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DISTRIBUTION MAPPING OF GLYPHOSATE-RESISTANT *Eleusine indica* IN SERDANG BEDAGAI REGENCY

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Abstract. The presence of *Eleusine indica* from oil palm plantations in Serdang Bedagai Regency has never been overall reported glyphosate-resistant. This study aims to distribution mapping and resistance classification of *E. indica* population to glyphosate herbicide of oil palm plantations in Serdang Bedagai Regency. This research was conducted on Weed Research Center Land in Faculty of Agriculture, Universitas Sumatera Utara in October 2016 to August 2017. This research used glyphosate herbicide with the recommended dose at 720 g ai ha^{-1} and three replications. Population ESU_0 (from Politeknik Negeri Medan Ball Field) as a comparison. Data analysis using IBM SPSS Statistics 20 software. The results showed there *E. indica* population classified as glyphosate-resistant amount 89.36% (42 population), classified as glyphosate-resistant moderate amount 10.64% (5 population) and there is no population glyphosate-susceptible on oil palm plantations in Serdang Bedagai Regency of recommended dose at 720 g ai ha^{-1}.

Keywords*:* *Eleusine indica*, Glyphosate, Resistant, Serdang Bedagai.

I INTRODUCTION

Eleusine indica is a C4 plant that grows very rapidly at full sunlight intensity, but its growth is less developed in the shaded condition [1]. In addition, *E. indica* weed is self-pollination, classified as the annual weed, have 2 sets of chromosomes (diploid), and relatively small genome size around 8.03 x 10^8 bp [2]. These weeds can produce up to 140,000 seeds per plant [3]. Full sunlight intensity conditions in oil palm plantations in Indonesia are at the seedling stage until immature. Based on the observation of experimental field, *E. indica* has fast growth and development from germination until seed production only takes ±2 mo. This effect on the presence of *E. indica* populations in the nursery until immature was more dominant than mature so as to decrease oil palm production. In general, weed control of oil palm plantations uses chemical control (herbicide) with rotation once in 3-4 mo. One of the most commonly used herbicides of oil palm plantations to weeds controlling is glyphosate. The glyphosate herbicide has an active ingredient namely N-(phosphonomethyl) glycine which is a derivative of glycine amino acid and phosphonic acid. Glyphosate is generally formulated as salt and water soluble. Salt on glyphosate is used as a non-selective, post-emergence herbicide that can control various types of annual and perennial weeds. Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme that occurs in plant chloroplast tissue and converts shikimate-3-phosphate (S-3-P) to enolpyruvylshikimate-3-phosphate (EPSP) and leads to the production of amino acids, phenylalanine, and tyrosine, as well as tryptophan. Shikimate-3-phosphate (S-3-P) cannot be converted to EPSP because S-3-P is unstable, then quickly converted to shikimate more stable and accumulated [4]. The use of herbicides with similar active ingredients be repeated can to resistance. If the weeds are already resistant then it is more difficult to control [5]. This activity will enhance the evolution *E. indica* of herbicide-resistant in oil

palm plantations in Sumatera Utara Province. Several research was reported that *E. indica* has been glyphosate-resistant of oil palm plantations in Sumatera Utara, especially in Serdang Bedagai Regency. According to [6] has reported *E. indica* populations from Adolina Estate has been glyphosate-resistant amount 7-fold compared susceptible population. [7] reported *E. indica* populations from Adolina Estate have been shown to develop into multiple resistance to glyphosate and paraquat. The resistance index value to glyphosate amount 7.5-fold compared susceptible population. In addition, [8] also reported *E. indica* populations was glyphosate-resistant from Adolina Estate is 16.7-fold, *E. indica* from Galang Estate also have been glyphosate-resistant is 5.2-fold, *E. indica* from Rambutan Estate was glyphosate-resistant is 5.8-fold. [9] also reported *E. indica* populations from blocks 1 to 9 of afdeling on Adolina Estate was been 57 glyphosate-resistant populations (98.28%) and 1 population developed resistant (1.72%) from 58 *E. indica* populations. However, this study is only a few estates from oil palm plantations in Serdang Bedagai Regency. Therefore has never been the mapping of *E. indica* populations overall glyphosate-resistant from oil palm plantations in Serdang Bedagai Regency. Serdang Bedagai Regency in 2015 has oil palm land area of government estate (25,878 ha), private and foreign estate (23,549 and 18,743 ha) and smallholder (12,661 ha) [10]. It is the necessary source of information in controlling *E. indica* populations to distribution mapping. This study aims to distribution mapping and resistance classification of *E. indica* to glyphosate herbicide from oil palm plantations in Serdang Bedagai Regency.

