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Physiological responses of *Centella asiatica* to the herbicides of glyphosate and 2,4-D

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Abstract. *Amiati GS, Solichatun, Anggarwulan E. 2019. Physiological responses of* Centella asiatica *to the herbicides of glyphosate and 2,4-D. Cell Biol Dev 3: 69-80.* The objective of this study was to determine the physiological response of *Centella asiatica* (L.) Urb. or *gotu kola* to the glyphosate herbicides and 2,4-Diclorophenoxyacetic acid (2,4-D). This study used a Completely Randomized Design (CRD) with two treatment factors: the type of herbicide and the dose of herbicide (0 $L.ha^{-1}$; 0.25 $L.ha^{-1}$; 0.5 $L.ha^{-1}$; 0.75 $L.ha^{-1}$; 1 $L.ha^{-1}$). The quantitative data obtained were examined by analysis of variance (ANOVA) to determine whether there was an effect of the treatments and followed by the DMRT (Duncan's Multiple Range Test) at a 5% level to determine the significant difference between the treatments. The t-test determined the effectiveness of glyphosate and 2,4-D. The results showed that the glyphosate herbicide at a dose of 0.25 to 1 L/ha did not inhibit the number of leaves, dry weight, and nitrate reductase. The glyphosate dose of 0.25 to 0.75 L/ha did not inhibit the formation of gross weight, chlorophyll content, and respiration rate. The glyphosate dose of 0.25 to 0.5 L/ha did not inhibit the leaf area and carotenoid content of *C. asiatica*. Herbicide of 2,4-D at a dose of 0.25 to 1 L/ha did not inhibit chlorophyll, respiration rate, and nitrate reductase. The glyphosate dose of 0.25 to 0.5 L/ha did not inhibit the formation of the number of leaves, leaf area, gross weight, dry weight, and carotenoid content. The glyphosate herbicide gave a lower inhibitory effect than the herbicide of 2,4-D in terms of growth: number of leaves, leaf area, gross weight, and dry weight. Cultivation of *C. asiatica* is more suitable for using the glyphosate herbicide because the growth of *C. asiatica* generally remains good. The glyphosate herbicide at a dose of 0.25 $L.ha^{-1}$ gave the highest yield on the formation of *C. asiatica* biomass.

Keywords: 2,4-Diclorophenoxyacetic acid, *Centella asiatica*, glyphosate, physiological

INTRODUCTION

Gotu kola (*Centella asiatica* (L.) Urb.) is a well-known medicinal plant worldwide. *C. asiatica* belongs to the Umbelliferae family, which is known as daun kaki kuda, pegagan, or antanan in Indonesia. *C. asiatica* is a cosmopolitan plant because it has a wide distribution area, particularly in the tropics and subtropics (Dalimartha 2000). *C. asiatica* is originally from tropical Asia and grows wild at an altitude of 1 to 2,500 m above sea level, in low shade fertile soil, foggy locations, along rivers, between rocks, meadows, yards, and on roadsides (Sudarsono et al. 2002).

Centella asiatica is often regarded as a neglected weed. However, many people have used *C. asiatica* as a medicinal plant. In West Java, *C. asiatica* leaves are also served as fresh vegetables, which cleanse the blood and cure digestive disorders (Steenis 1997); some even mix it as pickles (Dalimartha 2000). *C. asiatica* is also a cover crop to prevent erosion (Musyarofah 2006).

All parts of the *C. asiatica* can be used as traditional medicine. The *C. asiatica* herb cleanses the blood, improves blood circulation, is diuretic, antipyretic, hemostatic, improves memory nerves, anti-bacterial, anti-inflammatory, hypotensive, insecticidal, and inhibits excessive scar tissue (Sudarsono et al. 2002). In Brasilia, known as paardevoet, it is used to cure colon cancer. In Australia, known as *C. asiatica*, it is useful as an anti-senile and anti-stress. In China, known as ji xue cao, it is useful for improving blood circulation, and it is even believed to be more useful than ginko biloba or ginseng in Korea (Januwati and Yusron 2005).

The many benefits of *C. asiatica* seem to be related to the high content of active compounds, including asiaticoside, thankuniside, isothankunicide, madecasoside, brahminoside, brahmic acid, madekasitic acid, hydrocotyledon, mesoinoside, centellose, carotenoids, mineral salts (such as potassium, sodium, magnesium, calcium, iron salts), bitter substance vellarine and tannin (Dalimartha 2000). This plant is in great demand by traditional herbal medicine companies that process it into herbal ingredients. One herbal medicine factory requires approximately 100 tons of *C. asiatica* per year (Januwati and Yusron 2005); if only depending on the natural harvest, it is not enough to meet the demands of this plant. These conditions encourage the development of large-scale *C. asiatica* cultivation. A study on *C. asiatica* cultivation at the Center for Agricultural Research and Development resulted in total production of around 15-25 tons/ha or equivalent to 1.5-2.5 tons/ha of the dry plant. Further research in cultivation, such as the effect of harvesting system, the effect of shade level, type, and dose of fertilizer, has been carried out, while the growth of weeds and diseases has not been reported (Januwati and Yusron 2005).

Weed control in *C. asiatica* cultivation is currently only done manually, which has many weaknesses, such as requiring more workforce, especially on large lands with high weed populations; it must be carried out more than once because manual control does not kill weeds, so production costs are increasing (Januwati and Yusron 2005). Chemical control also has negative effects, including types of herbicides that are not selective; in addition to killing weeds, it can also kill cultivated plants; the presence of herbicide residues left in the soil can cause plants to become poisoned even die (Anwar 2002).

