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Single and Multiple Resistance of *Eleusine indica* from Asahan Regency, Indonesia

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ABSTRACT

Eleusine indica population at oil palm estate in the Asahan Regency has never been reported to being resistant to glyphosate and paraquat. This research was aimed to the resistance classification of *E. indica* population to glyphosate from one of oil palm estate in the Asahan Regency and to find out of glyphosate-resistant *Eleusine* (GRE) biotype has been also resistant to paraquat. This research was conducted on Weed Research Center Land, Faculty of Agriculture, Universitas Sumatera Utara, Medan from September 2017 until May 2018. Single resistance evaluation of *E. indica* population was performed using glyphosate at the dose 720 g a.i.ha^{-1}. However the multiple resistance of GRE biotype was performed using paraquat at rates of 0, 50, 100, 200, and 400 g a.i.ha^{-1} and glyphosate at rates of 0, 720, 1440, 2880, and 5760 g a.i.ha^{-1}. Susceptible-population (ESU-0) was taken at ball field of Politeknik Negeri Medan (Medan City) as a comparison. The data were analyzed by ANOVA and followed by Duncan test at level 5% using IBM SPSS Statistics software. The results showed that *E. indica* populations from one of oil palm estate in the Asahan Regency had survival of 81.48% (classified as glyphosate-resistant) and the dry weight of 24.35 g. Glyphosate at the dose 2880 g a.i.ha^{-1} and paraquat at the dose 200 g a.i.ha^{-1} were effectively (100%) controlled survival, tillers.pot^{-1}, fresh weight.pot^{-1}, dry weight.pot^{-1} and mortality of GRE biotype. The resistance index value of GRE biotype to glyphosate and paraquat of 4.91- and 1.26-fold, respectively compared to susceptible plants.

Introduction

Weeds presence causes losses >32% compared to pests (18%) and disease (15%). These losses could reach 69.80% if there is no chemical, biological or mechanical control (Oerke and Dehne, 2004). In general, weeds on oil palm plantations were conducted by chemical controlling rotation

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once in 3-4 months. Since oil palm plantations using herbicides, especially glyphosate and paraquat, these two herbicides application dominate to weed control. One of the annual weeds at oil palm plantation to difficult controlling is goosegrass.

Goosegrass (*Eleusine indica* (L.) Gaertn) has been characteristics flowering throughout in year and can produce until 140000 seeds per plant (Chin, 1979) and has been reported resistant to several herbicides from oil palm plantations on several regencies in the North Sumatra Province (Lubis et al. 2012; Hambali et al. 2015; Dalimunthe et al. 2015; Rahmadhani et al. 2016; Syahputra et al. 2016). Tampubolon and Purba, (2018a) reported that 8 of 22 *E. indica* populations and 5 of 6 *E. indica* populations were resistant to glyphosate at the dose 2 $l.ha^{-1}$ in the Padang Lawas and in the South Tapanuli Regency, respectively. Tampubolon and Purba, (2018b) reported that 8 of 19 *E. indica* populations were resistant to glyphosate at the dose 2 $l.ha^{-1}$ in the Langkat Regency. Also, Tampubolon et al. (2018a) reported that 89.36% *E. indica* population were resistant to glyphosate at the dose 2 $l.ha^{-1}$ in the Serdang Bedagai Regency. In another study, Tampubolon et al. (2018b) reported that 12 of 23 *E. indica* populations were resistant to glyphosate at the dose 2 $l.ha^{-1}$ in the Deli Serdang Regency. Herbicides application with similar active ingredients for a long time tends to lead resulting from resistant weeds and more difficult to control (Purba, 2009). Asahan Regency in 2017 has been oil palm plantations of 72416 ha consisting of government, smallholder, national private and foreign private (Directorate General of Estate Crops Indonesia, 2017). Oil palm plantations cannot be separated from glyphosate and paraquat herbicides to weed control. *E. indica* from Asahan Regency had never been reported resistant or susceptible to glyphosate and paraquat herbicides. It is necessary for screening and dose-response test as a source of initial information to weeds control. This research was aimed to obtain the resistance classification of *E. indica* population to glyphosate from one of oil palm estate in the Asahan Regency and to find out of glyphosate-resistant *Eleusine* (GRE) biotype has been also resistant to paraquat.