II MATERIALS AND METHODS

Collecting seeds of glyphosate-resistant *E. indica* populations were taken the mature seeds are marked in panicles already tanned as many as 50 plants from oil palm estates in Serdang Bedagai Regency (Table 1). Collecting seeds of *E. indica* glyphosate susceptible population (ESU_0) were taken from Politeknik Negeri Medan Ball Field, where the herbicide was never applied [11]. Collecting seeds was conducted in September until December 2017. Seeds of *E. indica* are soaked in potassium nitrate solution (KNO_3) with 0.2% concentration for 30 min [12]. Soaking aims to break *E. indica* seed dormancy. The seedling medium used is topsoil and manure with a volume ratio of 1:1. After thoroughly mixed, the medium was put on a temperature 100ºC for 3 h [11] and then inserted into the germination trays measures 33 × 24 cm. After 2-3 leaf stage, seedlings from each species were transplanted into pots with topsoil, sand and manure medium was filtrated with volume 1:1:1 ratio. Transplanted 10 plants into pots. The pots were arranged in a randomized block design (RBD) non-factorial with three replications.

Before spraying first performed calibration (292 l/ha). Glyphosate spraying is were made at 3-4 leaf stage [13]. The sprayed with glyphosate herbicide recommended dose at 720 g ai ha^{-1} (Round-up 486 SL, PT. Menagro Kimia). Parameters observed among *E. indica* survival, dry weight and resistance classification. Observation of *E. indica* survival was made at 21 days after application (21 DAA) for pot respectively [14]. The percentage of *E. indica* survival (% ES) was calculated using the formula of sum *E. indica* survival (SES) per sum *E. indica* was planted (SEP) x 100%. The mortality of *E. indica* (MEi) was calculated using the formula of sum *E. indica* die (SED) per sum *E. indica* was planted (SEP) x 100%. Observation dry weight of goosegrass was made at 6 weeks after application (6 WAA). Above-ground shoots were harvested and dried in the oven (65°C) for 72 h [14] for dry weight measurements.

$$\% \text{ ES} = \frac{\text{SES}}{\text{SEP}} \text{x } 100\% \quad (1)$$

$$\text{MEi} = \frac{\text{SED}}{\text{SEP}} \text{x } 100\% \quad (2)$$

Resistance classification measured by the percentage of survival populations. Populations were classed as resistant (20% or more survival), as developing resistance (2–20% survival), or as susceptible (less than 2% survival) [15]. Data analysis using mean at the level of 5% with IBM SPSS Statistics v. 20 software (New York: United States).

III RESULT AND DISCUSSION

Population *E. indica* survival on glyphosate dose at 720 g ai ha^{-1} from oil palm plantations in Serdang Bedagai Regency (Figure 1). *E. indica* population was glyphosate-resistant ranged from 23.33 to 100%. *E. indica* population as highest glyphosate-resistant (100%) was found in 2 afdeling of Paya Mabar Estate ($ESU_{4.3}$), 6 and 7 afdeling of Adolina

Estate ($ESU_{3.11}$ and $ESU_{3.12}$). The *E. indica* resistance to glyphosate form oil palm plantations in Serdang Bedagai Regency because continued use of glyphosate to control weeds. Glyphosate can inhibit the enzyme in chloroplast so that it can decrease chlorophyll content and increase accumulation of shikimic acid. This is consistent with [16] which states the EPSPS enzyme on biotype *E. indica* glyphosate-resistant from Southern China rapidly responds to glyphosate herbicide 12 hours after exposure to glyphosate. The expression of mRNA and protein from biotype *E. indica* the glyphosate-resistant increased constantly as glyphosate concentration increased. In addition, [17] which states on leaf biotype *E. indica* glyphosate-resistant from Chengdu and Guangzhou, China has decreased chlorophyll content by a small amount. [18] state that increased levels of shikimic acid on leaf *E. indica* exposed to glyphosate indicate an increase in resistance five to eight-fold compared with susceptible populations from Washington County, Mississippi.

