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Resistance of *Eleusine indica* to non-selective herbicides in Indonesian oil palm plantation

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Abstract. *Umiyati U, Kurniadie D, Widianto R, Widayat D, Nasahi C. 2023. Resistance of* Eleusine indica *to non-selective herbicides in Indonesian oil palm plantation. Biodiversitas 24: 4661-4667.* Indonesia is the world's largest producer of oil palm. One biological constraint that may reduce the national oil palm production is *Eleusine indica* (L.) Gaertn (goosegrass). This weed is usually controlled chemically using non-selective herbicides, such as glyphosate, paraquat, and ammonium glufosinate. Although these herbicides are widely used, there are minimal case reports of weed resistance in Indonesia. This study aimed to map and quantify the herbicide resistance of goosegrass in Indonesian oil palm plantations. Weed mortality after herbicide application was used to determine the resistance. The study collected samples from Indonesia's six primary oil palm regions: West Sumatra, North Sumatra, Riau, Lampung, Belitung, and West Kalimantan. The sample collection process was supplemented by conducting interviews to get information on the herbicide application practices of palm oil growers. The results showed that 15 samples of goosegrass from various locations were resistant to glyphosate, 17 were resistant to paraquat, and one showed developing resistance to ammonium glufosinate. The most resistant goosegrass was found in North Sumatra, with a resistant index of 3.32 and 3.81 to glyphosate and paraquat, respectively. At the same time, the resistance to ammonium glufosinate could not be measured because the low doses of ammonium glufosinate were sufficient to control the weeds.

Keywords: *Eleusine indica,* herbicide, North Sumatra, palm oil, resistance

INTRODUCTION

Indonesia is the world's largest oil palm producer, with 14.5 million hectares under cultivation and a total production of nearly 50 million tons in 2021 (Directorate General of Estate Crops 2021). The extensive use of herbicides for weed control in Indonesia is linked to the vast area of oil palm cultivation. Glyphosate, paraquat, and glufosinate are the most widely used non-selective herbicides in Indonesian plantation crops, particularly oil palm. Glyphosate was first registered for use in the United States by Monsanto in 1974 (Benbrook 2016), and it remains one of the world's most commonly used herbicides, including in Indonesia. Paraquat is another commonly used non-selective herbicide in Indonesia that has been on the market for over 60 years and used for over 100 different types of cultivated plants (Stuart et al. 2023). Due to the emergence of glyphosate-resistant weeds, ammonium glufosinate has emerged as an alternative herbicide. Ammonium glufosinate can be an alternative to glyphosate-resistant weeds such as *Eleusine indica* (L.) Gaertn (goosegrass) (Kurniadie et al. 2023).

Weed resistance can develop in various weed species and against various herbicide classes or mechanisms of action (Yu and Powles 2014). Herbicide resistance is a consequence of the genetic variability of the weed, which occurs through target-site or non-target-site alterations. With repeated herbicide use, resistant weeds that initially appear as isolated plants or patches in a field can quickly spread to dominate the weed populations (Deng et al. 2022). Over time, goosegrass has become a multiple resistance, making it harder to control. Herbicide-resistant weed is a significant issue in agriculture because it reduces the effectiveness of herbicides, which are essential in weed management. When a weed population develops herbicide resistance, it spreads quickly and becomes difficult to control, resulting in lower crop yields and farmer revenues (Vrbničanin et al. 2017).

Goosegrass is a diploid annual grass species native to Africa and Asia and found in most tropical and subtropical regions worldwide (Li et al. 2022). It causes a decrease in the production of 46 different cultivated plant species in over 60 countries, making it one of the five most challenging weeds globally (Ma et al. 2015). It will compete with plantation crops such as palm oil, causing yield losses (Woittiez et al. 2017). Furthermore, its seeds are buried at a depth of 20 cm and retain 79% viability after two years; its rapid growth and competitive nature result in reduced oil palm yields by competing for light, water, and nutrients (Chuah and Lim 2015). Moreover, goosegrass's ability to adapt and survive in diverse environments has contributed to its status as a significant concern for farmers and land managers. Goosegrass has evolved multiple resistance to herbicide classes, including glyphosate, paraquat, ammonium glufosinate, and ACCase inhibitor, making their resistance a severe problem (Jalaludin et al.

2014). The resistance of goosegrass to herbicides results from weed genetic variability, which occurs through target-site or non-target-site alterations (Deng et al. 2022).

