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Full Length Article

Histological, Physiological and Agronomic Characters of Glyphosate-Resistant *Eleusine indica* Biotypes

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Abstract

The presence of glyphosate-resistant *E. indica* biotypes cause weeds control failure in oil palm plantation areas. Histological, physiological and agronomic characters of different *E. indica* biotypes treated with different herbicides and their doses were recorded in the current study. Different herbicides used were; glyphosate, paraquat, glufosinate ammonium and triclopyr, whereas the doses of these herbicides included in the study were; 0, 0.25, 0.50, 1, 2, 4 and 8-fold of the recommended dose. The size of upper epidermis of GR-ESU under 2-fold dose, 3 days after herbicides application was greater than lower epidermis. Glufosinate ammonium and triclopyr at 2 to 8-fold dose effectively (100%) suppressed chlorophyll (SPAD values), survival, number of tillers and fresh and dry biomass of glyphosate resistant *E. indica* (GR-ESU). The resistance index values in biotypes 03, 12 and 29 decreased with glufosinate ammonium by 2.02, 0.20 and 0.33-fold and 1.47, 0.25 and 1.84-folds, respectively with triclopyr compared glyphosate and paraquat. Therefore, it is concluded that *E. indica* can be controlled with glufosinate ammonium and triclopyr in oil palm plants with confirmed resistance to glyphosate.
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Keywords: Glufosinate ammonium; Glyphosate; Paraquat; Resistant; Triclopyr

Introduction

Immature oil palm was cultivated on an area of 180,513 ha in the North Sumatra province of Indonesia during 2017. This cultivated area includes 21,750 ha of foreign estates, 77,005 ha of private estates, 37,043 ha of government estates and 44,715 ha of smallholders (Directorate General of Estate Crops, 2017). Increased weed infestation is observed in immature and nursery oil palms compared to mature oil palms, where goosegrass [*Eleusine indica* (L.) Gaertn] is commonly observed. Ampong-Nyarko *et al.* (1992) stated that *E. indica* is classified as a C4 plant and grows very fast in daylight intensity.

Generally, oil palm plantations in North Sumatra use glyphosate and paraquat herbicides for weed control with a rotation at 3–4 months interval. The use of herbicides with same mode of action to control *E. indica* can lead to the evolution of herbicide resistance and failure in weed control (Purba, 2009). Knezevic *et al.* (2017) stated that herbicide-resistant weeds can develop survival methods after herbicide application. The intensive and continuous use of herbicides having similar modes of action over the past few decades has resulted in the evolution of herbicide-resistant weeds. These evolutions are usually caused by gene mutations or changes in plant metabolism that cause resistance to specific herbicides or groups of herbicides with similar mode of action.

The prevalence of glyphosate-resistant *E. indica* (GR-ESU) biotypes make weed control unsuccessful in oil palm cultivations. Multiple resistance (MR) prevails in *E. indica*; therefore, finding lethal dose 50 (LD_{50}) and the resistance index value (RIV) of different herbicides could help to devise effective management practices against this weed. Lubis *et al.* (2012) stated that the RIV of GR-ESU biotype as 56, 1.5 and 7-fold for paraquat, glufosinate ammonium, and glyphosate compared to susceptible biotypes at Adolina Estate in the Serdang Bedagai Regency. Hambali *et al.* (2015) reported that the RIV of GR-ESU biotypes of 6.4-fold for paraquat at Adolina Estate in the Serdang Bedagai Regency. Dalimunthe *et al.* (2015) reported RIV of GR-ESU biotypes as 5.5 and 7.5-fold for paraquat and glyphosate at Adolina Estate, Serdang Bedagai Regency. Rahmadhani *et al.* (2016) reported RIV of *E. indica* populations as 16.7, 5.2, 5.8, 6.3 and 5.1-fold, and 4.5, 3.3, 2.6, 4.3 and 2.3-fold compared for glyphosate and paraquat, respectively. Tampubolon *et al.* (2019) also reported that 65.56% of *E. indica* populations were resistant to glyphosate in the North Sumatra Province, Indonesia.

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The mode of action of herbicides in multiple resistance has a different mechanism in influencing the histological, physiological and agronomic characters of *E. indica* at oil palm plantations in the North Sumatra. Specific assessment about the histological and physiological responses of GR-ESU to multiple resistance has never been reported at oil palm plantations in the North Sumatra, Indonesia. This research was aimed to study the histological, physiological, and agronomic characters of *E. indica* biotypes to different herbicides and determine their effective doses whichg could control the weed in North Sumatra.

Materials and Methods

Selection of Source Region for GR-ESU Biotypes

The seeds of GR-ESU biotypes used in the study had confirmed resistant to glyphosate (2 L ha^{-1}). The seeds of these biotypes were collected from oil palm plantations in three regencies of North Sumatra. The seeds of biotype 03 were collected from afdeling 1 of Bagerpang Estate in the Deli Serdang Regency (Tampubolon *et al*., 2018a). Similarly, the seeds of biotype 12 were collected from afdeling 2 of Rambung Sialang Estate in the Serdang Bedagai Regency (Tampubolon *et al*., 2018b). Likewise, seeds of biotype 29 were collected from the main nursery of Hapesong Estate in the South Tapanuli Regency (Tampubolon and Purba, 2018). Glyphosate-susceptible *E. indica* seeds were collected from soccer field of Politeknik Negeri Medan (Medan city) which had no herbicide use history for comparison. The seeds of GR-ESU biotypes were collected through November 2018 and March 2019.