Materials and Methods

Seed Collecting

Mature seeds of *E. indica* population were collected from between rows of oil palm at the afdeling 5 of Bandar Selamat Estate, PTPN III (ESU-1). Glyphosate-susceptible *E. indica* population (ESU-0) was collected at ball field of Politeknik Negeri Medan, whereas herbicide was never applied. This research was conducted in September until December 2017.

Single Resistance of E. indica Population

E. indica seeds were soaked with KNO_3 solution at 0.2% concentration for 30 minutes (Ismail et al. 2002). The seedling media uses topsoil and manure were mixed with the 1: 1 ratio then was sterilized 100^0C for 3 hours to prevent seedbank (Tampubolon and Purba, 2018a) and into germination trays of 33 cm × 24 cm. After the 2-3 leaf stage, ten seedlings were transplanted into pots with topsoil, sand and manure media were mixed with the 1: 1: 1 ratio. The pots were arranged on non-factorial randomized block design (RBD) with three replications. The spray volume was calibrated at 292 l.ha^{-1}. The glyphosate spraying at the dose 720 g a.i.ha^{-1} (Round-up 486 SL, PT Menagro Kimia) was conducted *E. indica* have 3-4 leaf stage (Hess et al. 1997).

The parameters include survival, resistance classification of *E. indica* at 21 days after application (DAA) (Jalaludin et al. 2015). The dry weight measurement of *E. indica* at 6 weeks after application (6 WAA) then were dried at 65°C for 72 hours (Jalaludin et al. 2015). Resistance classification were measured by survival percentage. Populations were classified as glyphosate-resistant (≥ 20% survival), classified as glyphosate-resistance moderate (2 until < 20% survival), or classified as glyphosate-susceptible (< 2% survival) (Owen and Powles, 2009).

Multiple Resistance of Glyphosate-resistant E. indica Biotype

Seeds of *E. indica* survival on single resistance was harvested then dried and given a description of glyphosate-resistant *E. indica* (GRE) biotype. Seed germination and seedlings same as described to single resistance. Glyphosate-susceptible *E. indica* population (GSE) seeds use on multiple resistance from the same location. Glyphosate application at the dose 0; 720; 1440; 2880; and 5760 g a.i.ha^{-1} (Round-up 486 SL, PT Menagro Kimia). Paraquat application at the dose 0; 50; 100; 200; and 400 g a.i.ha^{-1} (Gramoxone 276 SL, PT Syngenta Indonesia). The pots of 13.5 cm x 10.5 cm were arranged on factorial randomized block design (RBD) with three replications. The parameters include survival, mortality, and tillers.pot^{-1} at 1, 2, and 3 WAA. The fresh weight.pot^{-1}, dry weight.pot^{-1}, lethal dose 50 (LD_{50}) and the resistance index (RI) value of *E. indica* at 6 WAA. The dry weight were dried 65°C for 72 hours (Jalaludin et al. 2015). The data were analyzed by ANOVA and followed by Duncan test at level 5%. The RI value was calculated of the resistant/susceptible ratio. The LD_{50} value was estimated by probit regression analysis using IBM SPSS Statistics v. 20 software. Survival, mortality, controlling Tillers of *E. Indica,* and resistance index value was calculated by following equations.

Equation 1. $$\text{Survival of } E.indica = \frac{\sum E.indica \text{ survival}}{\sum E.indica \text{ was planted}} \times 100$$

Equation 2. $$\text{Mortality of } E.indica = \frac{\sum E.indica \text{ died}}{\sum E.indica \text{ was planted}} \times 100\%$$

Equation 3. $$\text{Controlling Tillers of } E.indica = 100 - \frac{\text{Herbicides}-\text{sprayed}}{\text{non}-\text{spraying}} \times 100\%$$

Equation 4. $$\text{Resistance index value} = \frac{\text{GRE LD50}}{\text{GSE LD50}}$$

Results and Discussion

Single Resistance of E. indica Population

The single resistance of *E. indica* population to glyphosate at the dose 720 g a.i.ha^{-1} showed that 81.48% of *E. indica* was resistant to glyphosate and the dry weight amounted to 24.35 g (Table 1).