Using herbicides properly and correctly can reduce the negative effects of chemical control. The selection of the glyphosate herbicides and 2,4-D to control weeds in *C. asiatica* cultivation was based on the advantages of these two herbicides. The advantages of the herbicide of glyphosate include: (i) it is non-selective with a broad spectrum, post-emergence systemic, which can control seasonal and annual weeds until their roots die after the plant grows (Moenandir 1988a,b), (ii) improving physical and chemical properties of soil because the use of the herbicide of isopropyl amine glyphosate can increase the rate of permeability, availability of P and CEC (Cation Exchange Capacity) of the soil, (iii) increasing the availability of organic N and C as well as soil microbes (Niswati et al. 1995), (iv) able to control narrow and broadleaf weeds that usually grows around *C. asiatica*. The advantages of herbicide of 2,4-D include: (i) it is selective and systemic in pre-emergence, (ii) it does not damage the environment because it is one of the auxin hormones belonging to phenoxy, (iii) able to control broadleaf weeds, and grass-like plants that usually grow around *C. asiatica* (Tjionger's 2002).

To optimize yields in *C. asiatica* cultivation, information on the plant's response to the herbicides of glyphosate and 2,4-D is required. Based on the information, the herbicide with the most appropriate type and dose can be selected to optimize the cultivation results in the cultivation practice. The objectives of this study were: (i) to determine the physiological response of gotu kola (*C. asiatica*) to the glyphosate herbicide. (ii) to determine the physiological response of *C. asiatica* to the herbicide of 2,4-D.

MATERIALS AND METHODS

Location and time of the study

The experimental study was carried out at the Green House of Biology Sub Lab, Central Laboratory of Mathematics and Natural Sciences of Universitas Sebelas Maret, Indonesia.

Materials

The main material was *C. asiatica* stolon obtained from the Gondang Village area, Tawangmangu Sub-district, Karanganyar District, Central Java, Indonesia. Other ingredients include the herbicide of glyphosate and the herbicide of 2,4-D.

Research design

This study used a Completely Randomized Design (CRD) which was arranged in a factorial manner with 2 treatment factors consisting of 5 levels with 5 replications as follows:

The first factor is the dose of the herbicide glyphosate consisting of 5 levels, including:

H_1K_1: 0 L.ha^{-1} of glyphosate (without herbicide)

H_1K_2: 0.25 L.ha^{-1} of glyphosate (equal to 0.875 mL/polybag)

H_1K_3: 0.5 L.ha^{-1} of glyphosate (equal to 1.75 mL/polybag)

H_1K_4: 0.75 L.ha^{-1} of glyphosate (equal to 2.625 mL/polybag)

H_1K_5: 1 L.ha^{-1} of glyphosate (equal to 3.5 mL/polybag)

The second factor is the dose of the herbicide 2,4-D consisting of 5 levels, including:

H_2K_1: 0 L.ha^{-1} of 2,4-D (without herbicide)

H_2K_2: 0.25 L.ha^{-1} of 2,4-D (equal to 0.875 mL/polybag)

H_2K_3: 0.5 L.ha^{-1} of 2,4-D (equal to 1.75 mL/polybag)

H_2K_4: 0.75 L.ha^{-1} of 2,4-D (equal to 2.625 mL/polybag)

H_2K_5: 1 L.ha^{-1} of 2,4-D (equal to 3.5 mL/polybag)

So, there are 10 treatments in total.

Procedure

Preparation of the growing media

Dried soil and compost were mixed with the ratio of soil: compost = 2:1 (i). 2 kg of the soil-compost mixture was taken and then put in a polybag Φ 30 cm (ii).

Preparation of seeds and treatments

Centella asiatica stolons, with 2 buds with a size of ± 2cm, were cut and then planted on the media provided (i). The herbicides were administered once for each treatment, which was done 7 before planting (Suwarni 2000) (ii).

Watering

Watering according to field capacity was carried out every day until new buds appeared and were 1 month old.

Harvest

Harvesting was done after *C. asiatica* had been grown for 2 months.

Observed variables

Leaf area

Observation of leaf area was carried out using a leaf area meter or gravimetric calculations. Leaf area was calculated by estimating by weight ratio (gravimetry). This can be done by, first, putting the leaf to be estimated on a piece of paper and producing a replica of the leaf. The leaf replica is cut out of paper whose weight and area are measured. The leaf area is then estimated based on the ratio of the weight of the leaf replica to the total paper:

$$LD = Wr/Wt \times LK$$

Where:

Wr: leaf replica paperweight

Wt: total papepaperweight

LK: total paper area (Sitompul dan Guritno 1995)

Number of leaves

The number of leaves is calculated at the end of the observation by calculating each sample plant's total number of leaves.

Gross weight

Observations are made by removing and cleaning the plants from the soil attached to the roots and then weighing them. Gross weight is weighed at the end of the observation using a balance.

Dry weight

Harvested crops cleaned of soil residues are put in paper bags and heated in the oven (temperature 50°C) for 4-5 days until a constant weight is reached. The constant weight achieved after being put in the oven is the dry weight of the plant.

Chlorophyll and carotenoid contents

Analyses of chlorophyll and carotenoid follow the method of Hendry and Grime (1993).

Nitrate reductase

Nitrate reductase analysis follows the Listyawati method (1994).