Procedure to Grow Seedlings

The topsoil and manure were mixed in a 1:1 volume ratio to prepare germination media. The germination media were steamed at 100ºC for 180 minutes (Tampubolon and Purba, 2018) and then filled into the germination trays (37 cm × 19.5 cm). The 2-3 leaved seedlings of *E. indica* were transplanted to pots (10 plants pot^{-1}) filled with topsoil, sand, and manure media in 1: 1: 1 ratio. The seedlings were grown at Weed Research Center Land, Faculty of Agriculture, Universitas Sumatera Utara.

Application of Herbicides

The spray volume was calibrated at 277.78 l ha^{-1}. Four herbicides with different modes of action, *i.e.*, glyphosate (Round-up 486 SL, PT. Menagro Kimia), glufosinate ammonium (Basta 150 SL, PT. Bayer Indonesia), paraquat (Gramoxone 276 SL, PT. Syngenta Indonesia) and triclopyr (Garlon 670 EC, Dow AgroSciences) with 0, 0.25, 0.50, 1, 2, 4 and 8-fold of the recommended dose were used in the study. The experiment was laid out according to Randomized Block Design (RBD) with factorial arrangement and four replications. The herbicides were applied when seedlings had 3-4 leaves (Hess *et al.*, 1997), during full light at 31°C, 73% moisture, and 1,002 h Pa air pressure.

Agronomic, Histological, and Physiological Characters

The agronomic characters included survival pot^{-1} at 3 weeks after spraying (WAS), tillers pot^{-1} at 3 and 6 WAS, fresh and dry biomass pot^{-1} at 6 WAS, LD_{50}, GR_{50}, RIV and growth reduction. The dry biomass was recorded by oven drying the plants at 65ºC for 72 h (Jalaludin *et al*., 2015). The growth reduction was calculated using the dry biomass (Mohamad *et al*., 2010). The histological characters included upper epidermis tissue, mesophyll and lower epidermis, which were taken from two-leaflet leaves with recommended dose at 3 days after spraying (DAS). Measurements of the histological characters were made using transverse incision with the paraffin method (Johansen, 1940) and image capture using the AxioVision 4.8 applications with a 10×10 magnification. The physiological characteristics included chlorophyll (SPAD values) of GR-ESU biotypes which were taken by two-leaflet leaves at 1, 3, 5, 7, 14 DAS using the SPAD 502 plus chlorophyll meter. The equations for calculating different parameters are given below:

$$\% \text{ Controlling histology tissue} = \frac{\text{Size histology } E.indica \text{ sprayed} - \text{unsprayed}}{\text{Size histology } E.indica \text{ unsprayed}} \times 100\% \quad (1)$$

$$\% \text{ Histology tissue R/S} = \frac{\text{Size histology } E.indica \text{ resistant} - \text{susceptible}}{\text{Size histology } E.indica \text{ susceptible}} \times 100\% \quad (2)$$

$$\% \textit{ E. indica} \text{ survival} = \frac{\sum E.indica \text{ survive}}{\sum E.indica \text{ was planted}} \times 100\% \quad (3)$$

$$\% \text{ Controlling tillers } \textit{E. indica} = 100 - \frac{\sum \text{Tillers } E.indica \text{ herbicide} - \text{sprayed}}{\sum \text{Tillers } E.indica \text{ unsprayed}} \times 100\% \quad (4)$$

$$\% \text{ Growth reduction} = 100 - \frac{\text{Dry weight } E.indica \text{ herbicide} - \text{sprayed}}{\text{Dry weight } E.indica \text{ unsprayed}} \times 100\% \quad (5)$$

$$\text{Resistance index value} = \frac{LD50\ E.indica \text{ herbicide} - \text{resistant}}{LD50\ E.indica \text{ herbicide} - \text{susceptible}} \quad (6)$$

$$\text{R/S ratio of } GR_{50} = \frac{GR50\ E.indica \text{ herbicide} - \text{resistant}}{GR50\ E.indica \text{ herbicide} - \text{susceptible}} \quad (7)$$

$$Ym = a + b1X \text{ or } Ydw = a + b2X \quad (8)$$

Where Ym is probit regression of mortality, Ydw is probit regression of dry weight, a is intercept, b1 is coefficient regression of mortality, b2 is coefficient regression of dry weight and X is log10 (dose). The physiological and agronomic characters were analyzed using ANOVA and the means were followed by DMRT at 5% probability level. The LD_{50} and GR_{50} were analyzed using probit regression from the comparison of susceptible and resistant populations using IBM SPSS Statistics v.20 software. The RIV and R/S ratio of GR_{50} were calculated by comparing LD_{50} and GR_{50} resistant and susceptible populations.