Table 1. Survival, dry weight and resistance classification of *E. indica* population to glyphosate at the dose 720 g a.i.ha^{-1}.

Sample	*E. indica* Survival (%) ± SE*	Dry weight (g) ± SE*	Resistance classification**
ESU-0	0.00 ± 0.00	0.00 ± 0.00	S
ESU-1	81.48 ± 32.08	24.35 ± 5.95	R

Note : * SE = Standard Error
** Population was classified as resistant (≥ 20% survival). classified as moderate resistance (≤ 20% survival) or classified as susceptible (less than 2% survival).

Table 1 showed that use of glyphosate herbicide on oil palm plantations has been intensively used to weed control. Its means use of glyphosate herbicide at oil palm estate in the Asahan Regency frequently at the dose 720 g a.i.ha^{-1} or equivalent 2 l.ha^{-1}. If using more frequent of glyphosate to weed control on one of oil palm estate, in the Asahan Regency resulted in failure of *E. indica* control, whereas glyphosate inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and result in the shikimic acid accumulation in chloroplast tissue. This is consistent to Syahputra et al. (2016) stated that *E. indica* at oil palm plantations with the different Regency, 98.28% of glyphosate-resistant and 1.72% of glyphosate-resistant developing at the dose 480 g a.i.ha^{-1} at Adolina Estate, in the Serdang Bedagai Regency. In addition, Dalimunthe et al. (2015) reported that 100% and 76.67% of *E. indica* was glyphosate-resistant at the dose 480 and 960 g a.i.ha^{-1}, respectively compared to susceptible at Adolina Estate, in the Serdang Bedagai Regency. Monaco et al. (2002) stated that increased shikimate in chloroplast tissue caused by glyphosate. The accumulation of shikimate acid was caused by glyphosate inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). EPSPS is an enzyme in aromatic amino acid biosynthesis pathway that converts shikimate-3-phosphate (S-3-P) to enolpyruvylshikimate-3-

phosphate (EPSP) and ultimately leads to the production of amino acids, phenylalanine, tyrosine, and tryptophan. Shikimate is formed in the glyphosate treatment because S-3-P cannot be converted to EPSP and since S-3-P is unstable, it is converted to a more stable and accumulated shikimate.

Multiple Resistance of E. indica Biotype

Survival of Glyphosate-Resistant *Eleusine* (GRE) biotype and Glyphosate-Susceptible (GSE) to glyphosate and paraquat on multiple resistance can be seen in Table 2.

Table 2. Survival of *E. indica* Biotype (%).

Herbicide (g a.i.ha^{-1})	Survival of *E. indica* biotype (%)					
	1 WAA		2 WAA		3 WAA	
	GRE	GSE	GRE	GSE	GRE	GSE
Glyphosate						
0	100.00 d	100.00 c	100.00 c	100.00 c	100.00 b	100.00 c
720	46.67 b	0.00 a	66.67 b	0.00 a	76.67 b	0.00 a
1440	20.00 a	0.00 a	20.00 a	0.00 a	20.00 a	0.00 a
2880	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
5760	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Paraquat						
0	100.00 d	100.00 c	100.00 c	100.00 c	100.00 b	100.00 c
50	100.00 d	96.67 c	100.00 c	96.67 c	100.00 b	96.67 c
100	76.67 c	10.00 b	80.00 b	10.00 b	86.67 b	10.00 b
200	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
400	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a

Note: Means within a column followed by the same letter are not significantly different by Duncan's Multiple Range Test at level 5%.

