

## Glyphosate resistance of *Eleusine indica* populations from North Sumatra, Indonesia

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**Abstract.** Tampubolon K, Purba E, Basyuni M, Hanafiah DS. 2019. Glyphosate resistance of *Eleusine indica* populations from North Sumatra, Indonesia. *Biodiversitas* 20: 1910-1916. Controlling of *Eleusine indica* populations in oil palm plantations using a similar mode of action of herbicide may cause resistant weed. Overall *E. indica* populations from oil palm plantations in North Sumatra, Indonesia have never been reported to be glyphosate-resistant. This research was aimed to determine the survival percentage and to classify the resistance of *E. indica* to glyphosate from oil palm plantations in 11 districts in North Sumatra Province. The research was conducted in the Weed Research Center Field Laboratory, Faculty of Agriculture, Universitas Sumatera Utara from October 2016 to August 2017. This research was arranged in a Randomized Block Design with the glyphosate-treatment using a recommended dose of two l ha<sup>-1</sup> and three replications. The ESU<sub>0</sub> population was collected in the soccer field of Politeknik Negeri Medan (Medan city) was used as a check, and has never been exposed to the herbicide. Parameters were analyzed using one-way ANOVA, and the means were compared using DMRT at a probability level of 5%. The results showed that 276 (65.56%) of 421 populations were classified as glyphosate-resistant, 88 populations (20.90%) were classified as glyphosate-resistant developing, and 57 populations (13.54%) were classified as glyphosate-susceptible. The highest range of dry weight of glyphosate-resistant *E. indica* populations were found in 5 districts including South Labuhanbatu District followed by the Serdang Bedagai, Simalungun, Asahan, and North Labuhanbatu Districts. The observed resistance level and dry weight range in the present study indicated the failure in controlling *E. indica* due to continued use of glyphosate and the potency of *E. indica* distribution into other oil palm estates due to its high biomass production capability.

**Keywords:** *Eleusine indica*, glyphosate, North Sumatra, oil palm plantations, resistance

### INTRODUCTION

*Eleusine indica* is a fast growing plant under high sunlight intensity and classified as C<sub>4</sub> plant (Ampong-Nyarko and De Datta 1992). *E. indica* has 2 sets of chromosomes (diploid), self-pollinated, annual weeds, and its genome size is relatively small approximately 8.03 x 10<sup>8</sup> bp (Ganeshaiah and Umashaanker 1982; Mysore and Baird 1997). This weed can produce up to 140,000 seeds plant<sup>-1</sup> (Chin 1979). The high sunlight intensity condition at oil palm plantations in Indonesia during the pre-and main-nursery to the immature plant (IP) enables *E. indica* has a more rapid growth as compared to the mature plant (MP). Based on field observations, *E. indica* needs only two months to grow and develop from germination to the generative phase. *E. indica* populations on oil palm nursery, both in the pre-and main-nursery until immature plant (IP) is more dominant than a mature plant (MP), which in turn reduces plant production. In general, weed control in oil palm plantations is carried out using herbicide once in 3-4 months. Glyphosate is one of the most often used herbicides for controlling weeds in oil palm plantations.

Glyphosate, *N*-(phosphonomethyl) glycine, is a derivative of glycine amino acids and phosphonic acid.

Glyphosate is generally formulated as salt in soluble water and is a non-selective herbicide which can control various types of annual and perennial weeds. Glyphosate inhibits the enzyme *5-enolpyruvylshikimate-3-phosphate synthase* (EPSPS) in the plant chloroplast tissue which converts *shikimate-3-phosphate (S-3-P) into enolpyruvylshikimate-3-phosphate* (EPSP) leading to the production of amino acids, phenylalanine, tyrosine, and tryptophan (Monaco et al. 2002). The repeated use of herbicides with similar active ingredients for long periods can cause weeds to become more difficult to control (Purba 2009). According to Knezevic et al. (2017), herbicide-resistant weeds are the weed species that have the ability to develop and keep surviving after being exposed to herbicides. The use of the same herbicides intensively and continuously over the past few decades may have resulted in the evolution of herbicide-resistant weeds. These evolutionary event is usually caused by gene mutations or changes on plant metabolism that cause resistance to specific or groups from a similar *mode of action*. There are 3 levels of herbicide resistance, namely: (i) single resistance that occurs when weeds are resistant to only one group of herbicides and or one *mode of action* (e.g. resistant to glyphosate), (ii) cross-resistance that occurs when weeds are resistant to one or

more herbicide groups with similar mode of action (e.g. resistant to sulfonylurea and imidazolinone herbicides, both included on Acetolactate synthase (ALS) mode of action), (iii) multiple resistance that occurs when weeds are resistant to more than two groups of herbicides and more than two modes of action herbicides (e.g. resistance to Pursuit (ALS) and glyphosate).