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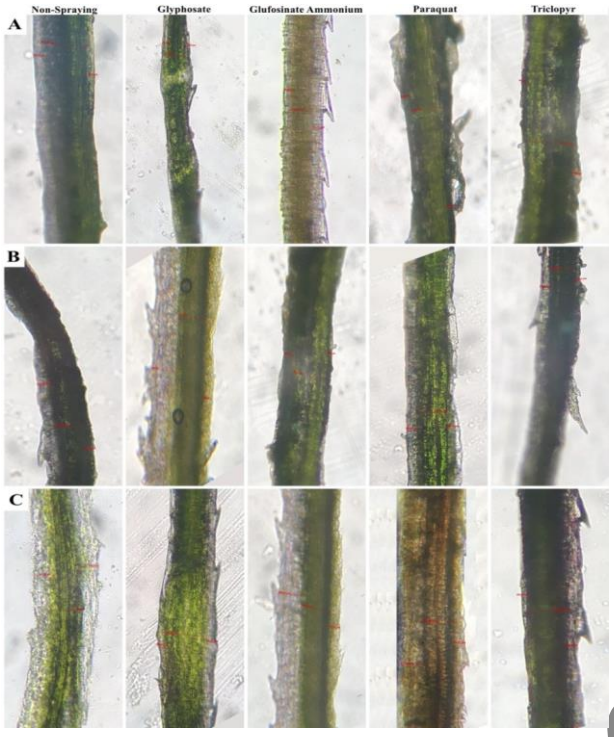

Fig. 1: Transverse incision of upper epidermis size, mesophyll, and lower epidermis of GR-ESU biotypes and susceptible population on herbicides sprayed at the 2-fold dose recommendation at 3 DAS. (A = biotype 03); (B = biotype 12); (C = biotype 29)

Results

The herbicides and their tested doses significantly (P<0.05) influenced the agronomic and physiological characters of GR-ESU biotypes (Table 1). Glyphosate, glufosinate ammonium, paraquat and triclopyr significantly influenced chlorophyll (SPAD values) from the first until 14 DAS. Likewise, survival, tillers, fresh weight, and dry weight of biotypes were also affected by applied herbicides.

Histological Characters

The upper epidermis size, mesophyll and lower epidermis of GR-ESU biotypes with 2-fold of the recommended dose at 3 DAS are presented in Fig. 1 and Table 2. The upper epidermis size of glyphosate-susceptible and resistant biotypes at 3 DAS was greater compared to the lower epidermis size. A decrease in the upper and lower epidermis was seen in biotype 03 but there was an increase in mesophyll size compared to herbicides-susceptible population. An increase in the upper epidermis and decrease in mesophyll and lower epidermis size was observed for biotype 12 compared to susceptible population. An increase in the upper and lower epidermis and decrease in mesophyll size was recorded for biotype 29.

Glyphosate and glufosinate ammonium with 2 L ha^{-1} dose significantly suppressed the upper epidermis size, lower epidermis and mesophyll in biotypes 03 and 29 compared to biotype 12. Paraquat and triclopyr suppressed the upper and lower epidermis, and mesophyll in biotypes 12 and 29 compared to biotype 03. The change of leaf histology size was dependent on the location of biotypes and active ingredient of herbicides.

Physiological Characters

Different herbicides included in the study significantly affected SPAD values of GR-ESU biotypes at 1, 3, 5, 7 and 14 DAS (Fig. 2). Glyphosate, glufosinate ammonium, and paraquat were effective in decreasing SPAD values in biotype 03 at 5 to 14 DAS, while triclopyr was effective at 7 to 14 DAS. Glufosinate ammonium and triclopyr decreased SPAD values in biotype 12 at 1 to14 DAS, while glyphosate and paraquat were unable to decrease SPAD values. Glyphosate effectively decreased SPAD values in biotype 29 at 5 to 14 DAS, glufosinate ammonium at 3 to 14 DAS, triclopyr at 7 to 14 DAS, and paraquat at 1 to3 DAS.

Agronomic Characters

Survival of GR-ESU biotypes: The applied herbicides significantly affected the survival of GR-ESU biotypes at 21 DAS (Fig. 3) and visual observations are presented in Fig. 4. The types and doses of herbicides were effective in controlling GR-ESU. Glufosinate ammonium with 300 to 1,200 g a.i ha^{-1} dose and triclopyr with 1,920 to 3,840 g a.i ha^{-1} dose effectively (100%) controlled GR-ESU biotypes compared to glyphosate and paraquat.

Controlling Tillers of GR-ESU biotypes: Different herbicides significantly altered the number of tillers of GR-ESU biotypes at 3 and 6 WAS (Fig. 5). An increase in the number of tillers was observed from 3 to 6 WAS for all biotypes. A decrease in the tillers of GR-ESU biotypes was observed with increased dose of glufosinate ammonium and triclopyr at 3 WAS. Glufosinate ammonium and triclopyr effectively (100%) reduced the tillers in biotypes 03 and 12 at 3 and 6 WAS compared to paraquat and glyphosate. Glufosinate ammonium, triclopyr and glyphosate effectively (100%) controlled the tillers in biotype 29 at 3 and 6 WAS compared to paraquat.

Fresh weight of GR-ESU biotypes: The tested herbicides significantly influenced fresh weight of GR-ESU biotypes at 6 WAS (Fig. 6). A decrease in the fresh weight was noted with increasing dose of glufosinate ammonium and triclopyr herbicides at 6 WAS in contrast to glyphosate and paraquat.