Table 1. *E. indica* populations were taken from oil palm plantations in Serdang Bedagai Regency

No.	Sampling Code	Afdeling	Estates
1	$ESU_{1.34}$	1	Melati Estate
2	$ESU_{1.35}$	2	Melati Estate
3	$ESU_{2.2}$	5	Sarang Ginting Estate
4	$ESU_{2.3}$	1	Rambutan Estate
5	$ESU_{2.4}$	2	Rambutan Estate
6	$ESU_{2.5}$	3	Rambutan Estate
7	$ESU_{2.6}$	4	Rambutan Estate
8	$ESU_{2.7}$	5	Rambutan Estate
9	$ESU_{2.8}$	8	Rambutan Estate
10	$ESU_{2.9}$	5	Silau Dunia Estate
11	$ESU_{2.10}$	3	Tanah Raja Estate
12	$ESU_{2.11}$	4	Tanah Raja Estate
13	$ESU_{2.12}$	5	Tanah Raja Estate
14	$ESU_{2.13}$	1	Gunung Monako Estate
15	$ESU_{2.14}$	2	Gunung Monako Estate
16	$ESU_{2.15}$	3	Gunung Monako Estate
17	$ESU_{2.16}$	4	Gunung Monako Estate
18	$ESU_{2.17}$	2	Gunung Pamela Estate
19	$ESU_{2.18}$	4	Gunung Pamela Estate
20	$ESU_{2.19}$	1	Gunung Para Estate
21	$ESU_{2.20}$	2	Gunung Para Estate
22	$ESU_{2.21}$	6	Gunung Para Estate
23	$ESU_{3.3}$	3	Pabatu Estate
24	$ESU_{3.4}$	6	Pabatu Estate
25	$ESU_{3.5}$	7	Pabatu Estate
26	$ESU_{3.6}$	1	Adolina Estate
27	$ESU_{3.7}$	2	Adolina Estate
28	$ESU_{3.8}$	3	Adolina Estate
29	$ESU_{3.9}$	4	Adolina Estate
30	$ESU_{3.10}$	5	Adolina Estate
31	$ESU_{3.11}$	6	Adolina Estate
32	$ESU_{3.12}$	7	Adolina Estate
33	$ESU_{4.1}$	6	Karya Hevea Indonesia Estate
34	$ESU_{4.2}$	4	Bangun Bandar Estate
35	$ESU_{4.3}$	2	Paya Mabar Estate
36	$ESU_{4.4}$	4	Paya Mabar Estate
37	$ESU_{4.5}$	1	Matapao Estate
38	$ESU_{4.6}$	2	Matapao Estate
39	$ESU_{4.7}$	3	Matapao Estate
40	$ESU_{5.6}$	1	Sibulan Estate
41	$ESU_{5.7}$	2	Sibulan Estate
42	$ESU_{5.8}$	1	Rambung Sialang Estate
43	$ESU_{5.9}$	2	Rambung Sialang Estate
44	$ESU_{5.10}$	3	Rambung Sialang Estate
45	$ESU_{5.11}$	6	Rambung Sialang Estate
46	$ESU_{11.1}$	1	Nusa Pusaka Kencana Estate
47	$ESU_{11.2}$	2	Nusa Pusaka Kencana Estate

Note : ESU (*Eleusine indica* from Sumatera Utara)