Table 2, showed that the ability of glyphosate at the dose 1440 to 5760 g a.i.ha^{-1} or equivalent at 4- to 16-fold dose recommendation up to 3 WAA were significantly in controlling survival of GRE biotype and different at the dose 720 g a.i.ha^{-1}. Glyphosate at 8- to 16-fold dose recommendation were effectively (100%) controlled survival in GRE biotypes. The ability of paraquat at the dose 200 to 400 g a.i.ha^{-1} or equivalent at 1- to 2-fold dose recommendation up to 3 WAA were significantly in controlling survival of GRE biotype different at the dose 50 to 100 g a.i.ha^{-1}. Paraquat at 1- to 2-fold dose recommendation were effectively (100%) controlled survival in GRE biotypes.

Mortality of GRE biotype and GSE to glyphosate and paraquat on multiple resistance are presented in Figure 1.

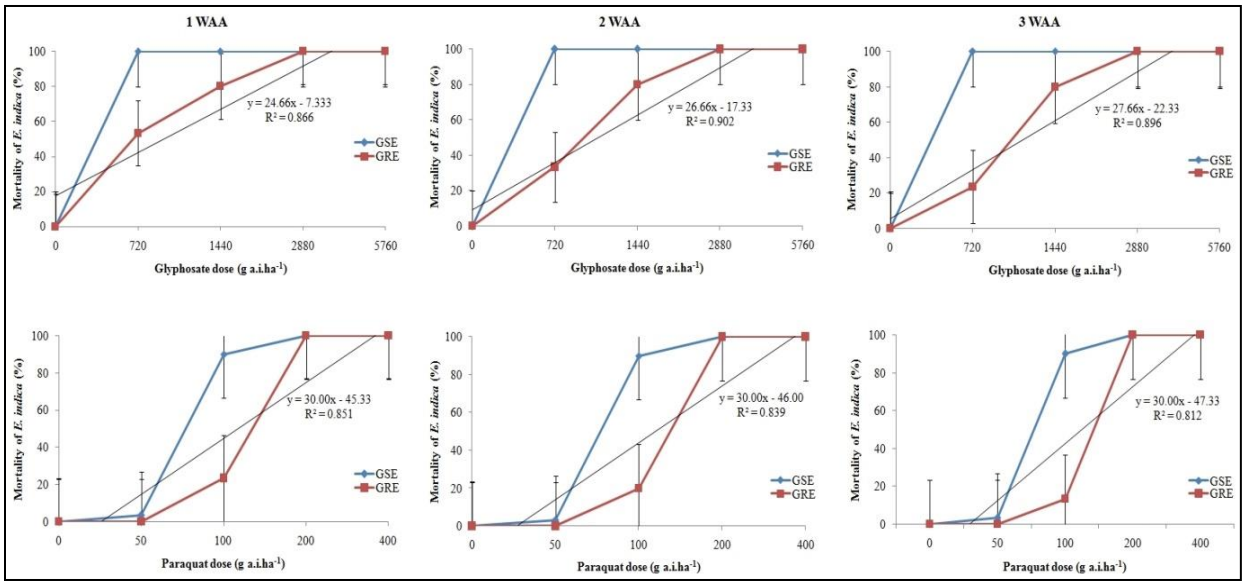

Figure 1. Mortality of glyphosate-resistant *Eleusine* (GRE) biotype and glyphosate-susceptible (GSE) on multiple resistance. Vertical bars indicate ± SE.

Figure 1, showed that the influence of glyphosate was classified as positive, direction and strongly in mortality of GRE biotype at 1, 2 and 3 WAA with the correlation value of 0.866; 0.902 and 0.896, respectively compared to paraquat had the correlation value of 0.851; 0.839 and 0.812, respectively. The correlation value of glyphosate-sprayed was higher compared to paraquat-sprayed. Although the correlation value of glyphosate was higher compared to paraquat in controlling of GRE biotype, effectiveness had used high doses. While paraquat is able to control of GRE biotype with the low dose (1-fold dose recommendation). This is linear to effectively of glyphosate and paraquat to control survival of GRE biotype of 8- to 16-fold and 1- to 2-fold dose recommendation (Table 1). Tillers.pot^{-1} of GRE biotype and GSE to glyphosate and paraquat on multiple resistance can be seen in Table 3.

