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Research Article

Dominance of *Cyperus kyllingia* Endl. at guava orchard and its possible resistance to glyphosate

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ABSTRACT

Weed resistance, indicated by increasing the level of herbicide for control, might arise due to the continuous use of similar herbicides in the long term without any herbicide rotation. The objective of this research was to evaluate the status of dominant weeds in a guava orchard to determine weed control strategies. The research was carried out from November 2020 to June 2021 at Agribusiness Technology Park IPB, Bogor. Vegetation analysis to define dominance was carried out at 4 blocks using the square method. In each block, five sampling points were randomly selected. Seeds of C. kyllingia were collected and planted as many as 25 seedlings in polybags. Glyphosate herbicide was applied onto C. kyllingia in 3 replications using 9 doses, i.e., 0 (D0), 60.75 (D1), 121.5 (D2), 243 (D3), 486 (D4), 972 (D5), 1944 (D6), 3888 (D7), and 7776 g ha^{-1} (D8). C. kyllingia mortality percentages were analyzed using linear regression to obtain LD_{50}. The results showed that the dominant weed was C. kyllingia. Block 4 showed the highest significant LD_{50} value at 759.11 g ha^{-1} with a resistance index of 2.67 (reduced sensitivity). The presence of dominant C. kyllingia was identified only at block 4, indicating the dominance could correlate with increasing resistance of the particular weed.

Keywords: doses; EPSPS; glyphosate; LD_{50}; sedges; vegetation analysis

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INTRODUCTION

Guava is one of the horticultural commodities that has great potential to be developed and cultivated in Indonesia as a source of Vitamin C. The content in guava is useful in boosting the body's immunity, anti-hypertension, anti-diabetes, antioxidants, and anti-cancer (Murthy & Bapat, 2020). One of the commercial guava varieties is crystal, a kind of seedless guava. According to statistics (BPS, 2018; BPS, 2020), guava production from 2018-2020 reached 230,697 tonnes to 396,268 tonnes.

However, high weed investments in guava orchards become important issues because it decreases fruit production and decreases maintenance effectiveness (Melo et al., 2019). The weed communities in the field vary due to variations in land topography and environment (Suryatini, 2018). One of the common weed control in guava orchards is using herbicides. Herbicides application is considered the easiest method with optimum results than other methods. The method also has more labor efficiency as compared to other methods, especially manual weeding (Kraehmer et al., 2014).

However, weed control using herbicides with the same active ingredient continuously in the long term without any rotation stimulates weed resistance to certain herbicides. Weed resistance might occur due to gene mutation (Beckie, 2020; Chen et al., 2017). Herbicide resistance is a phenomenon when they still survive and reproduce normally after the application of a particular herbicide at normal doses and the ability is inherited by offspring (Manik, 2019). Many cases of weed resistance have been reported, especially *Eleusine indica* (Manik, 2019; Tampubolon and Purba, 2018). To control resistance in weeds, higher level herbicides are required, therefore, weed resistance could imply financial orchard management.

The study area, Agribusiness Techno Park (ATP), maintains a guava orchard and to control weeds a herbicide of glyphosate is primarily used. Herbicides application in the ATP is 3 times a year. Each application uses a dose of 3 L ha^{-1} glyphosate solution (1458 g ha^{-1} of active ingredient) with a spraying volume of 400 L ha^{-1}. Although weed resistance evaluation has been widely carried out in many oil palm plantations in relation to frequent herbicide application, however resistance evaluation in guava orchards is rare. The objective of this study was to determine the dominant weed species in a guava orchard and to identify weed resistance to glyphosate.

MATERIALS AND METHODS

The research was conducted from November 2020 to June 2021 in ATP Farm IPB, Bogor, West Java. Identification of dominant weeds was conducted in the guava orchard by vegetation analysis in four blocks, namely Block 1, Block 2, Block 3, and Block 4 using the square (quadrat) method. Five sampling points were taken randomly in each block with a square sized 50 cm x 50 cm.