Control of *E. indica* with similar active ingredients of herbicide will cause the *E. indica* to evolve into herbicide-resistant. Research on oil palm plantations in North Sumatra Province has not overall reported that *E. indica* was resistant to glyphosate. Lubis et al. (2012) reported that 71.70% of *E. indica* populations from Adolina Estate in the Serdang Bedagai District were resistant to glyphosate at the dose of 720 g a.i ha<sup>-1</sup> and the resistance index value was 7-fold compared to the susceptible populations. Dalimunthe et al. (2015) reported that 76.67% of *E. indica* populations from Adolina Estate in the Serdang Bedagai District were resistant to glyphosate at the dose of 960 g a.i ha<sup>-1</sup> and the resistance index value was 7.5-fold compared to the susceptible populations. In addition, Rahmadhani et al. (2016) also reported that 73.30% of *E. indica* populations from Adolina Estate in the Serdang Bedagai District were glyphosate-resistant at the dose of 960 g a.i ha<sup>-1</sup> and the resistance index value were 16.7-fold compared to susceptible populations, *E. indica* populations from Sei Daun Estate in the South Labuhanbatu District, Sawit Seberang Estate in the Langkat District, and Rambutan Estate in the Serdang Bedagai District had also been a glyphosate-resistant of 53.30%; 36.70%; 33.30% and 40.00% respectively, at the dose of 480 g a.i ha<sup>-1</sup> and the resistance index values were 6.3; 5.1; 5.2; and 5.8-fold respectively. Syahputra et al. (2016) also reported that *E. indica* populations from the first to 9<sup>th</sup> afdeling blocks on

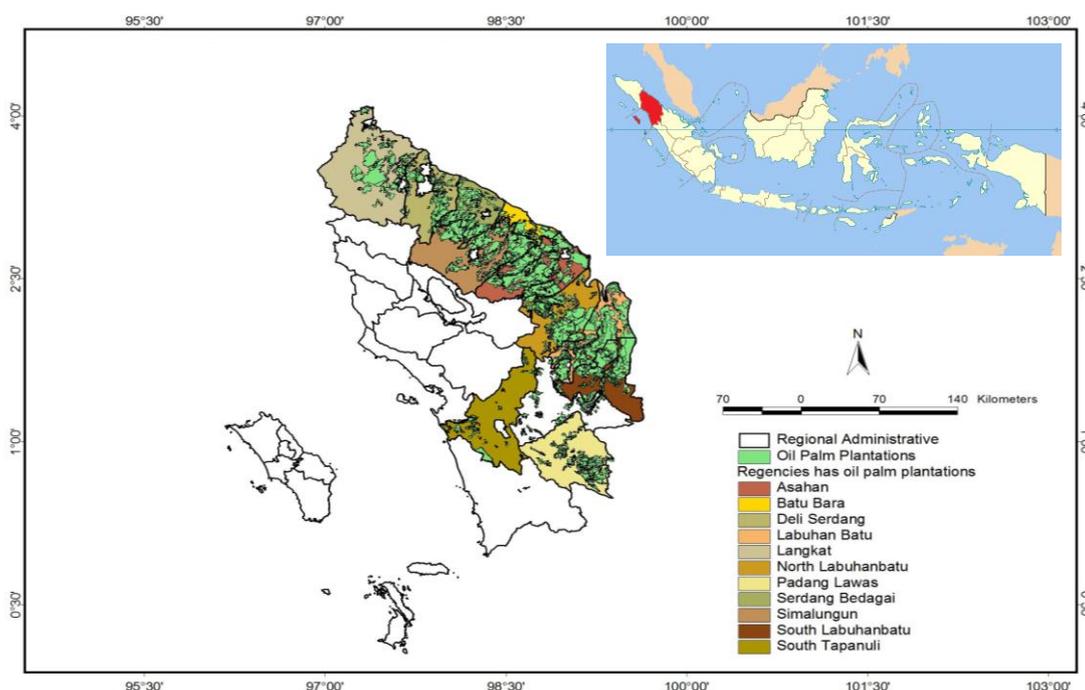
Adolina Estate in the Serdang Bedagai District was glyphosate-resistance at the dose of 480 g a.i ha<sup>-1</sup> amounted to 98.28%, glyphosate-resistant developing of 1.72%, and the glyphosate-susceptible population was absent