Table 3. Tillers.pot^{-1} of GRE Biotype and GSE on Multiple Resistance.

Herbicide (g a.i.ha^{-1})	Tillers.pot^{-1} and Controlling of non-spraying (%)					
	1 WAA		2 WAA		3 WAA	
	GRE	GSE	GRE	GSE	GRE	GSE
Glyphosate						
0	23.33 bc (0.00)	20.33 c	28.67 bc (0.00)	24.67 c	31.67 b (0.00)	34.33 c
720	17.67 bc (24.27)	0.00 a	25.67 bc (10.48)	0.00 a	26.33 b (16.85)	0.00 a
1440	4.33 a (81.43)	0.00 a	2.67 a (90.70)	0.00 a	9.33 a (70.53)	0.00 a
2880	0.00 a (100.00)	0.00 a	0.00 a (100.00)	0.00 a	0.00 a (100.00)	0.00 a
5760	0.00 a (100.00)	0.00 a	0.00 a (100.00)	0.00 a	0.00 a (100.00)	0.00 a
Paraquat						
0	25.67 c (0.00)	22.33 c	31.33 c (0.00)	28.67 c	34.67 b (0.00)	33.67 c
50	22.33 bc (13.00)	8.67 b	28.00 bc (10.63)	10.00 b	30.00 b (13.47)	14.67 b
100	16.33 b (36.37)	0.67 a	21.67 b (30.84)	0.67 a	23.33 b (32.70)	2.33 a
200	0.00 a (100.00)	0.00 a	0.00 a (100.00)	0.00 a	0.00 a (100.00)	0.00 a
400	0.00 a (100.00)	0.00 a	0.00 a (100.00)	0.00 a	0.00 a (100.00)	0.00 a

Note: Means within a column followed by the same letter are not significantly different by Duncan's Multiple Range Test at level 5%

Table 3, showed that glyphosate at the dose 1440 to 5760 g a.i.ha^{-1} were significantly in pressing tillers.pot^{-1} of GRE biotype until 3 WAA and different at the dose 720 g a.i.ha^{-1}. Glyphosate at 8- to 16-fold dose recommendation were effectively (100%) controlled tillers.pot^{-1} in GRE biotypes. Paraquat at the dose 200 to 400 g a.i.ha^{-1} were significantly in pressing tillers.pot^{-1} of GRE biotype until 3 WAA. The effect of paraquat at 1- to 2-fold dose recommendation were effectively (100%) controlled tillers.pot^{-1} in GRE biotypes. A decrease tillers.pot^{-1} of GRE biotype along with an increase at the dose glyphosate and paraquat at 1 until 3 WAA.

The fresh- and dry weight.pot^{-1} of GRE biotype and GSE to glyphosate and paraquat on multiple resistance can be seen in Figure 2.