Respiration rate

Respiration rate analysis follows the Suwarsono method (1987).

Data analysis

Observational data are analyzed by analysis of variance (ANOVA). If there is a significant difference, it is followed by Duncan's multiple distance test (DMRT) at a 5% level. The t-test can determine the effectiveness of the herbicides of glyphosate and 2,4-D.

RESULTS AND DISCUSSION

Based on the analysis of variance of all observation variables, it was obtained that 5 of 8 observational variables of the treatment of herbicide administration of 2,4-D showed significant differences. In comparison, 7 of 8 observational variables of glyphosate showed significant differences. Treatment with the herbicide of 2,4-D had a significant effect on the number of leaves, leaf area, gross weight, dry weight, and carotenoid content. Treatment with glyphosate significantly affected the number of leaves, leaf area, gross weight, dry weight, chlorophyll content, carotenoid content, and respiration rate. The response rates of *C. asiatica* on the treatment of the herbicides of 2,4-D and glyphosate are presented in Table 1.

Number of leaves

Observation of the number of leaves was required in addition to being an indicator of growth as well as supporting data to explain the growth processes that occurred, such as the formation of plant biomass. The number of leaves increased with the age of the plant.

The analysis of variance showed that the dose of the herbicides of 2,4-D and glyphosate had a significant effect on the number of leaves of each plant. The average number of leaves per plant in the treatment of the herbicide of 2,4-D and glyphosate is presented in Tables 2 and 3.

Table 2 shows that the effect of the administration of the herbicide of 2,4-D up to a dose of 0.5 $L.ha^{-1}$ was not significantly different from that of the control plant (0 $L.ha^{-1}$). Increasing the dose of herbicide of 2,4-D caused a decrease in the number of *C. asiatica* leaves. In the treatment of the herbicide of glyphosate (Table 3), herbicide doses of 0.5 $L.ha^{-1}$ to 1 $L.ha^{-1}$ were not significantly different compared to the control plant. The number of leaves in glyphosate treatment at a dose of 0.25 $L.ha^{-1}$ provided the highest yield and was significantly different compared to the control plant.

Based on Figures 1 and 2, it can be seen that an increase in the dose of the herbicide of 2,4-D caused the number of *C. asiatica* leaves to be reduced. According to Moenandir (1990), the herbicide of 2,4-D can cause weakening of the root cortex. The weakening of the root cortex results in the inhibition of the absorption of nutrients, especially nitrogen, which is used as the main ingredient for leaf formation.

Table 1. Responses of analysis of variance of *C. asiatica* to the herbicides of 2,4-D and glyphosate

Herbicide	NL	LA	GW	DW	CL	CR	RR	NR
2,4-D	*	*	**	*	ns	*	ns	Ns
Glyphosate	*	**	*	*	*	**	**	Ns

Notes: NL: number of leaves, LA: leaf area, GW: gross weight, DW: dry weight, CL: chlorophyll, CR: carotenoids, RR: respiration rate, NR: nitrate reductase enzyme, ns: non-significant, *: significant, **: high significant

Table 2. The average number of *C. asiatica* leaves on the administration of the herbicide of 2,4-D

Type of herbicide	Dose of herbicide				
	0 L/ha	0.25 L/ha	0.5 L/ha	0.75 L/ha	1 L/ha
2,4-D	39.33^{a}	30.33^{ab}	25.33^{abc}	21.67^{bc}	11.00^{c}

Table 3. The average number of *C. asiatica* leaves on the administration of the herbicide of glyphosate

Type of herbicide	Dose of herbicide				
	0 L/ha	0.25 L/ha	0.5 L/ha	0.75 L/ha	1 L/ha
Glyphosate	31.00^{b}	42.67^{a}	31.00^{b}	23.67^{b}	23.00^{b}

Note: Numbers followed by the same letter in the same row or column indicate no significant difference in the DMRT (Duncan's Multiple Range Test) at a 5% level

Abidin (1994) stated that 2,4-D acid is one of the growth regulators classified as auxin. The role of auxin is to stimulate the division and enlargement of cells in plant shoots and cause the growth of new shoots. The addition of a higher amount of auxin, or the addition of a more stable auxin, such as 2,4-D acid, tends to cause callus growth of explants and inhibit plant shoot regeneration (Wetherell 1982). Suryowinoto (1996) states that 2,4-D acid can create mutations at a certain dose. According to Wattimena (1988), 2,4-D acid has high phytotoxicity properties, so that it can be an herbicide.

The glyphosate herbicide (Figures 1 and 2) generally resulted in a higher number of leaves formed than the herbicide of 2,4-D. This is because the addition of glyphosate is considered to be able to increase the availability of nitrogen in the soil. According to Niswati et al. (1995), tillage cultivation with the application of the glyphosate herbicide led to the availability of organic N and C and increased soil microbes. This means that glyphosate can contribute total N to the soil because glyphosate contains NH_2^+ groups. As stated by Rinsema (1983), leaf formation is determined by the availability of nitrogen, so it is possible to increase the number of *C. asiatica* leaves depending on the supply of nutrients in the soil.

The results of the study on the effect of the herbicides of glyphosate and legin on the nodulation behavior of *Arachis hypogaea* L. showed that glyphosate up to a dose of 4.5 $L.ha^{-1}$ did not suppress plant growth and root nodule formation and showed the highest yield (Suwarni et al. 2000). A dose of 0.5 L.ha-1 to 1 L.ha-1 showed a decrease in the number of leaves. This is because administering glyphosate at high doses is not beneficial for root growth in absorbing nutrients in the soil. According to Suwarni et al. (2000), glyphosate inhibits root elongation because the entry of herbicides through the roots inhibits root growth and elongation and prevents lateral root growth. Active herbicides in the root system cause stunting and suppress lateral root growth (Moenandir 1993).