Weeds in the quadrant were cut at the ground level, then it was separated by species, and put into different paper bags. Weeds were oven-dried at 80°C for 2 x 24 hours (Bilkis et al., 2022). Weed relative density, relative frequency, and relative dominance were calculated to obtain summed dominance ratio (SDR) (Rahayu et al., 2018). Weed diversity in each block was evaluated using the coefficient community formula: C = 2W X 100% / (A + B), where C was the coefficient community; W was the sum of two quantities lowest to the kind of each community; A was the sum of SDR on the first community; and B was the sum of SDR on the first community (Yuniasih & Soejono, 2017).

The dominant weed for resistance testing was *Cyperus kyllingia* species. Seeds were taken from each block with as many as 20 sampling points representing the edges and center of the block.

Weed resistance testing was conducted using a one-factor completely randomized complete block design (RCBD) with three replications. The *C. kyllingia* of guava orchard were collected from Block 1, Block 2, Block 3, and Block 4, and susceptible populations. The seeds of susceptible *C. kyllingia* were collected from the roadside of IPB Darmaga (-6.556563, 106.730043), about 1 km from the guava orchard. Collected seeds were soaked in gibberellin solution (1000 ppm) for breaking the dormancy. Seeds were sown in pots, and 20 *C. kyllingia* seedling per pot was used. Pots were maintained every day by removing non-uniform seedlings and other weeds.

C. kyllingia in the pots were sprayed with glyphosate with eight levels, i.e., control 0 (D0), 60.75 (D1), 121.5 (D2), 243 (D3), 486 (D4), 972 (D5), 1944 (D6), 3888 (D7), and 7776 g ha^{-1} (D8). Glyphosate application was carried out using a knapsack sprayer with a blue nozzle with a spraying volume of 400 L ha^{-1}.

Fresh weed that survived at 7 days after application (DAA) was harvested by cutting biomass at ground level. Dried *C. kyllingia* biomass was obtained by oven-dried for 2x 24 hours at 80 °C. Plant biomass was then converted into the percentage of mortality using the calculation: Percentage of mortality (%) = (1 – biomass of fresh weeds due to treatments) x 100% / biomass of weed in D0 treatment. Lethal dose (LD_{50}) calculation was done by probit analysis based on the percentage of mortality against the log dose of herbicide. Probit analysis was done by converting the percentage of mortality into probit values (Y) and converting dose values into log dose (X). Linear regression equation (Y = aX + b) was obtained from probit values (Y) dan log dose (X). The Y value of the linear regression equation was then replaced with 5 to find the X value. LD_{50} could be found by antilog the X value (Guntoro & Fitri, 2013).

Data were statistically analyzed by ANOVA and Duncan Multiple Range Test (DMRT) P>0.05. The resistance index (RI) was obtained from the LD_{50} value of the resistant population divided by the LD_{50} value of the susceptible population. The resistance status was grouped as sensitive (RI <2), reduced sensitivity (RI = 2.0-2.9), low resistance (RI = 3.0-4.9), moderate resistance (RI = 5.0-9.9), high resistance (RI = 10.0-68.1), and very high resistance (RI > 68.2) (Stankiewicz-Kosyl et al., 2022).

RESULTS AND DISCUSSION

Vegetation analysis showed that evaluated blocks almost had the same number of weed species (Table 1). Weed species found in each block consisted of three groups, namely sedges, broadleaves, and grasses. Vegetation analysis found 9 species of grasses, 1 species of sedge, and 18 species of broadleaves. The highest number of species was obtained in Block 3, namely 18 weed species.

Table 1. Weed analysis at the guava orchard of ATP Farm.

