for quality seed production of crops weed prevention for quality seed production of crops. *SATSA Mukhapatra*, *18*, 48–57.

Finney, D. J., & Stevens, W. L. (1948). Probit analysis. *Biometrika*, *35*(1/2), 191. doi:10.2307/2332639

Hamamura, K., Muraoka, T., Hashimoto, J., Tsuruya, A., Takahashi, H., Takeshita, T., & Noritake, K. (2003). Identification of sulfonylurea-resistant biotypes of paddy field weeds using a novel method based on their rooting responses. *Weed Biology and Management*, *3*(4), 242–246. doi:10.1046/j.1444-6162.2003.00109.x

IBM SPSS Statistics for Windows, Version 19.0 [Computer Software]. Armonk, NY: IBM Corp.

Jugulam, M., & Godar, A. S. (2013). Understanding genetics of herbicide resistance in weeds: implications for weed management. *Advances in Crop Science and Technology*, *1*(4), 1–3. doi:10.4172/2329-8863.1000 115

Juraimi, A. S., Begum, M., Parvez Anwar, M., Shari, E. S., Sahid, I., & Man, A. (2012). Controlling resistant *Limnocharis flava* (*L.*) *Buchenau* biotype through herbicide mixture. *Journal of Food, Agriculture and Environment*, *10*(2), 1344–1348.

Kuk, Y. I., Jung, H. Il, Kwon, O. Do, Lee, D. J., Burgos, N. R., & Guh, J. O. (2003). Sulfonylurea herbicide-resistant *Monochoria vaginalis* in Korean rice culture. *Pest Management Science*, *59*(9), 949–961. doi:10.1002/ps.722

Minitab 17 Statistical [Computer Software]. State College, PA: Minitab, Inc.

Nandula, V. K. (2016). Herbicide resistance in weeds: survey, characterization and mechanisms. *Indian Journal of Weed Science*, *48*(2), 128. doi:10.5958/0974-8164. 2016.00033.2

Sereda, B., Erasmus, D. J., & Coetzer, R. L. J. (1996). Resistance of *Amaranthus hybridus* to atrazine. *Weed Research*, *36*(1), 21–30. doi:10.1111/j.1365-3180.1996.tb01797.x

Stanton, R., Storrie, A., Urwin, N., Pratley, J., Wagga, W., & Wales, N. S. (2004). Genetic relationship between glyphosate resistant annual ryegrass (*Lolium rigidum Gaud*.) populations from northern New South Wales. In B. Sindel & S. Johnson (Eds.), *Proceeding of the 14th Australian Weeds Conference* (pp. 445–448). Victoria, Australia: RG and FJ

Richardson.

Subdirectorate of Food Crops Statistics. (2018). *Harvested area and rice production in Indonesia*. Jakarta, Indonesia: BPS.

Vincent, K. (2008). *Probit analysis*. San Francisco, CA: San Fransisco State University. Retrieved from http://userwww.sfsu.edu/efc/classes/biol710/probit/ProbitAnalysis.pdf

Yang, Q., Deng, W., Li, X., Yu, Q., Bai, L., & Zheng, M. (2016). Target-site and non-target-site based resistance to the herbicide tribenuron-methyl in flixweed (*Descurainia sophia L*.). *BMC Genomics*, *17*(1), 1–14. doi:10.1186/s12864-016-2915-8

Zakaria, N., Ahmad-Hamdani, M. S., & Juraimi, A. S. (2018). Patterns of resistance to AHAS inhibitors in *Limnocharis flava* from Malaysia. *Plant Protection Science*, *54*(1), 48–59. doi:10.17221/131/2016-PPS

Zimdahl, R. L. (2018). *Fundamentals of weed science* (*5th ed*.). Cambridge, MA: Academic Press.

ZongZhi, L., ChaoXian, Z., JunFan, F., & GuiJun, L. (2009). Resistant *Monochoria korsakowii* biotypes to bensulfuron-methyl and their acetolactate synthase sensitivity. *Acta Phytophylacica Sinica*, *36*(4), 354–358.