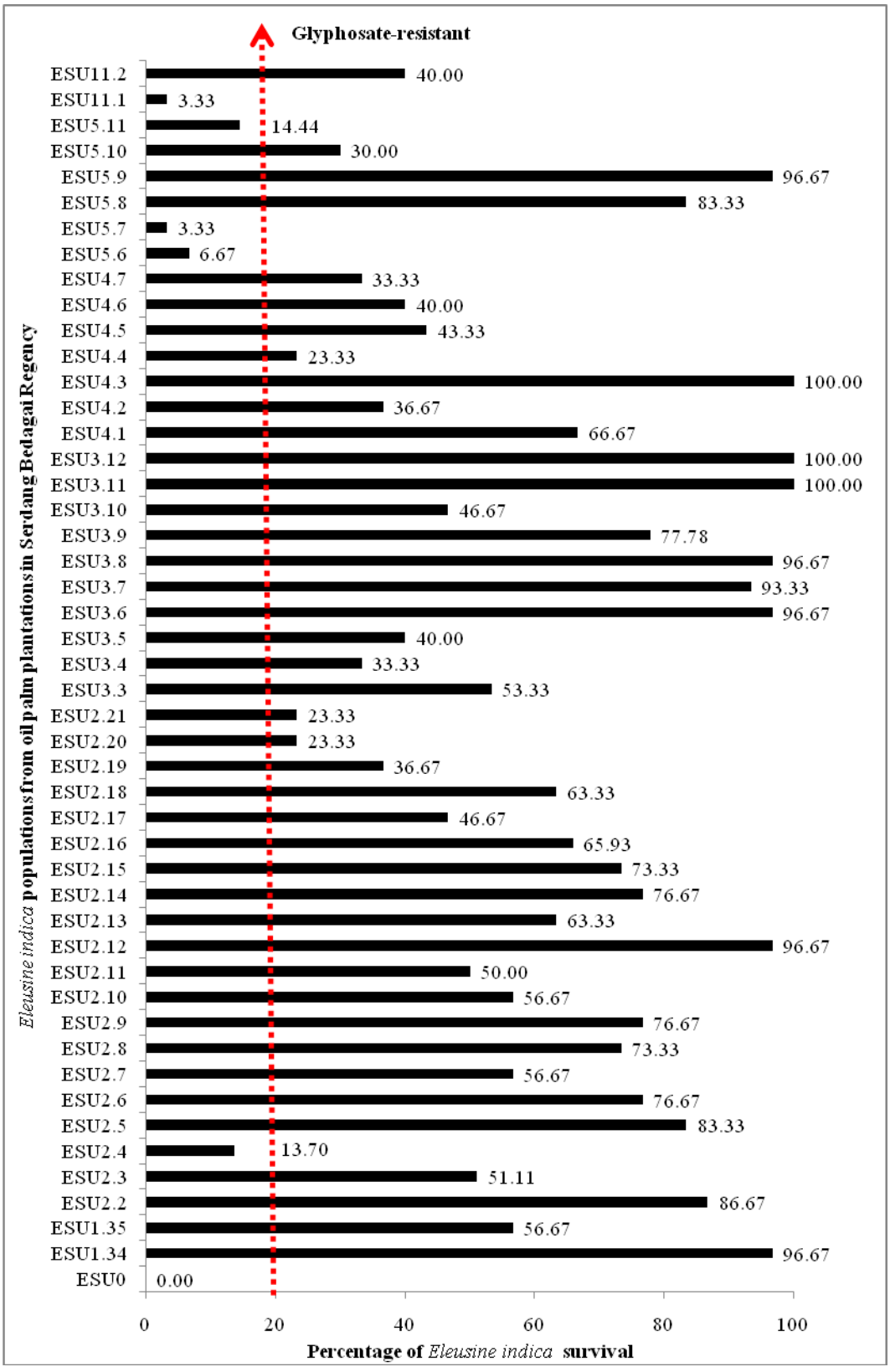

Figure 1. *E. indica* survival populations to glyphosate at 720 g ai ha^{-1} from oil palm plantations in Serdang Bedagai Regency

The average of *E. indica* mortality, dry weight and resistance classification from oil palm plantations in Serdang Bedagai Regency (Table 2). It was found the mortality and dry weight of 42 *E. indica* populations glyphosate-resistant on oil palm plantations in Serdang Bedagai