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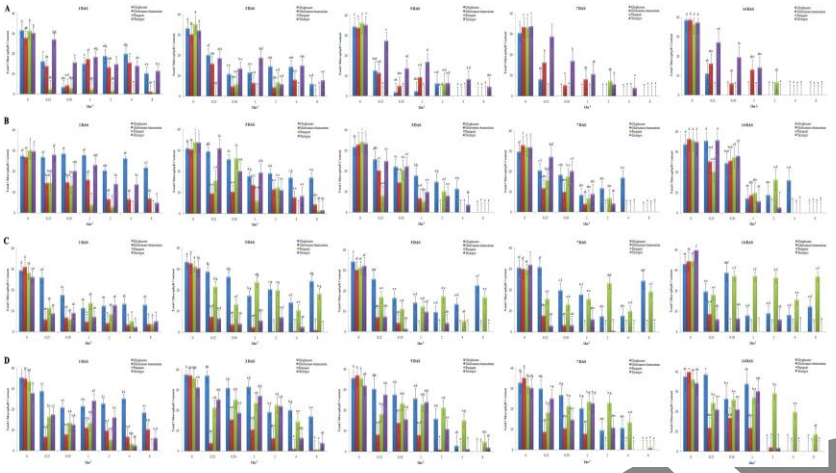

Fig. 2: Chlorophyll (SPAD) contents of GR-ESU biotypes and susceptible population at 1, 3, 5, 7, and 14 DAS. A= Susceptible, B= Biotype 03, C= Biotype 12, D= Biotype 29. Vertical bars indicate ± SE. Different lowercase letters mean significant difference by DMRT at $P < 0.05$

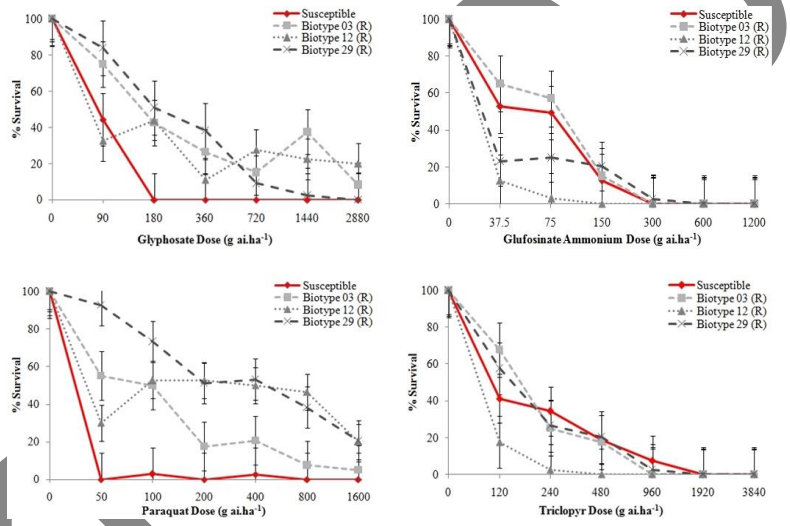

Fig. 3: Survival of GR-ESU biotypes and susceptible population at 21 DAS. Vertical bars indicate ± SE

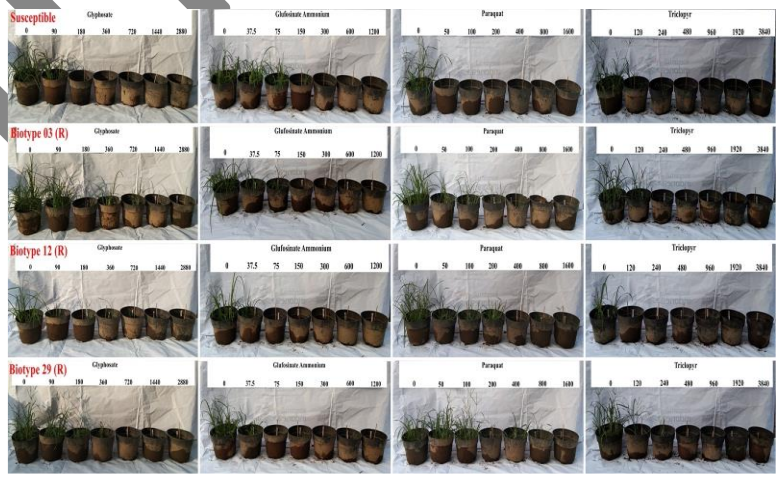

Fig. 4: Visual observation of GR-ESU biotypes and susceptible population at 3 WAS

Table 1: Mean square of ANOVA in GR-ESU biotypes and susceptible population

Characters	Mean Square of ANOVA			
	Susceptible	Biotype 03 (R)	Biotype 12 (R)	Biotype 29 (R)
Physiological (SPAD)				
Total chlorophyll 1 DAS	330.21*	261.47*	285.08*	341.36*
Total chlorophyll 3 DAS	421.77*	409.56*	331.71*	462.70*
Total chlorophyll 5 DAS	613.76*	568.70*	390.21*	598.96*
Total chlorophyll 7 DAS	600.21*	533.60*	391.66*	538.43*
Total chlorophyll 14 DAS	811.68*	877.67*	518.55*	833.20*
Agronomic				
Survival 3 WAS	5632.07*	5408.23*	4274.27*	5051.34*
Tillers 3 WAS	251.60*	223.94*	148.42*	158.20*
Tillers 6 WAS	544.11*	432.47*	242.89*	340.45*
Fresh weight 6 WAS	10682.37*	11962.09*	4623.58*	5501.45*
Dry weight 6 WAS	392.14*	422.62*	145.42*	203.90*