Based on the results of the *t*-test (Appendix IV), it can be seen that there was no significant difference in the area of *C. asiatica* leaves when administered the herbicides of 2,4-D and glyphosate. This means that *C. asiatica* has the same response to variations in the types and doses of herbicides.

Leaf area

Leaves function as light receivers and photosynthetic tools. The rate of photosynthesis per unit plant can be determined by leaf area (Sitompul and Guritno 1995). The analysis of variance showed that the herbicides of 2,4-D and glyphosate had a significant effect on the leaf area. The average leaf area per plant in the treatment variations of the herbicides of 2,4-D and glyphosate is presented in Tables 4 and 5.

Table 4 shows that the herbicide treatment up to a dose of 0.5 $L.ha^{-1}$ was not significantly different from those without the herbicide of 2,4-D. Leaf area at 0.75 $L.ha^{-1}$ and 1 $L.ha^{-1}$ herbicide treatments showed a significant decrease in leaf area compared to the control plant. On administering the glyphosate herbicide (Table 5), the herbicide dose of 0.25 $L.ha^{-1}$ was followed by an increase in leaf area per plant. The leaf area in herbicide treatment at a dose of 0.25 $L.ha^{-1}$ showed the highest significant yield compared to the control plant. At doses of 0.5 $L.ha^{-1}$ to 1 $L.ha^{-1}$ of glyphosate, the results were not significantly different compared to the plant without herbicide administration.

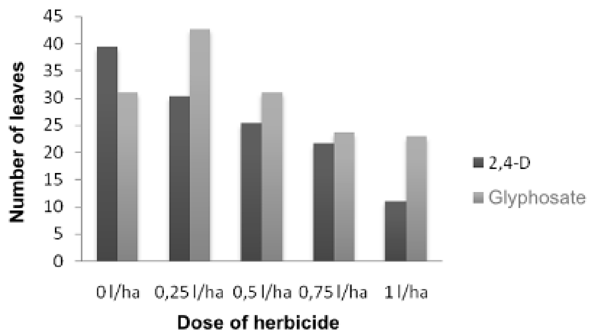

Figure 1. The effect of the herbicides of 2,4-D and glyphosate on the number of *C. asiatica* leaves

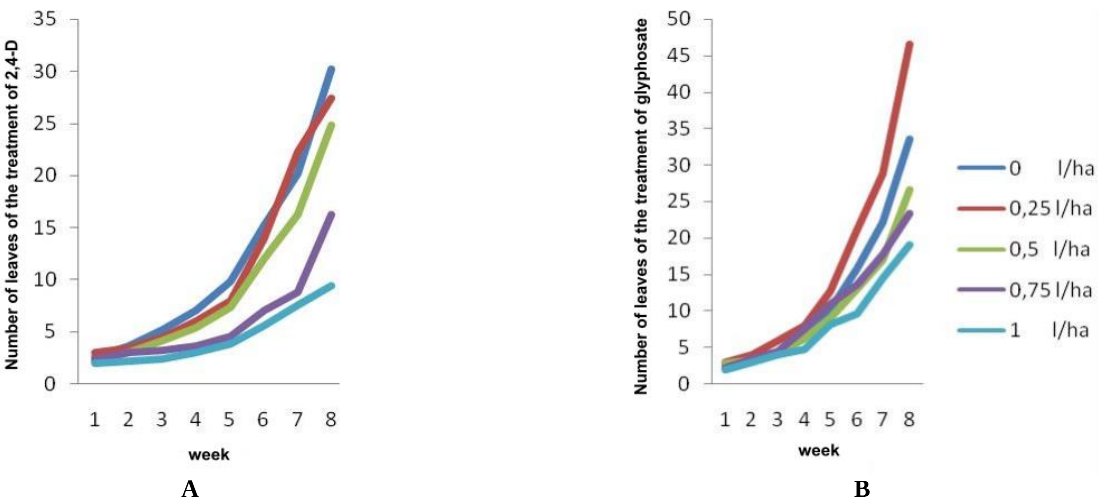

Figure 2. The effect of herbicides of 2,4-D (A) and glyphosate (B) on the number of *C. asiatica* leaves every week

Table 4. The average leaf area (cm^2) of *C. asiatica* on the administration of the herbicide of 2,4-D

Type of herbicide	Dose of herbicide				
	0 L/ha	**0.25 L/ha**	**0.5 L/ha**	**0.75 L/ha**	**1 L/ha**
2,4-D	381.149^{a}	300.207^{a}	213.972^{ab}	96.884^{b}	78.359^{b}

Table 5. The average leaf area (cm^2) of *C. asiatica* on the administration of the herbicide of glyphosate

Type of herbicide	Dose of herbicide				
	0 L/ha	**0.25 L/ha**	**0.5 L/ha**	**0.75 L/ha**	**1 L/ha**
Glyphosate	338.800^{a}	381.890^{a}	286.200^{ab}	164.270^{bc}	79.328^{c}

Note: Numbers followed by the same letter in the same row or column indicate no significant difference in the DMRT (Duncan's Multiple Range Test) at a 5% level

Auxin activity that is high enough causes abnormal growth in *C. asiatica*. It treated with 2,4-D was stunted with paler leaves. This is due to the location of the main activity of the herbicide of 2,4-D, which can change the growth pattern rapidly, so that the parenchyma cells of the roots swell, resulting in callus tissue and expansion of root primordia. Root elongation stops, and root tips swell.