Previous studies had reported glyphosate resistance in *E. indica* only in Serdang Bedagai District but report on the glyphosate resistance of *E. indica* populations in oil palm plantations of the whole North Sumatra Province was not available at present. In 2017, North Sumatra Province had oil palm area as a government estate of 324,938 ha, private and foreign estate of 720,009 ha and smallholder of 429,951 ha (Directorate General of Estate Crops 2017). Therefore, it is a need to collect information on population distribution and to determine the glyphosate resistance of *E. indica* populations from oil palm plantations in North Sumatra. This research was aimed to assess the distribution of *E. indica* population and glyphosate resistance level in oil palm plantations in 11 districts in North Sumatra Province, Indonesia.

## MATERIALS AND METHODS

### Seed collection of resistant and susceptible *Eleusine indica*

A simple random sampling method was used to collect mature seeds of 50 panicles of *E. indica* growing between rows of oil palm from several afdeling oil palm plantations in 11 districts in North Sumatra Province (Table 1 and Figure 1) (Tampubolon and Purba 2018a). Seeds of glyphosate-susceptible *E. indica* (ESU<sub>0</sub>) was collected at soccer field of Politeknik Negeri Medan (Medan city), where the herbicide has never been applied. Collection of *E. indica* seeds was conducted from October 2016 until February 2017.

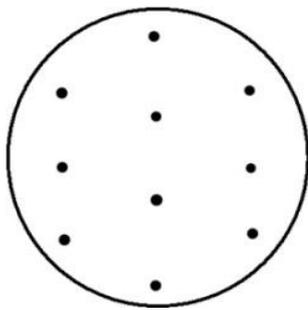


**Figure 1.** Map of *Eleusine indica* seeds collected in 11 districts in North Sumatra Province, Indonesia

**Table 1.** Oil palm plantations area and the total sample of *Eleusine indica* populations in 11 districts in North Sumatra Province, Indonesia

Districts	Area (ha)*		Sample
	Government estate	Private and foreign estates	
Langkat	42,090	58,585	19
Deli Serdang	14,373	33,171	23
Serdang Bedagai	25,878	42,292	47
Batu Bara	2,401	0	12
Asahan	43,891	66,255	107
Simalungun	65,408	18,127	90
Labuhabatu	53,838	247,680	18
North Labuhanbatu	3,287	53,108	28
South Labuhanbatu	49,840	71,207	49
Padang Lawas	6,542	0	22
South Tapanuli	7,637	42,619	6
		Total	421

Note : \*Directorate General of Estate Crops (2017)



**Figure 2.** Illustration of planting pot containing 10 *Eleusine indica* seedlings. The black dot represents the position of the seedling in the pot.

### Germination and transplanting

*E. indica* seeds were soaked in 0.2% KNO<sub>3</sub> for 30 min (Ismail et al. 2002) which aimed to break the dormancy of *E. indica* seeds. Topsoil and manure were mixed with the 1:1 volume ratio for germination media put in pots. The media was sterilized using an autoclaved at 100°C temperature for 3 hours (Tampubolon and Purba 2018a) and was then filled into the germination trays of 33 cm x 24 cm size. Insecticide was sprayed at 3 days after germinated to prevent it from red imported fire ant (*Solenopsis invicta*) attack. Seedlings of *E. indica* were transplanted into pots of 13.5 cm x 10.5 cm size filled with the media composed of mixture of topsoil, sand, and manure (1: 1: 1 volume ratio). Each pot was planted with ten seedlings of *E. indica* (Figure 2). The seedling preparation and transplantation were conducted in the Weed Research Center Field Laboratory, the Faculty of Agriculture, the Universitas Sumatera Utara.