No.	Species of weed	Type of weed	Summed dominance ratio (%)			
			Block 1	Block 2	Block 3	Block 4
1	*Cyperus kyllingia* Endl.	Sedge	30.02	27.69	15.73	34.56
2	*Ageratum conyzoides* L.	Broadleaf	-	3.06	5.91	7.31
3	*Amaranthus dubius* Mart. Ex Thell	Broadleaf	-	-	0.81	-
4	*Arthraxon hispidus* (Thunb.) Merr.	Broadleaf	-	1.77	-	-
5	*Asystasia gangetica* (L.) T. Anderson	Broadleaf	5.60	13.81	13.50	14.82
6	*Borreria alata* (Aubl.) DC.	Broadleaf	-	-	6.57	-
7	*Calystegia sepium* (L.) R.Br.	Broadleaf	-	-	3.60	-
8	*Cleome rutidosperma* D.C.	Broadleaf	-	-	2.24	1.63
9	*Commelina benghalensis* L.	Broadleaf	4.80	-	13.26	5.34
10	*Eclipta prostata* L.	Broadleaf	-	2.23	-	-
11	*Erigeron sumatrensis* Retz.	Broadleaf	2.29	8.43	-	1.53
12	*Mimosa pudica* L.	Broadleaf	-	-	0.75	-
13	*Oxalis barrelieri* L.	Broadleaf	2.06	-	-	-
14	*Parietaria judaica* L.	Broadleaf	-	-	-	1.81
15	*Peperomia pellucida* (L.) Kunth	Broadleaf	-	-	2.35	1.29
16	*Phyllanthus niruri* Linn.	Broadleaf	-	-	1.50	5.45
17	*Richardia brasiliensis* Gomes	Broadleaf	-	-	2.28	-
18	*Synedrella nodiflora* (L.) Gaertn.	Broadleaf	10.99	7.27	5.31	5.24
19	*Trilium cernuum*	Broadleaf	-	-	-	0.78
20	*Digitaria adscendens* (Kunth)	Grass	-	-	1.04	-
21	*Digitaria ciliaris* (Retz.) Koel.	Grass	13.41	2.30	-	-
22	*Digitaria sanguinalis* (L.) Scop.	Grass	-	8.66	3.73	2.74
23	*Echinochloa colona* (L.) Link.	Grass	-	-	-	0.78
24	*Eleusine indica* (L.) Gaertn.	Grass	1.88	-	-	-
25	*Ottochloa nodosa* (Kunt) Dandy	Grass	14.16	-	3.44	3.83
26	*Paspalum conjugatum* Berg.	Grass	9.04	15.53	-	-
27	*Rottboelia exaltata* L.f.	Grass	3.92	7.91	16.26	12.90
28	*Setaria plicata* (Lam.) T.Cooke	Grass	-	-	1.71	-
29	Others	-	1.83	1.33	-	-

Cyperus kyllingia (sedges group) had the highest SDR value in Blocks 1, 2, and 4 at 30.02%, 27.69%, and 34.56% respectively, while *Rottboellia exaltata* was the most dominant weed species in Block 3 with SDR value was 16.26% (Table 1). The dominance of particular weeds is influenced by many factors, e.g., weed control methods, herbicide resistance weeds, cropping system, climate change, temperature, rainfall, and micro-climate (Mainardis et al., 2020; Nguyen and Liebman, 2022; Ramesh et al., 2017; Storkey & Neve, 2018). A field is defined as having agronomic and environmental sustainability when its weed community has a high level of diversity (Storkey & Neve, 2018).

In general, *C. kyllingia* was the most dominant weed in the guava orchard at ATP Farm with an SDR value of 27.0%, followed by *Asystasia gangetica* with SDR was 11.9% and *R. exaltata* SDR was 10.3%. *Digitaria sanguinalis* was the weed with the lowest SDR of 3.8% (Figure 1). The occupation of *C. kyllingia* that dominate the field could mean as resistance weed. Application of herbicides with the same active ingredient caused selection so that resistant weeds would dominate the field (Gómez de Barreda et al., 2021). The frequency of spraying glyphosate herbicide in the ATP IPB orchard is every four months. Continuous spraying of glyphosate in the ATP Farm is suspected to stimulate resistance incidents (Marochi et al., 2018). The dominance of *C. kyllingia* in the guava orchard could be due to resistance to glyphosate.