Figure 3 shows that with an increase in the dose of the herbicide of 2,4-D, the area of the *C. asiatica* leaf formed decreased. The decrease in leaf area is caused by the lack of nutrients, especially nitrogen that plants can absorb, affecting plant growth, particularly leaf formation. Barriers in the process of nutrient absorption are caused by auxin activity, inhibiting root elongation, and swelling of root tips. According to Moenandir (1988a,b), the herbicide of 2,4-D treated on *Cyperus* sp (monocot) greatly reduced tissue differentiation and resulted in vacuolation with little cytoplasm. The size of the vascular system also decreases. In *Phaseolus* sp (dicot), leaf tissue is differentiated, and the replacement tissue has highly vacuolated cells without chloroplasts. 2,4-D also causes changes in morphology and internal structure of chloroplasts, damage to epidermal membrane cells, palisade, mesophyll, and changes in the metabolic system that greatly affect abscission events.

The herbicide of glyphosate (Figure 2) generally resulted in a wider leaf area than the herbicide of 2,4-D. This is because adding glyphosate can contribute total N into the soil derived from the NH_2^+ group of glyphosate. Added by Niswati et al. (1995), tillage cultivation with the application of the glyphosate herbicide leads to the availability of organic N and C and increased soil microbes. As Rinsema (1983) stated, leaf formation is determined by nitrogen availability. The increase in *C. asiatica* leaf area depends on the supply of nutrients in the soil. A dose of 0.5 $L.ha^{-1}$ to 1 $L.ha^{-1}$ showed decreased leaf area. This is because the absorption capacity of the soil has reached its maximum, so excess glyphosate accumulates in the area around the roots.

According to Moenandir (1993), glyphosate at high doses inhibits root elongation of sprouts because the entry of the herbicide of glyphosate into plants through roots inhibits root elongation and prevents lateral root growth. According to Thompson (1979), very little glyphosate herbicide is free in groundwater and immediately degraded by soil microorganisms. Adding a dose of glyphosate will inhibit the bacteria *Rhizobium* and *Pseudomonas* sp. in degrading glyphosate. This is because these microorganisms have EPSPS enzymes to produce aromatic amino acids in their bodies. Meanwhile, glyphosate inhibits the action of the EPSPS enzyme (5-enolpyrufil-shikamat-3-phosphate synthase) in plant tissues. Therefore, glyphosate also kills most soil microorganisms with the EPSPS enzyme, which forms aromatic amino acids, such as tryptophan, tyrosine, and phenylalanine (Wardoyo 2008).

Based on the results of the *t*-test (Appendix IV), it can be seen that there was no significant difference in the area of *C. asiatica* leaves when administrated the herbicides of 2,4-D and glyphosate. This means that *C. asiatica* has the same response to variations in the types and doses of herbicides.

Gross weight

The number and area of leaves will affect the fresh weight of the plant. Fresh weight is also affected by water uptake by plants (Sitompul and Guritno 1995). According to Gardner et al. (1991), fresh weight is reflected by plants' water absorption in the soil. One of how plants absorb water depends on the soil's water.

Mass gain is often determined by harvesting the whole plant or desired part and weighing it quickly before too much water evaporates from the material. This fresh mass varies somewhat depending on the water status of the plant (Salisbury and Ross 1995). Fresh weight describes the water content and humidity of the plant at that time (Foth 1994). The average gross weight per plant in the treatment variations of the herbicides of 2,4-D and glyphosate is presented in Tables 6 and 7.

Analysis of variance (Table 6) showed that the application of the herbicides of 2,4-D and glyphosate significantly affected gross plant weight. In the treatment of the herbicide of 2,4-D at doses of 0.25 $L.ha^{-1}$ and 0.5 $L.ha^{-1}$, the dry weight was not significantly different from the control plant (0 $L.ha^{-1}$). Increasing herbicide doses of 0.75 $L.ha^{-1}$ and 1 $L.ha^{-1}$ showed a significant decrease in gross weight compared to the control plant. On administering the glyphosate herbicide (Table 7), the herbicide dose of 0.25 $L.ha^{-1}$ showed the highest yield of gross plant weight. The gross weight of the glyphosate herbicide treatment at doses of 0.75 $L.ha^{-1}$ and 1 $L.ha^{-1}$ showed a significant decrease in gross weight compared to no glyphosate herbicide.

Figure 4 shows that increasing the dose of 2,4-D caused the gross weight of *C. asiatica* produced to be lower. The herbicide of 2,4-D has an activity like auxin, which plants need for growth and development, in very small amounts. Administration of the herbicide of 2,4-D in high doses results in abnormal cell division and enlargement. According to Moenandir (1990), adding the herbicide of 2,4-D in seedlings will change the growth pattern quickly, where meristematic cells will stop dividing, and cell elongation stops long growth but continues radial expansion. In mature plants, the parenchyma cells swell and divide, resulting in callus tissue and expansion of root primordia. Root elongation stops, and root tips swell.