Regency ranged from 0.00 to 76.67% and 4.60 to 28.58 g. *E. indica* populations classified as developing resistance among $ESU_{2.4}$, $ESU_{5.6}$, $ESU_{5.7}$, $ESU_{5.11}$ and $ESU_{11.1}$. While *E. indica* populations other have been classified as glyphosate-resistant on recommended dose at 720 g ai ha^{-1} or equivalent with 2 L ha^{-1} and there no susceptible population. Glyphosate-resistant *E. indica* populations because of high accumulation of shikimate acid in chloroplast tissue. The *E. indica* resistance in Serdang Bedagai Regency can be caused by a change target site (EPSPS enzyme). Glyphosate inhibits the EPSPS enzyme, resulting in reduced production of amino acids and secondary metabolites. Then interfere fixation carbon and biochemical pathway overall. Symptoms of damage caused relatively slow and sustained whole to weed tissue. Weed leaf has chlorosis from 5 to 10 days after glyphosate application then necrosis before weeds die. This is suitable literature [4] which states there is increased shikimate in chloroplast tissue caused by glyphosate. The accumulation of shikimate acid is caused by glyphosate inhibiting the enzyme EPSPS. EPSPS is enzyme in the aromatic amino acid biosynthesis pathway that converts shikimate-3-phosphate (S-3-P) to enolpyruvylshikimate-3-phosphate (EPSP) and leads to the production of amino acids, phenylalanine, tyrosine, and tryptophan. Shikimate is formed in the glyphosate treatment because S-3-P cannot be changed to EPSP and because S-3-P is unstable, it is converted to a more stable and accumulated shikimate.

From 42 glyphosate-resistant populations of oil palm plantations in Serdang Bedagai Regency (Table 2) was found the *E. indica* highest mortality amount 76.67% there on 4 afdeling of Paya Mabar Estate ($ESU_{4.4}$), 2 and 6 afdeling of Gunung Para Estate ($ESU_{2.20}$ and $ESU_{2.21}$) with average dry weight amount 4.60 g ($ESU_{4.4}$); 4.62 g ($ESU_{2.20}$) and 6.07 g ($ESU_{2.21}$) respectively. This show the use of glyphosate 720 g ai ha^{-1} or 2 L ha^{-1} can be suppressing the growth biotype *E. indica* glyphosate-resistant at 6 WAA (weeks after application) from oil palm plantations in Serdang Bedagai Regency with average dry weight ranges from 4.60 to 6.07 g. This is suitable research with [19] which states that glyphosate potassium herbicide dose at 330 g ai ha^{-1} (1.5 L ha^{-1}), 660 g ai ha^{-1} (3 L ha^{-1}), and 990 g ai ha^{-1} (4.5 L ha^{-1}) can suppress the growth of grasses weed (dry weight) at 6 weeks after planting with three soybean cultivars amount 2.80 g; 2.89 g; and 2.76 g respectively. In addition, [7] also stated the dry weight biotype *E. indica* glyphosate-resistant from Adolina Estate on the application of glyphosate dose at 480 g ai ha^{-1} amount 7.00 g pot^{-1} and decreased dry weight with dose at 960 and 1920 g ai ha^{-1} amount 5.37 and 0 g pot^{-1} respectively.

The percentage of *E. indica* resistance classification from oil palm plantations in Serdang Bedagai Regency (Figure 2). There are 42 populations of *E. indica* classified as glyphosate-resistant (89.36%), 5 populations classified as developing resistance (10.64%), and there is no glyphosate-susceptible population (0%) from oil palm plantations in Serdang Bedagai Regency. This resistant information describes the difficulty of oil palm plantations in controlling weeds especially *E. indica*. The higher percentage of *E. indica* glyphosate resistance, the more difficult to control the weeds. This condition is very disturbing the production and quality of oil palm. Glyphosate herbicides have been used intensely to controlling weeds on oil palm plantations at several regencies in Sumatera Utara. According to [11] reported the *E. indica* populations from oil palm plantations in Padang Lawas and Tapanuli Selatan Regency was the glyphosate-resistant dose at 720 g ai ha^{-1} or equivalent 2 L ha^{-1} amount 36.36% and 83.33% respectively. According to [9] reported the *E. indica* populations from Adolina Estate, Serdang Bedagai also glyphosate-resistant dose at 480 g ai ha^{-1} amount 98.28%. According to [11] reported the *E. indica* populations from oil palm plantations in Padang Lawas and Tapanuli Selatan Regency was the glyphosate-resistant dose at 720 g ai ha^{-1} or equivalent 2 L ha^{-1} amount 36.36% and 83.33% respectively. According to [9] reported the *E. indica* populations from Adolina Estate, Serdang Bedagai also glyphosate-resistant dose at 480 g ai ha^{-1} amount 98.28%. According to [21] reported the *E. indica* populations from Langkat Regency also glyphosate-resistant dose at 720 g ai ha^{-1} amount 42.11%.