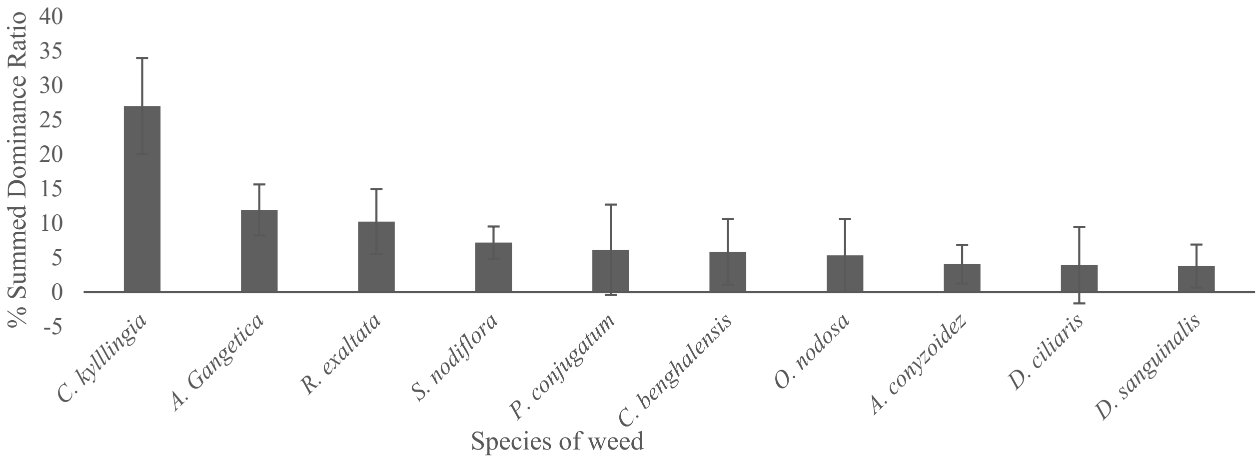

Figure 1. Average of summed dominance ratio (SDR) from all blocks of guava orchard at ATP Farm.

One of the parameters used to compare the vegetation diversity of two communities is the community coefficient. The four blocks that were analyzed, showed that the community coefficient value of each comparison was below 75%, which means that the level of homogeneity of vegetation was quite low (Table 2). The vegetation across communities is considered homogeneous with low vegetation diversity if the community coefficient value is more than 75% (Umiyati et al., 2019). The present study revealed that the highest weed similarity was between Block 3 and Block 4. Weed diversity was caused by canopy structure, crop density, types of fertilization, and chemical crop protection (Feledyn-Szewczyk et al., 2019). The community coefficient between Blocks showed low similarity, and the most dominant weed species was *C. kyllingia* species.

Table 2. Weed community comparison among blocks of guava orchard at ATP Farm.

Comparison of each blocks	Coefficient community (%)
Block 1 : Block 2	59.44
Block 1 : Block 3	38.80
Block 1 : Block 4	54.94
Block 2 : Block 3	49.24
Block 2 : Block 4	61.98
Block 3 : Block 4	69.22

Glyphosate dosage affected *C. kyllingia* biomass production (Table 3). Weed biomass decreased as the dose of glyphosate increased. *C. kyllingia* from Block 1, Block 2, and susceptible populations showed a significantly different; and *C. kyllingia* biomass decreased when up to 121.5 g ha^{-1} glyphosate was applied. A significantly different decrease in biomass occurred in the Block 3 and Block 4 populations starting at the 243 g ha^{-1} glyphosate treatment. The decrease in weed biomass occurred due to damaged leaf tissue after herbicide treatment. The difference in response to Block 3 and Block 4 indicates the potential for resistance. The highest glyphosate dose treatment showed that Blok 4 had the highest biomass than others, followed by Block 3.

Table 3. Biomass of *C. kyllingia* caused by glyphosate application.