Note: *The means indicates that is significantly different by DMRT at $P < 0.05$

Table 2: Upper epidermis size, mesophyll, and lower epidermis of GR-ESU and susceptible population on herbicides-sprayed at the 2-fold dose recommendation at 3 DAS

Herbicides-sprayed	Histology characters (µm) and compared to non-spraying (%)		
	Upper Epidermis	Mesophyll	Lower Epidermis
Susceptible	76.21	352.83	58.62
Biotype 03 (R)			
Non-Spraying	71.42 (0.94)*	373.23 (+5.78%)**	58.20 (-0.72%)*
Glyphosate	35.76 (-49.93%)	205.38 (-44.97%)	32.02 (-44.98%)
Glufosinate Ammonium	78.77 (10.29%)	175.83 (-52.89%)	66.45 (14.18%)
Paraquat	93.66 (31.14%)	326.65 (-12.48%)	68.38 (17.49%)
Triclopyr	96.46 (35.06%)	415.73 (11.39%)	64.65 (11.08%)
Biotype 12 (R)			
Non-Spraying	77.12 (+1.19%)**	203.22 (-42.40%)*	57.41 (-2.06)*
Glyphosate	282.00 (265.66%)	333.93 (64.32%)	110.00 (91.60%)
Glufosinate Ammonium	133.78 (73.47%)	366.37 (80.28%)	58.81 (2.44%)
Paraquat	80.06 (3.81%)	240.77 (18.48%)	64.01 (11.50%)
Triclopyr	76.34 (-1.01%)	217.82 (7.18%)	41.42 (-27.85%)
Biotype 29 (R)			
Non-Spraying	166.40 (+118.34)**	316.26 (-10.36)*	153.95 (+162.62)**
Glyphosate	77.37 (-53.50%)	328.39 (3.84%)	60.97 (-60.40%)
Glufosinate Ammonium	202.57 (21.74%)	274.23 (-13.29%)	84.51 (-45.11%)
Paraquat	178.44 (7.24%)	236.39 (-25.25%)	84.76 (-44.94%)
Triclopyr	66.19 (-60.22%)	285.78 (-9.64%)	44.80 (-70.90%)

Note: (– means pressing), (+ means non-pressing), (*means decrease of susceptible, (**means increase of susceptible)

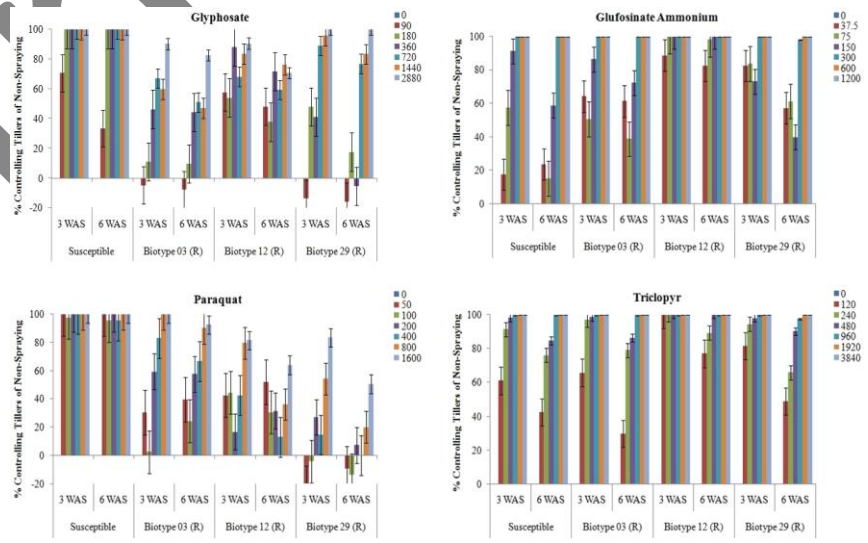

Fig. 5: Number of tillers of GR-ESU biotypes and susceptible population at 3 and 6 WAS. Vertical bars indicate ± SE

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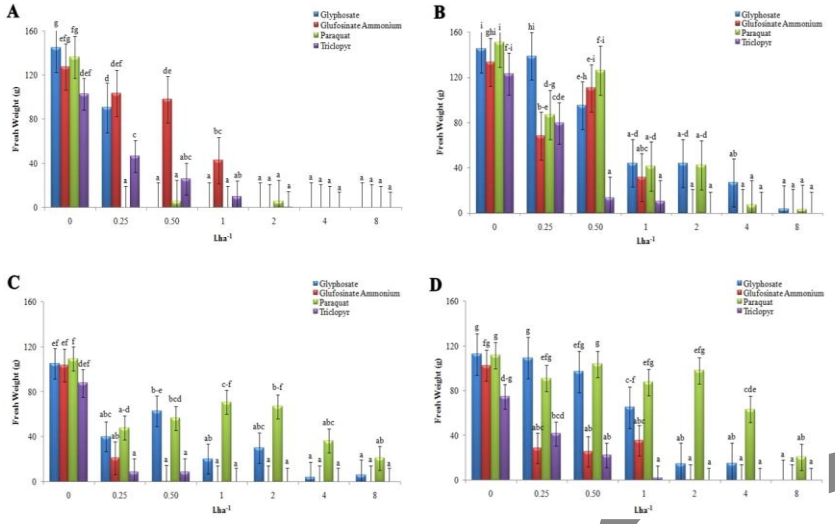

Fig. 6: Fresh weight of GR-ESU biotypes and susceptible population at 6 WAS. A= Susceptible, B= Biotype 03, C= Biotype 12, D= Biotype 29. Vertical bars indicate ± SE. Different lowercase letters mean significant difference by DMRT at $P < 0.05$

Glufosinate ammonium and triclopyr at 2 to 8-fold of recommended dose effectively (100%) reduced the fresh weight in biotypes 03 and 29 at 6 WAS compared to glyphosate and paraquat. Glufosinate ammonium and triclopyr at 1 to 8-fold of recommended dose effectively (100%) decreased the fresh weight in biotype 12 at 6 WAS compared to glyphosate, whereas paraquat was ineffective.