Although herbicide-resistant goosegrass is rare in Indonesia, it does not mean there are no cases of resistance. The lack of knowledge about resistant weeds means the potential for resistant weeds to emerge is very high; this is supported by oil palm farmers who complain about several herbicides that fail to control this weed. Two cases of goosegrass resistance in Indonesia have been documented against glyphosate and paraquat herbicides (Heap 2023). Goosegrass was also reported to be resistant to glyphosate in Bengkulu (Simarmata et al. 2023), and there are 276 glyphosate-resistant populations in North Sumatra, Indonesia (Tampubolon et al. 2019). Moreover, glufosinate resistance is possible, as demonstrated by other weeds such as *Lolium rigidum* Gaudin in Greece and *Amaranthus palmeri* S.Watson in the United States (Travlos et al. 2018; Carvalho-Moore et al. 2022). This indicates that a lack of information about resistance cases in Indonesia does not imply any resistance. Therefore, resistance cases must be monitored to prevent failure to control goosegrass and cause the development of resistance to multiple types of herbicides. This study aimed to investigate the cases of resistant goosegrass to glyphosate, paraquat, and glufosinate herbicides in Indonesian oil palm plantations.

MATERIALS AND METHODS

Plant materials

Weed samples of goosegrass were collected from the oil palm field from January to March 2022. An interview accompanied the sample collection on the palm oil farmers' herbicide application habits. Samples were taken from Indonesia's six largest oil palm regions: West Sumatra, North Sumatra, Riau, Lampung, Belitung, and West Kalimantan. Farmers complained that glyphosate, paraquat, and glufosinate were no longer effective in controlling goosegrass and were used to determine sampling locations. Then, the ripe goosegrass seeds were collected, dried in the sun for a week, threshed, and labeled according to each sample's coordinates. The coordinates of each weed biotype sampled are shown in Table 1.

Screening of goosegrass resistance

The experiment was organized in a completely randomized block design with three replicates. Goosegrass seeds were planted in pots that were 18 cm in diameter and filled with soil media that had been sterilized at 120º C for 6 hours, with 30 seeds per pot. After 25 days of planting, thinning, and embroidering were carried out, leaving 20 weed samples per pot. Glyphosate, paraquat, and ammonium glufosinate herbicides were applied 30 days after planting (weed height reaches 20-30 cm) at doses of 1,000, 400, and 300 g a.i. ha^{-1}, respectively. A semi-automatic back sprayer equipped with a T-jet nozzle was used to apply the herbicides at a pressure of 1 kg/cm (15-20 p.s.i), using 400 liters of water per ha. The resistance level was based on the survival of goosegrass 30 days after being applied to herbicides. The screening results were divided into three resistance categories, including susceptible (2% of survivors), developing resistance (2-19% of survivors), and resistant (>20% of survivors) (Owen and Powles 2009).

Dose-response experiments

The experimental design for each herbicide was a split-plot design (Split Plot Design) with two factors and three replicates. The first factor was the active herbicide dosage level, which had seven levels, and the second factor was the most resistant goosegrass biotype, which was determined based on screening results (susceptible biotype to glyphosate, paraquat, and glufosinate originated from Wampu Village, Langkat District, North Sumatra: 3°38'49.0"N 98°19'44.3" E). Herbicide doses used were 0x, 0.25x, 0.5x, 1x, 2x, 4x, and 8x of the recommended dose of each herbicide, which was 750 g a.i. ha^{-1} for glyphosate, 400 g a.i. ha^{-1} for paraquat, and 300 g a.i. ha^{-1} for ammonium glufosinate. Planting media preparation, planting, and herbicide application were similar to those used in the screening experiment, but the weeds were thinned out, leaving 10 samples per pot. The dose that causes a growth reduction with a 50% probability (GR50) was calculated using the dry weed weight obtained 30 days after planting. The Resistance Index (RI), used to determine the resistance level, was calculated using GR50. The levels of resistance were categorized as RI < 2 = susceptible, RI 2-6 = low resistance, RI > 6-12 = moderate resistance, and RI > 12 = high resistance (Ahmad-Hamdani et al. 2012).