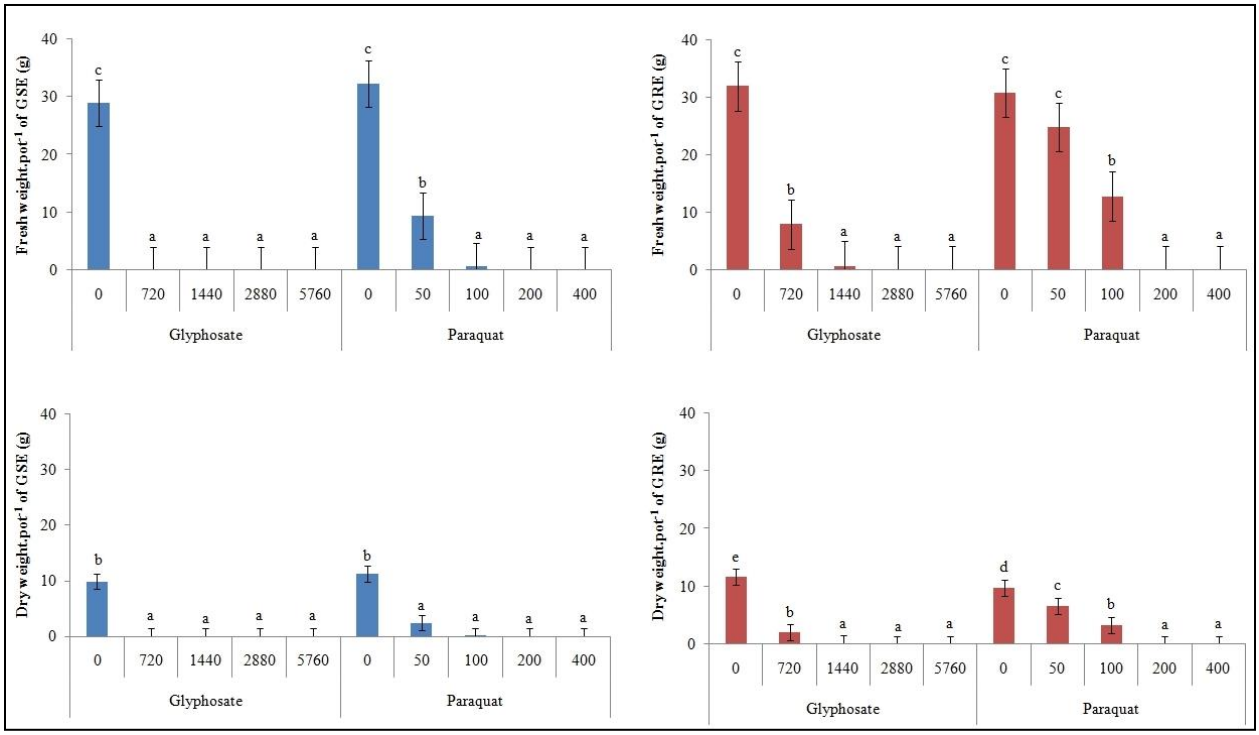

Figure 2. The fresh- and dry weight.pot^{-1} of GRE biotype and GSE on multiple resistance. Vertical bars indicate ± SE. Different lowercase letters mean significant difference by DMRT at level 5% ($P <$ 0.05).

Figure 2, showed that glyphosate at the dose 1440 to 5760 g a.i.ha^{-1} were significantly in controlling fresh- and dry weight.pot^{-1} of GRE biotype until 6 WAA and different at the dose 720 g a.i.ha^{-1}. Glyphosate at 8- to 16-fold dose recommendation were effectively (100%) controlled fresh- and dry weight.pot^{-1} in GRE biotypes. Paraquat at the dose 200 to 400 g a.i.ha^{-1} were significantly in controlling fresh- and dry weight.pot^{-1} of GRE biotype until 6 WAA. The effect of paraquat at 1- to 2-fold dose recommendation were effectively (100%) controlled fresh- and dry weight.pot^{-1} in GRE biotypes. A decrease fresh- and dry weight.pot^{-1} of GRE biotype along with an increase at the dose glyphosate and paraquat until 6 WAA.

The lethal Dose$_{50}$ of glyphosate and paraquat herbicide to GRE biotype and GSE can be seen in Table 4. It was found that LD$_{50}$ of GRE biotype to glyphosate and paraquat amounted to 987.47 and 98.81, respectively. It was showed that the resistance index value of GRE biotype at the afdeling 5 of Bandar Selamat Estate has been resistant to glyphosate and paraquat amounted 4.91 and 1.26-fold, respectively compared to GSE.

Table 4. Regression Equation Probit and LD_{50} value.