### Glyphosate application

*Eleusine indica* seeds from different locations were planted in pots arranged in non-factorial Randomized

Block Design (RBD) with three replications. Glyphosate herbicide was sprayed at the recommended dose of 720 g a.i ha<sup>-1</sup>. The glyphosate spraying was conducted when the *E. indica* was at 3-4 leaf stage (Hess et al. 1997). The spray volume was calibrated at 292 l ha<sup>-1</sup>. Spraying was conducted during sunny weather (29°C temperature and 74% moisture).

### Data analysis

The parameters observed in this research included mortality, resistance level, and dry weight of *E. indica*. The observation was done at 21 days after spraying (DAS) (Jalaludin et al. 2015). The dry weight of *E. indica* was collected at six weeks after spraying (WAS) after oven dried at 80°C for 48 hours (SERAS 1994). The mortality data were analyzed using one-way ANOVA, and the means were separated using Duncan Multiple Range post hoc Test at 5% significance level. The statistical analysis was performed using an IBM SPSS Statistics v.20 software. The percentage of mortality was calculated using the following formula:

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

The resistance level of *E. indica* was determined based on the percentage of mortality caused by glyphosate spray. *E. indica* with percent mortality of >98-100% was classified as glyphosate-susceptible (S), >80-98% was classified as glyphosate-resistant developing (DR), and ≤80% was classified as glyphosate-resistant (R) (Owen and Powles 2009).

## RESULTS AND DISCUSSION

*Eleusine indica* mortality collected from oil palm plantations on 11 districts in the North Sumatra were dominantly resistant to the recommended glyphosate dose of 720 g a.i ha<sup>-1</sup> (Figure 3). Glyphosate at recommended dose of 720 g a.i ha<sup>-1</sup> was significantly effective to control *E. indica* from oil palm plantations in Langkat District (11 populations), Deli Serdang District (7 populations), Asahan District (16 populations), Simalungun District (18 populations), North Labuhanbatu District (7 populations), South Labuhanbatu District (3 populations), Padang Lawas District (8 populations), and South Tapanuli District (one population), however the herbicide was less effective in the Serdang Bedagai, Batu Bara, and Labuhan Batu Districts (showed by notation in Figure 3). *E. indica* populations have become glyphosate-resistant due to frequent use of glyphosate at recommended dose of 720 g a.i ha<sup>-1</sup> in oil palm plantations in the North Sumatra. This is in line with the finding that glyphosate-susceptible *E. indica* ranged from 0 to 27.27% (Table 2). Chen et al. (2015) found that the mortality of 12 biotypes *E. indica* in the Chengdu and Guangzhou, China was classified as low level (less than 100%) at 14 days after glyphosate spray at the dose of 1,680 g a.i ha<sup>-1</sup> whereas 12 biotypes had been sprayed using glyphosate more than 3 times a year at the dose 840 g a.i

ha<sup>-1</sup>. Besides, Simarmata et al. (2005) also reported a 41% mortality of F<sub>1</sub> *Rigid ryegrass* from California at the glyphosate dose of 2.24 kg ha<sup>-1</sup> (2x recommended dosage).

The results showed that more than 50% populations of *E. indica* collected from oil palm plantations in 11 districts were resistant to glyphosate applied at the recommended dose of 720 g a.i ha<sup>-1</sup> (Table 2). Four hundred twenty-one populations of *E. indica* collected from North Sumatra (Table 1), the glyphosate-susceptible populations ranged from 0.00 to 27.27%, glyphosate-resistant developing ranged from 8.16 to 42.11%, and glyphosate-resistant ranged from 36.36 to 89.36%. *E. indica* populations that were classified as glyphosate-resistant were from eight of 11 districts in North Sumatra. The highest percentage of resistant populations was found in Serdang Bedagai District of 89.36% (Table 2), which presumably caused by the excessive use of glyphosate herbicide to control *E. indica* in oil palm plantations at a dose of 720 g a.i ha<sup>-1</sup> not only in nursery but also in immature and mature of oil palm plants. The increased number of glyphosate-resistant *E. indica* populations resulted in the failure of weed control in oil palm plantations. This failure do occur because the glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme the in plant chloroplasts tissue, which then resulted in the accumulation of shikimic acid. This is consistent to Molin et al. (2013) who stated that increased shikimic acid levels in *E. indica* leaf exposed to glyphosate indicated an increase in resistance level of 5 to 8-fold compared to susceptible populations from Washington County, Mississippi. Zhang et al. (2015) stated that the EPSPS enzyme in glyphosate-resistant *E. indica* biotypes from South China quickly responded to glyphosate herbicide at 12 hours after exposure. The mRNA and protein expression of glyphosate-resistant *E. indica* biotypes constantly increases with increasing glyphosate concentration. Chen et al. (2015) also stated that glyphosate-resistant *E. indica* biotype has a small decrease in chlorophyll content at the glyphosate dose of 1,680 g a.i ha<sup>-1</sup>.