Statistical analysis

Weed dry weight data were statistically analyzed using the F test (ANOVA) at the 5% significance level. When a significant difference was found, the Duncan Multiple Range Test (DMRT) was employed using IBM SPSS Statistics 19 software (IBM Corp 2010). Non-linear regression analysis using the log-logistic model determined the GR50 value (Seefeldt et al. 1995: Widianto et al. 2022). A sigmoidal growth curve using the non-linear log-logistic regression equation matched the data distribution on declining growth. Moreover, the regression analysis was carried out using the Origin Pro version 2018 (Originlab Corporation 2018). The log-logistic regression curve formula of the non-linear model is as follows:

$$y = \mathrm{C} + \frac{\mathrm{D} - \mathrm{C}}{1 + (x/\mathrm{I}_{50})^{\mathrm{b}}}$$

Where:

C is the lower limit of the data range, D is the upper limit of the data range, b is the slope, and I50 is the dose that produces a 50% response.

RESULTS AND DISCUSSION

Growth reduction percentage

The results of this study (Table 2) indicated that six samples of goosegrass were susceptible, nine were developing resistance, and 15 were resistant to glyphosate. For paraquat, three samples were susceptible, 10 were developing resistance, and 17 were resistant. For glufosinate, 17 samples were susceptible, and 13 were developing resistance. Biotypes A and D from North Sumatra showed the highest resistance to these three non-selective herbicides than the other biotypes based on the highest average of goosegrass survival to glyphosate, paraquat, and glufosinate.

The ANOVA test revealed an interaction between the location of the weeds and the dosage levels of glyphosate herbicide on the average dry weight of goosegrass. Table 3 shows that goosegrass from different locations exhibited varying glyphosate, paraquat, and glufosinate responses. Glyphosate treatment at the recommended dose (1,000 g ai. ha^{-1}) to 8 times the recommended dose (8,000 g ai. ha^{-1}) was not significantly different in its effect on the average dry weight of susceptible goosegrass but was significantly lower when compared to the dry weight of resistant biotypes at the same dose. From the lowest dose of paraquat herbicide (100 g ai. ha^{-1}) to 8 times the recommended dose (3,200 g a.i. ha^{-1}) was unable to completely control the resistant biotypes, as evidenced by the lower and significantly different dry weight values of susceptible weeds at the same dose level. The dry weight value of weeds reaching 0 g (death) at the recommended dose (300 g a.i. ha^{-1}) up to 8 times the recommended dose (2,400 g a.i. ha^{-1}) demonstrates that glufosinate herbicide can control the susceptible and the resistant biotypes.

Table 4 shows the results of the resistance level test. Goosegrass biotype A from North Sumatra had low resistance to glyphosate and paraquat herbicides. Goosegrass-resistant biotypes had a glyphosate GR_{50} value of 1,777.27 and a paraquat GR_{50} value of 1,283.08 based on the results of the non-linear log-logistic regression test (Figure 1).

Table 1. The sampling site coordinates goosegrass biotypes

Province	Biotype	Coordinate
West Sumatra	A	0°01'26.8"N 99°50'19.7"E
	B	0°01'26.6"N 99°50'19.6"E
	C	0°02'45.2"N 99°50'00.9"E
	D	0°02'48.9"N 99°50'10.0"E
	E	0°01'26.4"N 99°49'54.4"E
Lampung	A	5°17'29.2"S 105°10'19.5"E
	B	5°09'28.0"S 105°06'01.0"E
	C	4°41'37.0"S 104°59'13.0"E
	D	5°10'07.0"S 105°06'12.0"E
	E	4°49'19.46"S 105°30'41.7"E
Bangka Belitung	A	3°0'38"S 107°55'15"E
	B	3°1'23"S 107°52'23"E
	C	2°56'35"S 107°50'3"E
	D	2°58'11"S 107°50'33"E
	E	2°56'9"S 107°53'55"E
West Kalimantan	A	1°25'05.2"S 110°11'36.2"E
	B	1°28'11.3"S 110°12'25.3"E
	C	1°26'11.5"S 110°11'34.9"E
	D	1°26'42.7"S 110°13'41.6"E
	E	1°28'28.1"S 110°12'23.4"E
North Sumatra	A	1°11'51.0"N 99°06'54.0"E
	B	1°12'15.0"N 99°06'57.0"E
	C	3°38'49.046"N 98°19'34.458"E
	D	3°32'32.952"N 98°25'20.871"E
	E	3°38'1.259"N 98°22'29.663"E
Riau	A	0°9'2.797"S 102°13'23.764"E
	B	0°9'3.183"S 102°13'28.156"E
	C	0°9'2.678"S 102°12'19.955"E
	D	0°9'22.439"S 102°12'51.997"E
	E	0°9'15.56"S 102°12'20.569"E

Table 2. The percentage of survival and resistance classification of goosegrass biotypes to glyphosate, paraquat, and glufosinate