Table 6. The average gross weight (g) of *C. asiatica* on the administration of the herbicide of 2,4-D

Type of herbicide	Dose of herbicide				
	0 L/ha	**0.25 L/ha**	**0.5 L/ha**	**0.75 L/ha**	**1 L/ha**
2,4-D	21.078[a]	19.167[a]	17.011[a]	5.047[b]	3.143[b]

Table 7. The average gross weight (g) of *C. asiatica* on the administration of the herbicide of glyphosate

Type of herbicide	Dose of herbicide				
	0 L/ha	**0.25 L/ha**	**0.5 L/ha**	**0.75 L/ha**	**1 L/ha**
Glyphosate	16.414[ab]	22.062[a]	15.574[ab]	10.861[bc]	5.158[c]

Note: Numbers followed by the same letter in the same row or column indicate no significant difference in the DMRT (Duncan's Multiple Range Test) at a 5% level

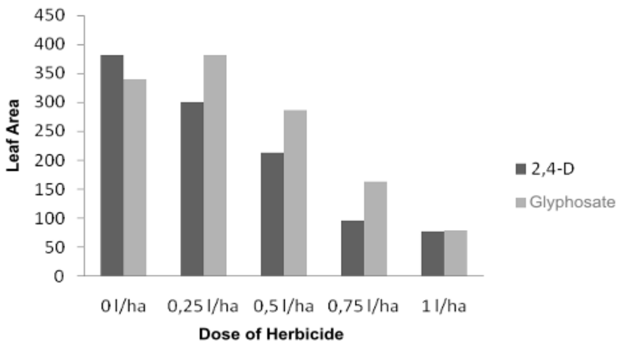

Figure 3. The effect of herbicides of 2,4-D and glyphosate on the surface area of *C. asiatica* leaves

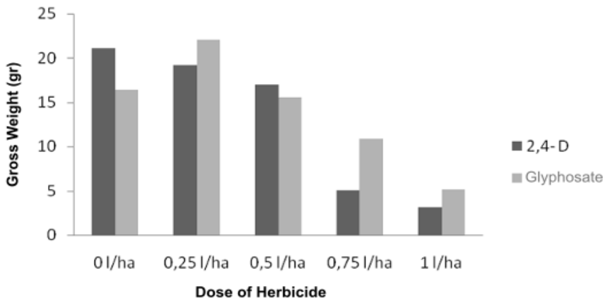

Figure 4. The effect of the herbicides of 2,4-D and glyphosate on the gross weight of *C. asiatica*

Swelling of the root tips will inhibit the absorption of water and nutrients from the soil resulting in disturbed plant metabolism. Thus, the fresh weight of the plant will also be reduced. The value of gross weight is influenced by tissue moisture content, nutrients, and metabolism (Salisbury and Ross 1995).

The glyphosate herbicide (Figure 3) generally resulted in a higher gross weight yield than the herbicide of 2,4-D. It is suspected that the dose of glyphosate increases nitrogen in the soil, so the absorption of nitrogen nutrients increases. The increased absorption of nitrogen nutrients will also increase the nitrogen content in the leaves. The nitrogen content of the leaf tissue will stimulate an increase in metabolic rate (Salisbury and Ross 1995). Added by Haryadi (1991), the enlargement of plant cells will form large cell vacuoles so that they can absorb large amounts of water. Besides, the formation of plant protoplasm will increase so that it can cause an increase in fresh weight and fresh yield of plants.

The increase in fresh weight is related to the number of leaves and leaf area. This is because increasing the number of leaves and leaf area on the plant will increase the fresh weight of the plant. According to Dwijoseputro (1993), fresh plant weight is influenced by nutrients in plant tissue cells. With the formation of roots and leaves, the physiological activities of plants in absorbing nutrients, water, and sunlight for the photosynthesis process can take place well in subsequent growth. The rapid growth of roots and leaves causes the absorption of nutrients, water, and light for more optimal photosynthesis; the resulting assimilation is used for faster plant development and the formation of more shoots so that the plant's fresh weight increases. At a dose of 0.5 $L.ha^{-1}$ to 1 $L.ha^{-1}$, there was a decrease in fresh weight due to the addition of glyphosate at high doses, which could cause damage to the root system could inhibit the absorption of nutrients from the soil.

Based on the results of the t-test (Appendix IV), it can be seen that there was no significant difference in the gross weight of *C. asiatica* in the administration of the herbicides of 2,4-D and glyphosate. This means that *C. asiatica* had the same response to variations in the types and doses of herbicides.

Dry weight

To measure plant productivity, it is more relevant to use plant dry weight to measure growth (Salisbury and Ross, 1995). According to Lakitan (1993), the dry weight of plants reflects the accumulation of organic compounds that plants have successfully synthesized from inorganic compounds, especially water and carbon dioxide.

Dry weight is a balance between CO_2 uptake (photosynthesis) and CO_2 removal (respiration). If respiration is higher than photosynthesis, these plants lose dry weight and vice versa (Gardner et al. 1991). Added by Dwijoseputro's (1993) statement that 90% of plant dry matter results from photosynthesis and growth analysis expressed in dry weight. Table 8 shows the results of the average dry weight of *C. asiatica* plants.

Analysis of variance showed that the treatment with the herbicides of 2,4-D and glyphosate had a significant effect on the dry weight variable. Table 8 shows that the dry weight of plants in the treatment without the herbicide 2,4-D up to a dose of 0.5 $L.ha^{-1}$ was not significantly different. The treatment with doses of 0.75 $L.ha^{-1}$ and 1 $L.ha^{-1}$ showed a significant reduction in dry weight compared to plants without the herbicide of 2,4-D. The dry weight of the herbicide treatment with glyphosate (Table 9) at a dose of 0.25 $L.ha^{-1}$ showed the highest significant increase in dry weight compared to the control plant. Increasing the dose of herbicide from 0.5 $L.ha^{-1}$ to 1 $L.ha^{-1}$ showed a decrease in dry weight compared to plants without herbicides.