Growth Reduction

The growth reduction of GR-ESU at 6 WAS is presented in Fig. 7. The highest growth reduction of GR-ESU biotypes was found on glufosinate ammonium and triclopyr at 2 to 8-fold dose. Paraquat at the 2 to 8-fold dose was able to achieve growth reduction of GR-ESU from 18.62% to 97.08%.

LD_{50}, GR_{50} and Resistance Index Value

The LD_{50}, GR_{50}, and RIV of GR-ESU biotypes are presented in Table 3. Glyphosate dose causing 50% mortality of GR-ESU (LD_{50}) in biotypes 03, 12 and 29 was 177.46, 95.00 and 218.79 g a.i. ha^{-1}, respectively. The R/S ratio of GR_{50} in biotypes 03, 12 and 29 for glyphosate was 2.52, 0.58 and 3.90-fold, respectively. The RIV in biotypes 03, 12, and 29 with glyphosate was 2.49, 1.33 and 3.07-fold, respectively. The R/S ratio of GR_{50} and RIV were decreased by glufosinate ammonium and triclopyr, in contrast an increase with paraquat sprayed.

Discussion

Overall, the herbicide types and their doses significantly influenced the suppression of the agronomic and physiological characteristics of GR-ESU biotypes (Table 1). A decrease in SPAD values, survival, tillers, fresh and dry weight of biotypes was recorded 6 WAS. This is linear to Simarmata *et al*. (2005) who stated that *Lolium rigidum* had decreased survival with increase glyphosate dose up to 8-fold in California. Kaundun *et al*. (2008) stated that *E. indica* had increased mortality and decreased biomass production with increasing glyphosate dose up to 16 kg a.i ha^{-1} at 21 and 35 DAS in the Davao island, Philippines. In addition, Huffman *et al*. (2016) stated that the dry weight of glyphosate-resistant *E. indica* biotypes was decreased with an increase in glyphosate dose and completely (100%) controlled with 1,680 to 6,720 g ha^{-1} dose in the Tennessee, United States.

Glyphosate and glufosinate ammonium with 2 l ha^{-1} dose proved effective in suppressing the upper and lower epidermis size and mesophyll in biotypes 03 and 29 compared to biotype 12 (Table 2). The effect of glyphosate showed the symptoms of chlorosis in the leaf tissue which might be due to the inhibition of EPSPS enzymes. Glufosinate ammonium can inhibit electron flow in photosynthesis and glutamine synthesis (GS) enzymes; thus, which increased ammonia level quickly by 10-fold at 4 h after sprayed compared to non-spraying. Paraquat and triclopyr suppressed the upper and lower epidermis, and mesophyll in biotypes 12 and 29 compared to biotype 03. The paraquat application showed the symptoms of necrosis a few hours after sprayed on leaf tissue. Triclopyr had slow response translocated to phloem tissue. According to Monaco *et al*. (2002) the symptoms of glyphosate were classified as slow and continuous requiring 5 to 10 DAS for showing the chlorosis, which turn into necrosis and ultimately mortality. Glyphosate had low mobility in xylem and phloem tissues. Paraquat had the ability to accept electrons from photosystem I during electron flow in the photosynthesis and become free radicals, which can stop electron transport to Nicotinamide Adenine Dinucleotide Phosphate; thus, cell membrane damage occurs at few hours after spraying.

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Table 3: LD_{50}, GR_{50}, and resistance index value of GR-ESU biotypes

Biotypes	LD_{50}			GR_{50}		
	Regression Equation	g a.i. ha^{-1}	Resistance Index value	Regression Equation	g a.i. ha^{-1}	R/S ratio of GR50
Glyphosate-MR						
Susceptible	Y = -6.21 + 3.35X	71.26		Y = 12.74 - 6.59X	86.01	
Biotype 03 (R)	Y = -2.26 + 1.01X	177.46	2.49	Y = 3.02 - 1.29X	217.03	2.52
Biotype 12 (R)	Y = -2.01 + 1.01X	95.00	1.33	Y = 1.77 - 1.04X	49.72	0.58
Biotype 29 (R)	Y = -5.62 + 2.40X	218.79	3.07	Y = 5.76 - 2.28X	335.25	3.90
Glufosinate Ammonium-MR						
Susceptible	Y = -4.25 + 2.49X	51.36		Y = 5.28 - 2.74X	84.07	
Biotype 03 (R)	Y = -11.16 + 5.53X	103.82	2.02	Y = 3.56 - 2.03X	56.29	0.67
Biotype 12 (R)	Y = -2.13 + 2.11X	10.20	0.20	Y = 3.25 - 2.66X	16.54	0.20
Biotype 29 (R)	Y = -1.62 + 1.32X	16.91	0.33	Y = 2.09 - 1.62X	19.50	0.23
Paraquat-MR						
Susceptible	Y = -0.35 + 1.13X	6.05		Y = 1.08 - 1.32X	6.65	
Biotype 03 (R)	Y = -2.32 + 1.27X	68.17	11.27	Y = 2.67 - 1.36X	92.36	13.89
Biotype 12 (R)	Y = -0.23 + 0.18X	19.39	3.20	Y = 2.07 - 1.12X	69.75	10.49
Biotype 29 (R)	Y = -3.23 + 1.26X	371.99	61.49	Y = 3.03 - 1.21X	317.01	47.67
Triclopyr-MR						
Susceptible	Y = -3.53 + 1.72X	113.13		Y = 3.17 - 1.72X	70.20	
Biotype 03 (R)	Y = -6.14 + 2.77X	166.21	1.47	Y = 8.75 - 4.18X	124.08	1.77
Biotype 12 (R)	Y = -2.48 + 1.71X	27.98	0.25	Y = 2.53 - 1.76X	27.71	0.39
Biotype 29 (R)	Y = -4.70 + 2.17X	208.65	1.84	Y = 6.72 - 3.16X	133.88	1.91