Province	Bio-type	Resistance category to glyphosate (% survival)	Resistance category to paraquat (% survival)	Resistance category to glufosinate (% survival)
West Sumatra	A	S (1.67)	D (18.33)	D (6.67)
	B	S (0.00)	R (23.33)	D (8.33)
	C	S (0.00)	R (21.67)	D (11.67)
	D	S (1.67)	D (16.67)	D (8.33)
	E	D (11.67)	D (11.67)	D (11.67)
Lampung	A	S (0.00)	D (3.33)	S (0.00)
	B	S (0.00)	D (6.67)	S (0.00)
	C	D (5.00)	D (11.67)	S (0.00)
	D	D (6.67)	D (10.00)	S (1.67)
	E	R (46.67)	R (20.00)	S (0.00)
Bangka Belitung	A	R (53.33)	D (16.67)	D (8.33)
	B	R (56.67)	R (26.67)	D (3.33)
	C	R (45.00)	D (16.67)	S (0.00)
	D	R (55.00)	S (1.67)	D (3.33)
	E	R (38.33)	S (0.00)	D (3.33)
West Kalimantan	A	R (46.67)	S (1.67)	S (0.00)
	B	D (10.00)	R (55.00)	S (0.00)
	C	D (16.67)	R (46.67)	S (0.00)
	D	D (10.00)	R (48.33)	S (0.00)
	E	R (48.33)	R (43.33)	S (0.00)
North Sumatra	A	R (43.33)	R (60.00)	D (15.00)
	B	R (23.33)	R (46.67)	D (18.33)
	C	R (41.67)	D (3.33)	D (8.33)
	D	R (25.00)	R (80.00)	D (13.33)
	E	D (10.00)	R (78.33)	S (0.00)
Riau	A	R (30.00)	R (36.67)	S (0.00)
	B	R (43.33)	R (30.00)	S (0.00)
	C	R (40.00)	R (30.00)	S (0.00)
	D	D (10.00)	R (36.67)	S (0.00)
	E	D (16.67)	R (43.33)	S (0.00)

Note: S: Susceptible; D: Developing resistant; R: Resistant

Table 3. The effect of different doses of glyphosate, paraquat, and glufosinate herbicides on the dry weight of goosegrass

Recommended Dosage (g ai ha^{-1})	Glyphosate		Paraquat		Glufosinate	
	S	R	S	R	S	R
0 times	25.95 a A	27.72 a A	25.96 a A	27.91 a A	27.69 a A	27.36 a A
0.25 times	20.27 b B	23.47 b B	17.86 b B	26.95 a A	4.83 b A	0.50 b B
0.5 times	13.47 c B	21.11 b A	6.91 c B	19.04 b A	0.77 c A	0.00 b A
1 time	2.81 d B	13.16 c A	5.77 c B	9.93 c A	0.00 c A	0.00 b A
2 times	0.40 d B	11.4 c A	0.11 d B	9.14 c A	0.00 c A	0.00 b A
4 times	0.00 d B	7.92 d A	0.00 d B	8.74 c A	0.00 c A	0.00 b A
8 times	0.00 d B	1.12 e A	0.00 d B	8.09 c A	0.00 c A	0.00 b A

Note: S: Susceptible; R: Resistant (North Sumatra A biotype). The average value marked with lowercase letters (vertical direction) and uppercase letters (horizontal direction), which is the same in each column, shows no significant difference at the 5% level according to the Scott-Knott Test

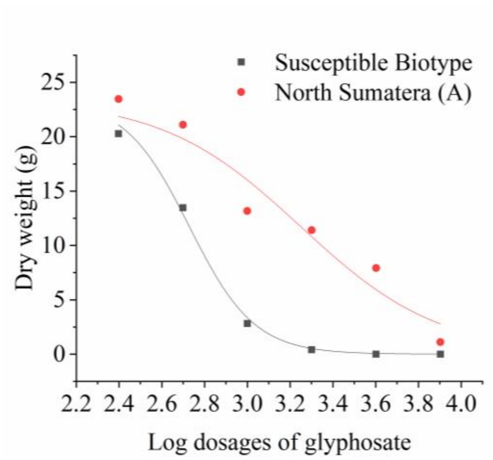
Table 4. GR_{50} and weed resistance levels goosegrass against glyphosate, paraquat, and glufosinate herbicides