Glyphosate dose (g ha^{-1})	Biomass of *Cyperus kyllingia* (g)[a]				
	Block 1	Block 2	Block 3	Block 4	Susceptible
0.00	2.06a	2.09a	1.92a	2.07a	1.97a
60.75	2.02a	1.95a	1.75a	1.96a	1.89a
121.50	1.41b	1.70b	1.53ab	1.90a	1.66b
243.00	1.22b	1.53b	1.23bc	1.48b	1.08c
486.00	0.66c	1.05c	0.93cd	1.27c	0.43d
972.00	0.62c	0.54d	0.75de	0.76d	0.27de
1,944.00	0.35d	0.37de	0.44ef	0.54e	0.23de
3,888.00	0.29d	0.30de	0.30f	0.26f	0.18e
7,776.00	0.11d	0.17e	0.20f	0.25f	0.14e

Note: [a] Means within columns followed by different letters are significantly different according to Duncan's multiple range test (DMRT) where P≤0.05. Data of each block were analyzed apart.

Herbicide application affected the percentage of mortality (Table 4). The higher the dose of glyphosate applied, the higher the percentage of mortality. *C. kyllingia* from Block 1 and susceptible populations started to show a significant difference in the percentage of mortality after 121.5 g ha^{-1} glyphosate treatment. Block 2, Block 3, and Block 4 populations began to show significant differences starting from the 243 g ha^{-1} treatment. The 486 g ha^{-1} treatment gave a different percentage of mortality in all weed populations. Block 4 showed the lowest mortality percentage of 38.98% while susceptible showed the highest mortality percentage of 77.96%. The difference in response at the same dose indicated the potential for resistance in the weed population from the ATP Farm.

Table 4. Percentage of *C. kyllingia* mortality caused by glyphosate application.

Glyphosate dose (g ha^{-1})	Percentage of mortality (%)[a]				
	Block 1	Block 2	Block 3	Block 4	Susceptible
60.75	2.04e	6.92e	8.99f	5.59f	4.28e
121.50	31.28d	18.54de	20.00ef	8.19f	16.17d
243.00	40.60d	26.81d	35.98de	28.66e	45.18c
486.00	67.44c	49.89c	51.34cd	38.98d	77.96b
972.00	69.83bc	74.23b	60.51bcd	63.35c	86.23ab
1,944.00	82.97ab	85.50ab	76.86abc	73.98b	88.50ab
3,888.00	85.88a	91.84a	84.41ab	87.94a	90.69a
7,776.00	94.52a	82.47ab	89.59a	87.53a	93.97a

Note: [a] Means within columns followed by different letters are significantly different according to Duncan's multiple range test (DMRT) where P≤0.05. Data of each block were analyzed apart.

Cyperus kyllingia from all blocks of the guava orchard showed higher LD_{50} values than the those of susceptible population (Table 5). Based on Table 5, Block 4 population showed the highest LD_{50} (759.11 g ha^{-1}) among other populations. The other blocks showed higher LD_{50} values than susceptible ones but they still belonged to the sensitive group since their resistance index was below 2, otherwise, Block 4 belonged to the reduced sensitivity group because the resistance index was 2.67 (Stankiewicz-Kosyl et al., 2022). The LD_{50} value in the four blocks which was higher than the sensitive was presumably due to herbicide selection so that the weeds were able to reproduce after routine application of glyphosate. Here, the sensitive population was never been exposed to glyphosate, therefore, selection by herbicides was estimated absent. On the other side, the higher generation of *C. kyllingia* in the guava orchard was presumably due to the existence of selection by herbicide application, as shown by increasing LD_{50} value. The difference in LD_{50} in each weed block could also be expected due to differences in weed generation (Norsworthy et al., 2021).

Application dose of 486 g ha^{-1} glyphosate on the sensitive population caused the highest percentage *C. kyllingia* mortality by 77.96% as compared to other blocks, namely, Block 1 by 67.85%, Block 2 by 49.89%, Block 3 by 51.34%, and Block 4 by 38.98% (Table

5). The high weed mortality in the sensitive block inline with low LD_{50} value. The LD_{50} value of each block will then be presented in Table 5 based on the calculation of probit analysis. The lethal dose is the dose value of a herbicide needed to kill weeds as much as 50% of the weed population. LD_{50} value is needed to determine the effective dose of a herbicide that can suppress target weeds by 50% of the weed population (Guntoro & Fitri, 2013). The calculation of the resistance index and resistance category followed by previous research (Barroso et al., 2018; Stankiewicz-Kosyl et al., 2022).