Note : Y = Probit value from mortality and dry weight. X = Log Dose

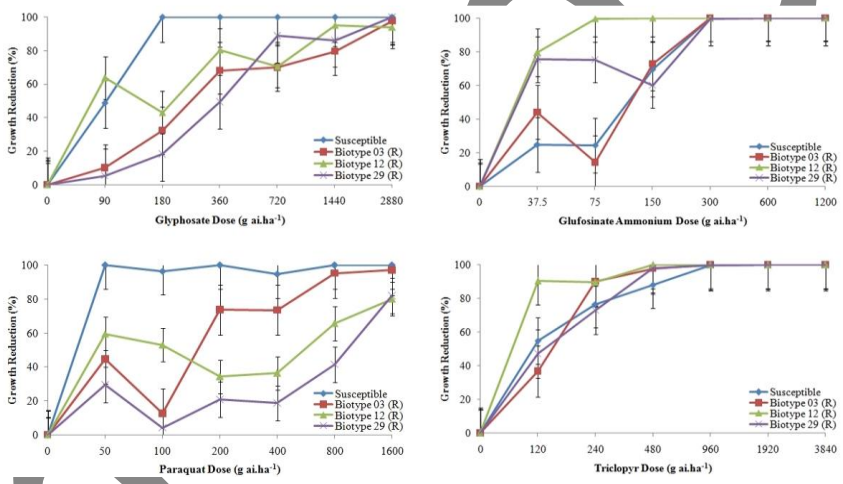

Fig. 7: Growth reduction of GR-ESU biotypes at 6 WAS. Vertical bars indicate ± SE

Glufosinate ammonium can inhibit glutamine synthesis caused by a decrease in the levels of several amino acids such as glutamate, aspartate, asparagine, alanine and serine. Triclopyr inhibits growth regulators by translocating active ingredients slowly to phloem tissue and accumulating in the meristematic tissues (leaf and root). In addition, Saw (2011) stated that *E. indica* population had the upper epidermis tissue (length 4.4-6.1-9.9 µm x width 1.1-1.4-1.7 µm) in the North Dagon, Myothit (Myanmar) which was greater than lower epidermis.

Glyphosate at the dose 2,880 g a.i. ha^{-1}, glufosinate ammonium at the dose 300 to 1,200 g a.i. ha^{-1} and paraquat at the dose 800 to 1,600 g a.i. ha^{-1} effectively (100%) decreased total SPAD in biotype 03 at 5 to 14 DAS, while triclopyr at the dose 1,920 to 3,840 g a.i. ha^{-1} was effective at 7 to 14 DAS (Fig. 2). Glufosinate ammonium at the dose 150 to 1,200 g a.i. ha^{-1} and triclopyr at the dose 1,920 to 3,840 g a.i. ha^{-1} effectively (100%) lowered total SPAD in biotype 12 at 1 until 14 DAS, while glyphosate and paraquat were unable to decrease SPAD values. Glyphosate at the dose 2,880 g a.i. ha^{-1}, glufosinate ammonium at the dose 600 to 1,200 g a.i. ha^{-1}, and triclopyr at the dose 1,920 to 3,840 g a.i. ha^{-1} effectively (100%) suppressed total SPAD in biotype 29 at 5 to 14 DAS, at 3 until 14 DAS, at 7 until 14 DAS, respectively; however, paraquat at the dose 1,600 g a.i. ha^{-1} was only able to decrease total SPAD at 1 to 3 DAS (Fig. 5). Based on total SPAD in multiple resistances, it can be suggested to use of glufosinate ammonium at 1-fold dose as the first solution in management of GR-ESU. According to Chen *et al*. (2015), glyphosate at the dose 1,680 g a.i. ha^{-1} was able decrease total SPAD of glyphosate-resistant *E. indica* biotypes (ZC1, HD1, SL3, SL4 and SL6) from China at 2 until 10 DAS.