Herbicide	Goosegrass Biotype	GR_{50} (g ai ha^{-1})	Resistance Index	Resistance Level (Hamdani 2012)
Glyphosate	Control	535.52	-	Susceptible
	North Sumatra (A)	1,777.27	3.32	Low resistance
Paraquat	Control	336.67	-	Susceptible
	North Sumatra (A)	1,283.08	3.81	Low resistance
Glufosinate	Control	-	-	Susceptible
	North Sumatra (A)	-	-	Susceptible

Discussion

The *E. indica* from North Sumatra was determined to be resistant to glyphosate with an index value of 3.32, resistant to paraquat with an index value of 3.81, and susceptible to ammonium glufosinate. These results are consistent with a previous report from Tampubolon et al. (2019), which found that out of 22 populations of goosegrass in Padang Lawas District, North Sumatra, Indonesia, eight populations were resistant to glyphosate (36.36%), eight populations were developing resistance (36.36%); six populations were susceptible to glyphosate (27.27%). Most goosegrass did not significantly reduce dry weight after exposure to glyphosate and paraquat at high doses compared to the susceptible biotype. This is similar to the findings of a study by Kurniadie et al. (2021), who observed that *Monochoria vaginalis* (Burm.f.) C.Presl weed resistant to methyl bensulfuron only lost 17% of its dry weight at the appropriate dose and 45% at 16 times the recommended dose compared to controls. Chorismate is a precursor to the amino acid tyrosine, phenylalanine, and tryptophan, which may be inhibited by glyphosate, resulting in a significant loss in dry weight and even the death of weeds (Averesch and Krömer 2018). However, the resistant biotypes are unaffected because the herbicide cannot bind to and block the EPSPS enzyme.

Resistance to glyphosate occurred in the provinces of North Sumatra, Riau, Lampung, Bangka Belitung, and West Kalimantan. Goosegrass resisted glyphosate found in West Sumatra province, and glufosinate was found in West Sumatra, Bangka Belitung, and North Sumatra. This shows that goosegrass resistance to glyphosate has emerged in the largest oil palm plantation area in Indonesia. Glufosinate as a substitute herbicide must also be considered because it has been identified as developing resistance. Goosegrass from North Sumatra, Indonesia, was reportedly resistant to ammonium glufosinate with a resistance index of 2.4. This occurred because the application of ammonium glufosinate to control paraquat and glyphosate-resistant goosegrass was carried out without herbicide rotation (Kurniadie et al. 2023). Goosegrass originating from Malaysia is reported to be multiply resistant to glufosinate, glyphosate, paraquat and ACCase-inhibiting herbicides (Jalaludin et al. 2014), indicating that goosegrass has the potential to experience resistance to ammonium glufosinate and ACCase inhibitors if control of glyphosate and paraquat resistant goosegrass is not carried out correctly.



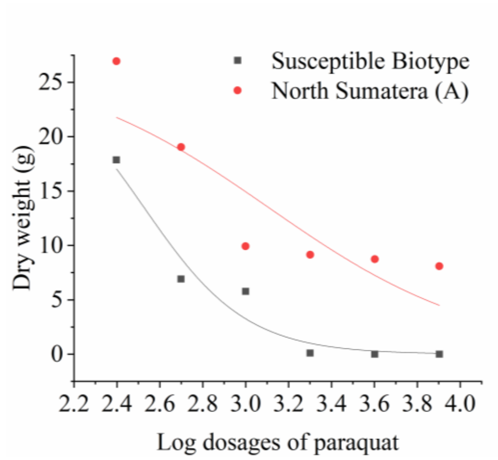


Figure 1. Fit of the dry weight curves of susceptible and resistant goosegrass weed biotypes after herbicide application to the dose-response log-logistic non-linear regression curve (Seefeldt et al. 1995; Widianto et al. 2022)

In addition to being resistant to glyphosate, goosegrass originating in the six provinces was also shown to be resistant to paraquat. This indicates that paraquat can no longer be used to control glyphosate-resistant goosegrass. Currently, 72 paraquat resistance cases and 10 weed resistance cases to ammonium glufosinate have been documented (Heap 2023). The ability of weed metabolism to adapt to herbicides, such as inhibition of absorption and translocation, detoxification, and other processes, is related to the mechanism of weed resistance to paraquat. This is because paraquat does not have target sites but binds electrons to produce radical molecules such as Reactive Oxygen Species (ROS) (Nazish et al. 2022). Although no glufosinate-resistant biotypes were discovered (Heap 2023), goosegrass developed resistance, indicating that goosegrass could become resistant if left untreated. The resistance mechanism to ammonium glufosinate is still unknown, although it is widely associated with inhibiting herbicide translocation (Carvalho-Moore et al. 2022). Furthermore, glufosinate resistance in this resistant population is not caused by glutamine synthetase mutations, increased activity, altered uptake, glufosinate translocation, or increased glufosinate metabolism (Jalaludin et al. 2014).