Table 5. The lethal dose of *C. kyllingia* to glyphosate herbicide and resistance status.

Population	Regression equation[a]	R^2 (%)	LD_{50} (g ha^{-1})[b]	RI	Status[c]
Susceptible	Y = 1.269X + 1.885	83.23	283.95	-	-
Block 1	Y = 1.114X + 2.225	96.93	309.66	1.09	Sensitive
Block 2	Y = 1.254X + 1.612	86.13	501.98	1.77	Sensitive
Block 3	Y = 1.156X + 1847	99.01	534.14	1.88	Sensitive
Block 4	Y = 1.427X + 0.890	95.65	759.11	2.67	Reduced sensitivity

Note: [a] Y, Probit value based on the percentage of weed damage; X, Log dose; [b] LD_{50}, effective glyphosate dose needed for 50%; RI, Resistance index, [c] Resistance index classification was based on the scale of (Stankiewicz-Kosyl et al., 2022).

From resistance index and its status in Table 5 indicates that among four blocks of guava orchard at ATP Farm, Block 4 showed resistance potential as the sensitivity to common glyphosate dose was decreased. Frequent herbicide spraying causes natural selection in weeds, where adaptive weeds will dominate the field (Norsworthy et al., 2021). The sensitivity of weeds to particular herbicides might decrease when the application dose is below the recommendation causing some weeds to develop adaptation mechanisms to survive (Saragih et al., 2021).

The present study shows that variation in *C. kyllingia* among blocks in the guava orchard of ATP Farm indicates that weed control carried out at the farm is fairly good. However, the presence of 'reduced sensitivity' of *C. kyllingia* implies the need to combine weed control methods such as manual clearing and chemical (herbicide) control to maintain sensitivity to glyphosate. The combination of weed control methods and herbicide rotation of different active ingredients is effective to reduce the incidence of weed resistance (Evans et al., 2016). It is important to note that sometime paraquat-based herbicide is used in blocks 1 to 3 of the guava orchard at ATP Farm. Although it is still preliminary, such a technique benefits maintaining weed, especially *C. kyllingia* was still responsive to glyphosate.

In general, weed resistance might occur through natural selection by herbicide treatment. Resistant weeds might survive under such natural selection, unlike sensitive ones. Weed alters herbicides' effectivity through two mechanisms, namely target-site resistance (TSR) and non-target-site resistance (NTSR) (Beckie, 2020). The TSR mechanism could occur due to mutations in the active site of the herbicide target, while the NTSR mechanism occurred when the plant forms defense mechanisms such as decreased herbicide absorption and translocation, increased herbicide metabolism, and others (Domínguez-Valenzuela et al., 2021; Jugulam & Shyam, 2019).

The cause of resistant weeds was the presence of alleles that were naturally able to withstand herbicide stress so herbicide selection made the presence of alleles continue to increase in each population and accumulate in each individual (Ghanizadeh & Harrington, 2017). Gene accumulation could occur in cross-pollinated plants. Sedges groups were generally pollinated with the help of wind. In addition, pollination assisted by insects could also occur, such as in the *Rhynchospora alba* species which was assisted by honeybees (Villa-Machío et al., 2022). In this case, *C. kyllingia* in the ATP garden is still in the sensitive category presumably because the frequency of herbicide application was rare (once every 2-3 months) so selection by herbicides rarely occurred. However, the cross-pollinated of many sedges made the potential for resistance in sedge species increase.

CONCLUSIONS

Cyperus kyllingia was the dominant weed at the guava orchard of ATP Farm as shown by the highest SDR value (27.0%). However, the resistance status of *C. kyllingia* in the study site was still sensitive to glyphosate predominantly at blocks 1, 2, and 3 because the resistance index value was still <2. Block 4 showed resistance potential due to the highest LD_{50} and resistance index at 2.67 which means reduced sensitivity. Frequent application of glyphosate at ATP Farm caused the LD_{50} values of the four blocks to be higher than the susceptible population that had never been exposed to glyphosate.

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