Herbicide	Regression Equation	LD_{50} (g a.i.ha^{-1})		Resistance Index value
		GRE	GSE	
Glyphosate	Y = -16.52 + 5.52 X	987.47	201.28	4.91
Paraquat	Y = -9.29 + 4.66 X	98.81	78.60	1.26

Note : Y = Probit value from mortality means. X = Log Dose

Table 4, indicated that the use of glyphosate and paraquat herbicide has been intensive to weed control at oil palm estate in the Asahan Regency. The use of glyphosate herbicide continuously at oil palm estate will result in economic and technical field losses. Multiple resistance showed that glyphosate at the dose 2880 to 5760 g a.i.ha^{-1} can be controlling survival, tillers.pot^{-1}, fresh weight.pot^{-1}, dry weight.pot^{-1}, and mortality of GRE biotype with the resistance index value of 4.91-fold compared to GSE. Therefore there was not change in the target site (EPSPS enzyme) in GRE biotype at 2880 to 5760 g a.i.ha^{-1} which shikimate acid level decreased. This is consistent to Rahmadhani et al. (2016) stated that 6.3-fold of *E. indica* was resistant to glyphosate at Sei Daun Estate, South Labuhanbatu Regency, 5.1-fold at Sawit Seberang Estate, Langkat Regency and at the Adolina Estate, Galang Estate, Rambutan Estate in the Serdang Bedagai Regency of 16.7-fold, 5.2-fold, 5.8-fold respectively compared to glyphosate-susceptible. According to Molin et al. (2013) stated that 5 to 8-fold of goosegrass was resistent to glyphosate from Mississippi then also increase shikimate levels. Alcantara et al. (2016) stated that shikimic acid levels increased from 12 to 96 hours after treatment on glyphosate at the dose 720 and 1440 g a.i.ha^{-1}. Additionally, Tampubolon et al. (2019) stated that the highest ranges of dry weight of glyphosate-resistant *E. indica* indicated that *E. indica* can produce higher biomass, which then followed by the production of seeds of the resistant *E. indica* populations and thus influence their distribution.

Multiple resistance showed that paraquat at the dose 200 to 400 g a.i.ha^{-1} can be controlling survival, tillers.pot^{-1}, fresh weight.pot^{-1}, dry weight.pot^{-1} and mortality of GRE biotype with the resistance index value of 1.26-fold compared to GSE. Dalimunthe et al. (2015) stated that 5.5 fold *E. indica* had been resistant to paraquat compared to susceptible population at the Adolina Estate, Serdang Bedagai Regency. Moreover, Rahmadhani et al. (2016) reported that 4.3- and 2.3 fold *E. indica* had been resistant to paraquat at the Sei Daun Estate South Labuhanbatu Regency, and at the Sawit Seberang Estate Langkat Regency. In addition, 4.5, 3.3, and 2.6 fold *E. indica* had been resistant to paraquat compared to susceptible population at the Adolina Estate, Galang Estate, and Rambutan Estate in the Serdang Bedagai Regency. This is consistent to Monaco et al. (2002) stated that paraquat inhibits photosystem I (PS I) have the ability to accept an electron from PS I during

electron flow in the photosynthesis and become free radicals (monocations). The formation of free radicals can stop electron transport to oxidized nicotinamide adenine dinucleotide phosphate (NADP). Therefore plant tissue will membrane damage and death.

Therefore, control of E. indica was expected to use the rotation mode of action other herbicides to break the resistance index to be lower. The resistance index value to be lower can reduce the cost of production compared to control using glyphosate is continuously or manually especially E. indica population which is difficult to control. This is research recommending the use of paraquat as different mode of action herbicide can be a solution in controlling of glyphosate-resistant E. indica biotype in one of oil palm estate in the Asahan Regency.

Conclusion

Survival and dry weight of *E. indica* population at the afdeling 5 of Bandar Selamat Estate, in the Asahan Regency of 81.48% and 24.35 g, respectively. *E. indica* was classified as resistant to glyphosate at the dose 720 g a.i.ha^{-1}. Glyphosate at the dose 2880 g a.i.ha^{-1} and paraquat at the dose 200 g a.i.ha^{-1} were effectively (100%) controlled the survival, tillers.pot^{-1}, fresh weight.pot^{-1}, dry weight.pot^{-1}, and mortality of GRE biotype. The LD_{50} of GRE biotype to glyphosate and paraquat of 987.47 and 98.81, respectively. The resistance index value of GRE biotype to glyphosate and paraquat of 4.91- and 1.26-fold, respectively compared to GSE (susceptible).

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Conflicts of Interest

No conflicts of interest have been declared.

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