The dry weight ranges of glyphosate-resistant *E. indica* collected in oil palm plantations in 11 districts in North Sumatra can be seen in Figure 4.

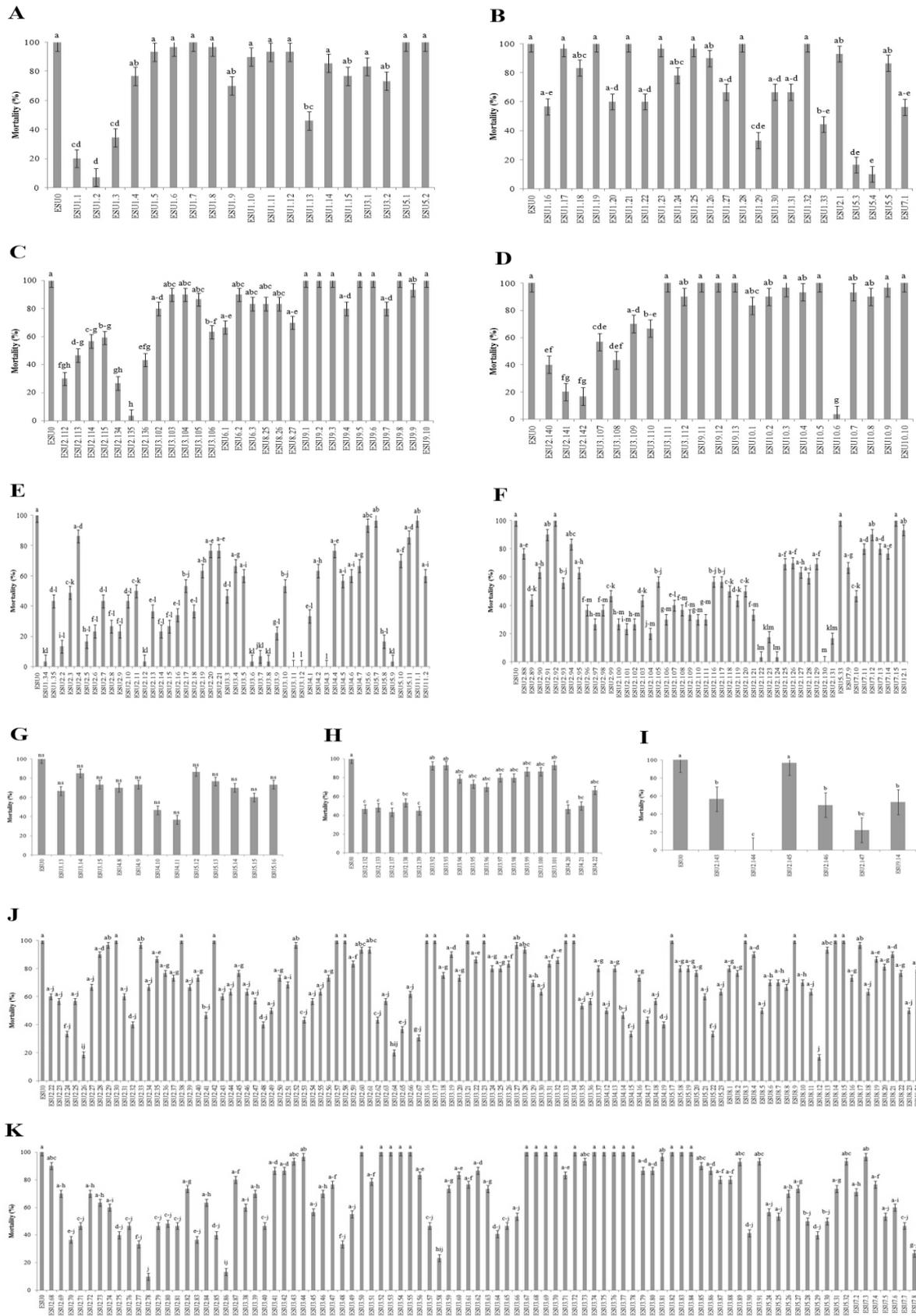
The dry weight ranges of glyphosate-resistant *E. indica* populations were found in 5 districts included South Labuhanbatu District followed by Serdang Bedagai District, Simalungun District, Asahan District, and North Labuhanbatu District. The increased in dry weight ranges indicated that *E. indica* can produce higher biomass, which then followed by the production of seeds of the resistant *E. indica* populations and, thus, influence their distribution. The high biomass production did occur because the active ingredient of glyphosate was unable to inhibit amino acid synthesis for protein formation in glyphosate-resistant *E. indica* populations. The observed glyphosate-resistant *E. indica* populations in 5 districts are highly alarming and a beneficial information for oil palm plantation companies to prevent increased cost for weed control. According to Widayat and Yustisiyanika (2015), glyphosate potassium herbicide at the dose of 330 g a.i ha<sup>-1</sup> (1.5 l ha<sup>-1</sup>), 660 g a.i ha<sup>-1</sup> (3 l ha<sup>-1</sup>), and 990 g a.i ha<sup>-1</sup> (4.5 l ha<sup>-1</sup>) were able to suppress the grasses dry weight at 6 weeks after planting (WAP) in soybean cultivation up to 2.80 g; 2.89 g; and 2.76 g respectively. In addition, Dalimunthe et al. (2015) also stated that glyphosate-resistant *E. indica* biotypes dry weight from Adolina Estate were 7.00 g pot<sup>-1</sup> with glyphosate application at the dose of 480 g a.i ha<sup>-1</sup>, while the dry weight was decreased to 5.37 and 0 g pot<sup>-1</sup> if the dose applied was at 960 and 1,920 g a.i ha<sup>-1</sup>, respectively.