Table 8. The average dry weight (g) of *C. asiatica* on the administration of the herbicide of 2,4-D

Type of herbicide	Dose of herbicide				
	0 L/ha	**0.25 L/ha**	**0.5 L/ha**	**0.75 L/ha**	**1 L/ha**
2,4-D	2.113[a]	1.691[a]	1.232[ab]	0.312[b]	0.215[b]

Table 9. The average dry weight (g) of *C. asiatica* on the administration of the herbicide of glyphosate

Type of herbicide	Dose of herbicide				
	0 L/ha	**0.25 L/ha**	**0.5 L/ha**	**0.75 L/ha**	**1 L/ha**
Glyphosate	1.387[ab]	2.196[a]	1.190[ab]	0.994[b]	0.409[b]

Note: Numbers followed by the same letter in the same row or column indicate no significant difference in the DMRT (Duncan's Multiple Range Test) at a 5% level

Figure 5 shows that the increase in the dose of the herbicide of 2,4-D caused the dry weight of the *C. asiatica* plant to decrease. According to Gardner et al. (1991), if respiration is higher than photosynthesis, the dry weight of the plant decreases, and vice versa. Figure 8 shows that the respiration rate of plants treated with high 2,4-D was the highest dose of the herbicide of 2,4-D. This is presumably due to a decrease in the rate of photosynthesis so that the dry weight produced is small at the increase in the highest herbicide dose. The increase in respiration rate aims to maintain the assimilate gradient (Moenandir 1990). The herbicide of 2,4-D causes damage to the phloem in the leaves. This results in the accumulation of assimilation in the leaves, so it is necessary to balance the assimilate gradient so that it is not excessive. Plants increase the rate of respiration with the aim that assimilation can be decomposed. Decomposition of assimilating in the form of starch aims to produce ATP or energy used for self-defense from excessive synthetic auxin activity. This causes the dry weight of *C. asiatica* to be produced a little at increasing the dose of the herbicide of 2,4-D.

The administration of the glyphosate herbicide (Figure 5) generally caused the dry weight of *C. asiatica* to remain higher than that of the herbicide of 2,4-D. This is presumably because the dose of glyphosate applied through the soil can increase nitrogen in the soil so that the absorption of nitrogen nutrients increases as well. The element N is always associated with an increase in the rate of photosynthesis. Chlorophyll and the enzyme ribulose bisphosphate carboxylase oxygenase (Rubisco) are the molecules that play a role in photosynthesis. Nitrogen is one element that plays a major role in synthesizing these two molecules. The increase in N levels in the soil due to glyphosate applied through the soil has a good effect on the photosynthesis process and will produce photosynthate, which is quite influential on the dry weight yield of plants. According to Al-Kaisi and Yin (2003), the overall response of N uptake by plants at various stages of growth has more effect on increasing plant dry weight than increasing N concentration in plant tissues.

The increase in dry weight at a dose of 0.25 $L.ha^{-1}$ occurred due to the rate of photosynthesis in the form of photosynthate, which is the end product of the metabolic process. The end product of the photosynthesis process is sugar. Sugar is the basic material for preparing organic matter in plant cells, such as structural, metabolic, and important food reserves. Plant cell parts, such as cytoplasm, cell nucleus, and cell wall, are composed of organic matter. This process results in the accumulation of plant dry matter (Salisbury and Ross 1995). At doses of 0.5 $L.ha^{-1}$ to 1 $L.ha^{-1}$ of glyphosate, there was a decrease in dry weight. According to Suwarni et al. (1990), an increase in the dose of the herbicide of glyphosate causes peanut (*A. hypogaea*) plants to stunt their growth and root length. Therefore, the absorption of element N is not optimal and affects the process of photosynthesis and the resulting photosynthate. As a result, the dry weight of the plant decreases.

Based on the results of the t-test (Appendix IV), it can be seen that there was no significant difference in the dry weight of *C. asiatica* in the administration of the herbicides of 2,4-D and glyphosate. This means that *C. asiatica* had the same response to variations in the types and doses of herbicides.

Chlorophyll

Chlorophyll is a very important pigment in photosynthesis. It is a magnesium porphyrin attached to proteins. The relative amount of chlorophyll varies typically from one plant species to another (Lehningher 1990). Salisbury and Ross (1995) classified 2 pigments found in the thylakoid membrane, including green chlorophyll, which consists of chlorophyll a and chlorophyll b, and carotenoids which are yellow to orange pigments.

The analysis of variance in Tables 10 and 11 shows that the administration of the herbicide of 2,4-D did not have a significant effect. In contrast, the administration of the glyphosate herbicide significantly affected chlorophyll content. Based on Table 10, it can be seen that the increasing administration of the herbicide of 2,4-D showed an increase in plant chlorophyll content, and a dose of 1 $L.ha^{-1}$ showed the highest average chlorophyll content but was not significantly different from the control plant. In administering the glyphosate herbicide (Table 11), herbicide doses of 0.25 $L.ha^{-1}$ to 0.75 $L.ha^{-1}$ showed no significant results from the control plant. Chlorophyll content at a dose of 1 $L.ha^{-1}$ showed a significant decrease compared to without glyphosate herbicide.