It is shown that glufosinate ammonium can inhibit glutamine synthesis enzyme in leaf tissue of glyphosate-resistant *E. indica* biotypes. According to Seng *et al*. (2010) glufosinate-resistant *E. indica* biotypes was completely (100%) controlled at 4-fold dose or 1.80 kg.ha^{-1} at 7 DAS. Avila-Garcia and Mallory-Smith (2011) stated that glyphosate-resistant *Lolium perenne* biotypes (OR1, OR2, and OR3) from Oregon at the dose 0.4 kg a.i. ha^{-1} of glufosinate ammonium at 0 h after spray (HAS) had ammonia levels ranged from 11.0 to 15.9 μg.g^{-1} fresh weight, then increased at 24, 48, 72 and 96 HAS and the lower compared to susceptible populations. Jalaludin *et al*. (2017) stated that [^{14}C]-glufosinate uptake of glufosinate ammonium-resistant *E. indica* biotypes had increased from 27.6 to 49.9% at 16 to 72 HAS at the dose 125 g a.i. ha^{-1} in Malaysia. In addition, Lewis *et al*. (2012) stated that use of triclopyr at the dose 1.12 kg.ha^{-1} controlled *E. indica* at 28 and 98 DAS of 48% and 73%, respectively in Tennessee, United States.

Seng *et al*. (2010) stated that control of *E. indica* from Air Kuning, Malaysia was decreased by 10% at 21 DAS with glufosinate ammonium and paraquat compared to 7 DAS and recovery occurred *E. indica* at 21 DAS. Lubis *et al*. (2012) also stated that tillers of glyphosate-resistant *E. indica* biotypes were completely (100%) controlled at the dose 330 to 660 g a.i. ha^{-1} glufosinate ammonium at 6 WAS.

According to Seng *et al*. (2010), the fresh weight of *E. indica* was decreased with an increase in the dose of glufosinate ammonium and effectively (100%) controlled at the 4-fold dose r in Malaysia. Mueller *et al*. (2011) stated that fresh and dry weight of glyphosate-resistant *E. indica* were decreased with an increase in the dose glyphosate at 3 WAS in Tennessee, United States. Gherekhloo *et al*. (2017) stated that the fresh weight were decreased in 4 biotypes of glyphosate-resistant *E. indica* along with an increase at the dose glyphosate at 3 WAS in Veracruz, Mexico. Simarmata (2009) also stated that the ability of glufosinate ammonium was higher in the control of glyphosate-resistant *Lolium rigidum* biotypes compared to glyphosate.

Seng *et al*. (2010) stated that the GR_{50} of *E. indica* biotypes with 0.17 kg ha^{-1} of glufosinate ammonium from Malaysia. Molin *et al*. (2013) stated that dry weight of glyphosate-resistant *E. indica* biotypes (GG14, GG16 and GG19) was decreased with an increase in glyphosate dose at 2 WAS in Mississippi. Huffman *et al*. (2016) stated that the dry weight of glyphosate-resistant *E. indica* in Tennessee, United States was decreased along with an increase in glyphosate dose and the highest (100%) was found with 1,680 to 6,720 g a.i. ha^{-1} glyphosate. Jalaludin *et al*. (2010) also stated that controlling *E. indica* was increased ranged from 0 to 85% along with an increase at the dose 495 to 3,960 g a.i.ha^{-1} glufosinate ammonium with the LC_{50} amounted to 2,297 g.ha^{-1} from oil palm nursery in the Jerantut, Malaysia.

The RIV and R/S ratio of GR_{50} showed that the difficulty to control *E. indica* which had been previously reported to be glyphosate-resistant (65.56%) at 2-fold dose in the North Sumatra (Tampubolon *et al*., 2019). Histological, physiological and agronomic characters of GR-ESU biotypes were suppressed by glufosinate ammonium and triclopyr compared to glyphosate and paraquat. Therefore, the use of glufosinate ammonium and triclopyr (different modes of action) are highly recommended for control of GR-ESU biotypes compared to glyphosate and paraquat in oil palm plantations of North Sumatra. Simarmata *et al.* (2003) stated that the shikimic acid of glyphosate-resistant *Lolium rigidum* biotype decreased 10-fold on glyphosate-sprayed at the dose 2.24 kg ha^{-1} compared to the susceptible population at 11 DAS in California, United States. Jalaludin *et al.* (2015) also stated that the use of glufosinate ammonium was ineffective in controling glufosinate-resistant *E. indica* biotypes compared to paraquat (GR_{50} and $RI_{glufosinate}$ > GR_{50} and $RI_{paraquat}$), it means that the rotation of mode of action herbicide (paraquat) effectively controlled glufosinate-resistant *E. indica* biotypes compared to the same herbicide (glufosinate ammonium).

Conclusions

The upper epidermis size of GR-ESU biotypes was greater than lower epidermis. Glufosinate ammonium and triclopyr at 2 to 8-fold of recommended dose effectively (100%) suppressed total SPAD, survival, tillers, fresh weight, and dry weight of GR-ESU biotypes compared to paraquat and glyphosate. The RIV and R/S ratio of GR_{50} in biotypes 03, 12, and 29 were decreased with the use of glufosinate ammonium (RIV = 2.02; 0.20; 0.33-fold and R/S ratio of GR_{50} = 0.67; 0.20; 0.23-fold) and triclopyr (RIV = 1.47; 0.25; 1.84-fold and R/S ratio of GR_{50} = 1.77; 0.39; 1.91-fold) and lower compared to glyphosate and paraquat. The use of glufosinate ammonium and triclopyr has shown that the rotation with different mode of action herbicides effectively controlled GR-ESU biotypes at oil palm plantations in the North Sumatra.

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