The resistance level of *E. indica* at oil palm plantations in 11 districts in North Sumatra can be seen in Figure 5. 276 *E. indica* populations (65.56%) were classified as glyphosate-resistant, 88 populations (20.90%) were classified as glyphosate-resistant developing and 57 populations (13.54%) were classified as glyphosate-susceptible.

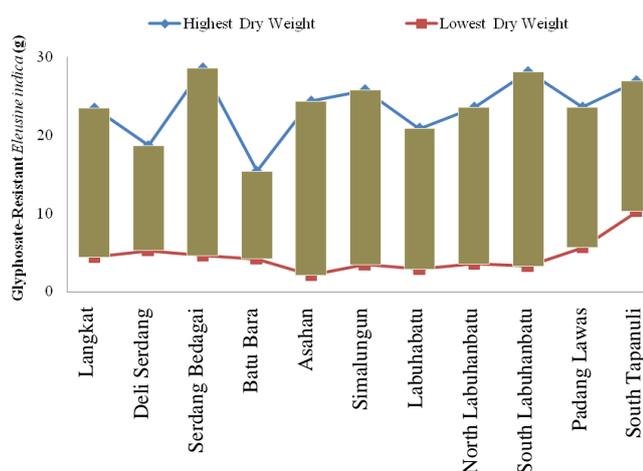
**Table 2.** The resistance level of *Eleusine indica* from oil palm plantations in 11 districts in North Sumatra at 21 days after spraying (DAS).

Districts	Resistance level of <i>Eleusine indica</i> populations		
	S	DR	R
Langkat <sup>a</sup>	3 pop. (15.79%)	8 pop. (42.11%)	8 pop. (42.11%)
Deli Serdang <sup>b</sup>	3 pop. (13.04%)	7 pop. (30.43%)	13 pop. (56.52%)
Serdang Bedagai <sup>c</sup>	0 pop. (0.00%)	5 pop. (10.64%)	42 pop. (89.36%)
Batu Bara <sup>e</sup>	0 pop. (0.00 %)	2 pop. (16.67%)	10 pop. (83.33%)
Asahan	17 pop. (15.89%)	21 pop. (19.63%)	69 pop. (64.49%)
Simalungun	18 pop. (20.00%)	19 pop. (21.11%)	53 pop. (58.89%)
Labuhanbatu	0 pop. (0.00%)	5 pop. (27.78%)	13 pop. (72.22%)
North Labuhanbatu	7 pop. (25.00%)	8 pop. (28.57%)	13 pop. (46.43%)
South Labuhanbatu	3 pop. (6.12%)	4 pop. (8.16%)	42 pop. (85.71%)
Padang Lawas <sup>d</sup>	6 pop. (27.27%)	8 pop. (36.36%)	8 pop. (36.36%)
South Tapanuli <sup>d</sup>	0 pop. (0.00%)	1 pop. (16.67%)	5 pop. (83.33%)