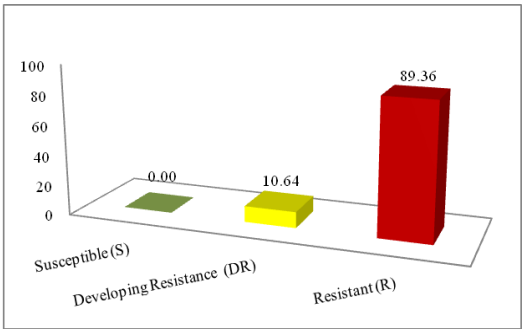

Figure 2. Percentage of *E. indica* resistance classification to glyphosate at 720 g ai ha^{-1} from oil palm plantations in Serdang Bedagai Regency.

Table 2. The average mortality, dry weight, resistance classification of *E. indica* from oil palm plantations in Serdang Bedagai Regency and susceptible population (ESU_0).

No	Sampling Code	Mortality (%)	Dry weight (g)	Resistance classification		
				S	DR	R
1	ESU_0	100.00	0.00	√	-	-
2	$ESU_{1.34}$	3.33	22.95	-	-	√
3	$ESU_{1.35}$	43.33	14.29	-	-	√
4	$ESU_{2.2}$	13.33	19.07	-	-	√
5	$ESU_{2.3}$	48.89	11.61	-	-	√
6	$ESU_{2.4}$	86.30	2.85	-	√	-
7	$ESU_{2.5}$	16.67	21.19	-	-	√
8	$ESU_{2.6}$	23.33	17.66	-	-	√
9	$ESU_{2.7}$	43.33	15.88	-	-	√
10	$ESU_{2.8}$	26.67	15.90	-	-	√
11	$ESU_{2.9}$	23.33	20.94	-	-	√
12	$ESU_{2.10}$	43.33	17.60	-	-	√
13	$ESU_{2.11}$	50.00	14.46	-	-	√
14	$ESU_{2.12}$	3.33	18.67	-	-	√
15	$ESU_{2.13}$	36.67	13.90	-	-	√
16	$ESU_{2.14}$	23.33	23.54	-	-	√
17	$ESU_{2.15}$	26.67	15.02	-	-	√
18	$ESU_{2.16}$	34.07	19.10	-	-	√
19	$ESU_{2.17}$	53.33	11.02	-	-	√
20	$ESU_{2.18}$	36.67	16.94	-	-	√
21	$ESU_{2.19}$	63.33	9.27	-	-	√
22	$ESU_{2.20}$	76.67	4.62	-	-	√
23	$ESU_{2.21}$	76.67	6.07	-	-	√
24	$ESU_{3.3}$	46.67	10.00	-	-	√
25	$ESU_{3.4}$	66.67	6.89	-	-	√
26	$ESU_{3.5}$	60.00	10.14	-	-	√
27	$ESU_{3.6}$	3.33	17.86	-	-	√
28	$ESU_{3.7}$	6.67	28.58	-	-	√
29	$ESU_{3.8}$	3.33	12.59	-	-	√
30	$ESU_{3.9}$	22.22	24.87	-	-	√
31	$ESU_{3.10}$	53.33	9.48	-	-	√
32	$ESU_{3.11}$	0.00	25.84	-	-	√
33	$ESU_{3.12}$	0.00	18.91	-	-	√
34	$ESU_{4.1}$	33.33	12.25	-	-	√
35	$ESU_{4.2}$	63.33	6.79	-	-	√
36	$ESU_{4.3}$	0.00	22.29	-	-	√
37	$ESU_{4.4}$	76.67	4.60	-	-	√
38	$ESU_{4.5}$	56.67	8.08	-	-	√
39	$ESU_{4.6}$	60.00	11.04	-	-	√
40	$ESU_{4.7}$	66.67	5.36	-	-	√
41	$ESU_{5.6}$	93.33	0.86	-	√	-
42	$ESU_{5.7}$	96.67	0.48	-	√	-
43	$ESU_{5.8}$	16.67	12.43	-	-	√
44	$ESU_{5.9}$	3.33	16.61	-	-	√
45	$ESU_{5.10}$	70.00	8.15	-	-	√
46	$ESU_{5.11}$	85.56	2.76	-	√	-
47	$ESU_{11.1}$	96.67	0.95	-	√	-
48	$ESU_{11.2}$	60.00	12.10	-	-	√