Based on interviews with oil palm farmers, glyphosate and paraquat herbicides have been the most widely used herbicides for more than ten years, and the dosage and frequency of herbicide use are appropriate and follow the recommended dosage on the product label. Although some weeds eventually failed to be controlled, these herbicides are preferred because they are effective. Weeds can become resistant due to repeated herbicide applications without herbicide rotation over a long period, even if the correct dosage is used. In the Italian region of Caserta, weeds in crop nurseries were controlled by making 3 to 4 applications of glyphosate per year at the recommended dose, but this still led to the emergence of glyphosate-resistant goosegrass weeds (Loddo et al. 2020). However, due to the absence of herbicide rotation, goosegrass has adapted and become resistant to glyphosate herbicides (Loddo et al. 2020). According to reports from Malaysia, goosegrass is also resistant to paraquat, glufosinate, and glyphosate. In Air Kuning, Perak, Malaysia, paraquat herbicide has been used since 1970, while glufosinate has been applied six times annually for five straight years. Therefore, goosegrass has evolved resistance to paraquat and glufosinate (Seng et al. 2010). Several variables, including overuse or misuse of herbicides, reusing herbicides with the same treatment, and genetic heterogeneity within weed populations, can lead to the development of herbicide resistance (Délye 2013). Therefore, using the same herbicides repeatedly over time is one of the primary causes of weed resistance to herbicides. When farmers use the same herbicide over time, the weed populations that survive the treatment can resist it, making it less effective (Upasani and Barla 2018).

Glyphosate and paraquat are two herbicides that provide a significant risk of establishing herbicide resistance. Currently, 296 weed species are herbicide-resistant, with glyphosate and paraquat-resistant weed species totaling 51 and 31, including goosegrass (Heap 2023). Resistance of goosegrass found in this study must be carefully addressed and managed because areas with good weed management can be susceptible to resistant weeds due to the spread of resistant weeds from the surrounding land area; because goosegrass is a self-pollinating species, the dissemination of glyphosate-resistant biotypes is modest via pollen movement (Loddo et al. 2020). Glyphosate-resistant biotypes can spread by seed movement via machinery, plant materials, or substrates (Loddo et al. 2020). Herbicide resistance in goosegrass poses a severe threat to agriculture and the environment. Herbicide-resistant goosegrass populations can live and reproduce following herbicide application, lowering herbicide effectiveness and raising weed management costs (Kurniadie et al. 2023). Herbicide-resistant goosegrass populations can overgrow, become dominant in weed communities, and are difficult to control (Devi et al. 2017). To prevent the development of goosegrass resistance, herbicides with distinct modes of action or combined herbicides might be used in rotation (Seng et al. 2010). Herbicides that inhibit ACCase enzymes, protoporphyrinogen oxidase, and photosystem II inhibitors have been shown to manage glyphosate-resistant goosegrass (Kurniadie et al. 2023), herbicide rotation is one way to help prevent the development of resistance. Farmers can lessen the selection pressure on weeds by employing various herbicides with various methods, slowing or even preventing resistance development (Délye 2013). However, if farmers do not rotate their herbicides or use other integrated pest management strategies, weeds can quickly develop resistance to the herbicides they use (Upasani and Barla 2018).

The findings of this investigation exhibit a notable difference compared to documented instances of goosegrass resistance in Indonesia. This discrepancy implies that resistance to glyphosate and paraquat among goosegrass has emerged throughout the six provinces boasting Indonesia's highest oil palm cultivation areas. Understanding the prevalence of herbicide resistance holds significant importance for multiple reasons. Integrating resistant populations into weed management systems can assist farmers and agricultural researchers. Until now, there is no absolute weed management program to reduce the negative impact of herbicide use, only limited to using herbicides at the correct dose and frequency. However, there is a reasonable probability of lowering weed evolution and resistance development by adopting various management strategies best suited to the conditions existing at a specific location (Neve et al. 2018; Khan et al. 2019). In addition, it can potentially improve global understanding of the evolution process and patterns of resistance spread. It helps consider resistant weed management to suppress the potential spread and evolution of resistant weeds, considering the limited new methods of herbicides (Moss et al. 2019).

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