Note : \*S: susceptible (*E. indica* mortality > 98-100%); DR: resistant developing (*E. indica* mortality > 80-98%); R: resistant (*E. indica* mortality ≤ 80%) (Owen and Powles 2009); pop.: populations. a (Tampubolon and Purba 2018b); b (Tampubolon et al. 2018a); c (Tampubolon et al. 2018b); d (Tampubolon and Purba 2018a); e (Tampubolon et al. 2018c).



**Figure 3.** Graphs showing the glyphosate-induced mortality of *Eleusine indica* collected in oil palm plantations in 11 districts in North Sumatra, Indonesia. A. Langkat, B. Deli Serdang, C. North Labuhanbatu, D. Padang Lawas, E. Serdang Bedagai, F. South Labuhanbatu, G. Batu Bara, H. Labuhan Batu, I. South Tapanuli, J. Asahan, K. Simalungun. Vertical bars indicate  $\pm$  SE. Bars having the same lowercase are not significantly different at 5% DMRT.



**Figure 4.** The dry weight ranges of glyphosate-resistant *Eleusine indica* were collected from oil palm plantations in 11 districts in North Sumatra.



**Figure 5.** The resistance level of *Eleusine indica* from oil palm plantations in 11 districts in North Sumatra at 3 weeks after spraying.

Information on the observed glyphosate-resistant *E. indica* populations illustrates the difficulty of oil palm plantations holders to control *E. indica*. The higher the percentage of glyphosate-resistant *E. indica* population, the more difficult the control of this weed. The resistant *E. indica* populations are very disturbing for oil palm plants in producing fresh fruit bunches (FFB) both in quality and quantity. These populations, under oil palm plantations, will produce more tillers making them stronger in competing with oil palm plants to obtain nutrients and water, which in turn inhibit the growth and development of oil palms, primarily the immature plant. The evidence of glyphosate-resistant *E. indica* on oil palm plantations in North Sumatra had been reported by previous research. Tampubolon and Purba (2018a) reported that 36.36% and 83.33% of *E. indica* from Padang Lawas and Tapanuli Selatan District were resistant to glyphosate at the dose of 720 g a.i ha<sup>-1</sup>. Tampubolon and Purba (2018b) reported that 42.11% of *E. indica* in Langkat District were glyphosate resistance at the dose of 720 g a.i ha<sup>-1</sup>. Syahputra et al.

(2016) reported that 98.28% of *E. indica* from Adolina Estate in the Serdang Bedagai District were glyphosate-resistant at the dose of 480 g a.i ha<sup>-1</sup>. Tampubolon et al. (2018a) reported that 56.62% of *E. indica* in the Deli Serdang District were glyphosate-resistant at the dose of 720 g a.i ha<sup>-1</sup>. Tampubolon et al. (2018b) reported that 89.36% of *E. indica* in the Serdang Bedagai District was glyphosate-resistant at the dose of 720 g a.i ha<sup>-1</sup>. Tampubolon et al. (2018c) also reported that 83.33% of *E. indica* in the Batu Bara District were glyphosate-resistant at the dose of 720 g a.i ha<sup>-1</sup>. Survey of glyphosate-resistant *E. indica* from oil palm plantations are urgently needed as initial information in making decisions weed control in the case of the spread of resistant biotypes. This decision will benefit the company in saving weed control costs for the top management in purchasing herbicides that are appropriate to the resistance level.

In conclusions, *E. indica* populations collected in oil palm plantations in 11 districts in North Sumatra Province were classified as glyphosate-resistant, glyphosate-resistant developing, and glyphosate-susceptible of 65.56% (276 populations), 20.90% (88 populations), and 13.54% (57 populations), respectively based on the recommended glyphosate dose of 720 g a.i ha<sup>-1</sup>. The highest ranges of dry weight of glyphosate-resistant *E. indica* populations were found in 5 districts including South Labuhanbatu District followed by Serdang Bedagai, Simalungun, Asahan, and North Labuhanbatu Districts.

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