Note : S = susceptible (< 2,00%); DR = developing resistance (2,00 - < 20,00%); R = resistant (≥ 20,00%)

Table 3. Report of *E. indica* weed glyphosate-resistant globaly

Country	Year	Situation	Active Ingredients	Site of Action (MoA)
Malaysia	1997	Orchards	Fluazifop-P-butyl, and Glyphosate	**Multiple Resistance: 2 Sites of Action** ACCase inhibitors EPSPS inhibitors
Colombia	2006	Coffee	Glyphosate	EPSPS inhibitors
Bolivia	2007	Soybean	Glyphosate	EPSPS inhibitors
Malaysia	2009	Oil Palm Nursery	Butroxydim, Fluazifop-P-butyl, Glufosinate ammonium, Glyphosate, Haloxyfop-methyl, and Paraquat	**Multiple Resistance: 4 Sites of Action** ACCase inhibitors PSI Electron Diverter EPSPS inhibitors Glutamine synthase inhibitors
China	2010	Orchards	Glyphosate	EPSPS inhibitors
Costa Rica	2010	Pejibaye palm	Glyphosate	EPSPS inhibitors
US (Mississippi)	2010	Cotton	Glyphosate	EPSPS inhibitors
US (Tennessee)	2011	Soybean	Glyphosate	EPSPS inhibitors
Argentina	2012	Corn (maize), Fallow, and Soybean	Glyphosate	EPSPS inhibitors
Indonesia	2012	Oil Palm Nursery	Glyphosate, and Paraquat	**Multiple Resistance: 2 Sites of Action** PSI Electron Diverter EPSPS inhibitors
Jepang	2013	Rice Paddy Levee	Glyphosate	EPSPS inhibitors
Brazil	2016	Corn (maize), Soybean, and Wheat	Glyphosate	EPSPS inhibitors
Brazil	2017	Beans, Corn (maize), Cotton, and Soybean	Fenoxaprop-P-ethyl, Glyphosate, and Haloxyfop-methyl	**Multiple Resistance: 2 Sites of Action** ACCase inhibitors EPSPS inhibitors

Source : [20]

The presence of *E. indica* weed has been widely reported glyphosate-resistant from 1997 to 2017 in various countries (Table 3). It is found the *E. indica* populations were first glyphosate-resistant in 1997 in Malaysia. However, the presence of *E. indica* of glyphosate-resistant from oil palm plantations was first reported in 2009 (oil palm nursery) in Malaysia and in 2012 in Indonesia (oil palm nursery). While the results of this study show the *E. indica* populations has been glyphosate-resistant amount 89.36% from oil palm plantations in Serdang Bedagai Regency. The *E. indica* populations classified as developing resistance is also feared to be evolution and potentially resistant to glyphosate herbicide if the herbicide is used continuously with rotation once in 3-4 months of oil palm plantations in Serdang Bedagai Regency. Therefore management of *E. indica* glyphosate-resistant is needed more appropriate so that production and quality of oil palm produced can be maximized.

CONCLUSION

There are *E. indica* populations classified as glyphosate-resistant amount 89.36% (42 populations), classified as developing resistance amount 10.64% (5 population), there is no glyphosate-susceptible population (0%). The *E. indica* highest glyphosate-resistant (100%) found on 2 afdeling of Paya Mabar Estate ($ESU_{4.3}$); 6 and 7 afdeling of Adolina Estate ($ESU_{3.11}$ and $ESU_{3.12}$) from oil palm plantations in Serdang Bedagai Regency on recommended dose at 720 g ai ha^{-1}.

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