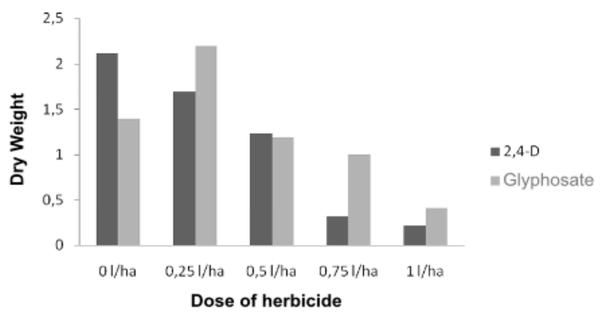

Figure 5. The effect of the herbicides of 2,4-D and glyphosate on the dry weight of *C. asiatica*

Table 10. The average chlorophyll content (mg/L) of *C. asiatica* on the administration of the herbicide of 2,4-D

Type of herbicide	Dose of herbicide				
	0 L/ha	0.25 L/ha	0.5 L/ha	0.75 L/ha	1 L/ha
2,4-D	67.712	73.340	74.544	75.815	76.915

Table 11. The average chlorophyll content (mg/l) of *C. asiatica* on the administration of the herbicide of glyphosate

Type of herbicide	Dose of herbicide				
	0 L/ha	0.25 L/ha	0.5 L/ha	0.75 L/ha	1 L/ha
Glyphosate	75.700[a]	75.452[a]	75.094[a]	73.004[a]	69.906[b]

Note: Numbers followed by the same letter in the same row or column indicate no significant difference in the DMRT (Duncan's Multiple Range Test) at a 5% level

Based on the graph of the average chlorophyll content of *C. asiatica* (Figure 6), it is shown that the increase in the dose of the herbicide of 2,4-D caused the total chlorophyll content of the *C. asiatica* plant to be significantly high. This may be because the administration of the herbicide of 2,4-D does not significantly affect the metabolic process in the formation of chlorophyll. The increase in the amount of chlorophyll is considered to be influenced by the number of carotenoids. By the opinion of Salisbury and Ross (1995), in addition to functioning as light-taking pigments that are useful for photosynthesis, carotenoids also function to protect chlorophyll from damage caused by oxidation by oxygen. Another factor affecting the high levels of chlorophyll is the amount of nitrate reductase (Figure 9).

The higher the nitrate reductase, the higher the chlorophyll count. Nitrate reductase converts nitrate into ammonia which can then be converted into ammonium in the presence of protons. Ammonium combines with glutamate to be converted with glutamine synthase to be glutamine. Glutamine then binds to α-ketoglutarate with the help of glutamate synthase to convert to glutamate. Glutamate will produce proline, arginine, and δ-aminolevulinate. δ-aminolevulinate is an intermediate in forming chlorophyll (Loveless 1991; Salisbury and Ross 1995).

The administration of the glyphosate herbicide (Figure 6) generally resulted in lower chlorophyll content of *C. asiatica* than the herbicide of 2,4-D. This shows a similar pattern to the growth parameters. Chlorophyll plays an important role as a medium for capturing energy from sunlight which in photosynthesis will produce ATP and NADPH. According to Sampson et al. (2003) and Fracheboud (2006), chlorophyll content can be used as a sensitive indicator of the physiological condition of a plant because it is positively correlated with leaf nitrogen content. It can be used as an indicator of the rate of photosynthesis. The highest chlorophyll content was at a dose of 0.25 $L.ha^{-1}$ because, at that dose, the N in the soil was available optimally due to the administration of glyphosate. Therefore, the amount of N that plants can absorb also increases. The high N absorbed by plants also increases chlorophyll content because chlorophyll molecules are composed of C, H, O, and N elements and one Mg atom (Gardner et al. 1991).

Based on the results of the *t*-test (Appendix IV), it can be seen that there was no significant difference in the chlorophyll content of *C. asiatica* in the administration of the herbicides of 2,4-D and glyphosate. This means that *C. asiatica* had the same response to variations in the types and doses of herbicides.

Carotenoids

Carotenoids are photosynthetic pigments divided into two groups: xanthophylls, such as lutein and zeaxanthin, and carotene, which consists of β-carotene and α-carotene (Zaripheh and Erdman 2002). Carotenoids have several functions for plants, including absorbing light and stabilizing the structure by disposing of excess energy. This component also protects plants from free radicals when the light intensity exceeds the capacity for photosynthesis (Frank and Codgell 1996; Havaux and Niyogi 1999). Carotenoid biosynthesis is influenced by pH, enzyme activity, light, oxidation, and water. The optimum temperature for carotenoid biosynthesis is around 30°C, while the required optimum pH is 7.4 (Salisbury and Ross 1995). The average chlorophyll content of *C. asiatica* in the treatment of the herbicides of 2,4-D and glyphosate is presented in Tables 12 and 13.

The analysis of variance (Tables 12 and 13) showed that the treatment with the herbicides of 2,4-D and glyphosate had a significant effect on carotenoid content. The treatment of the herbicide of 2,4-D (Table 12) at doses of 0.5 $L.ha^{-1}$ to 1 $L.ha^{-1}$ showed a significant increase in carotenoid content compared to the control plant. Chlorophyll contents at a dose of 0.25 $L.ha^{-1}$ showed that there were not significantly different from the control plant. In the administration of the glyphosate herbicide (Table 13), herbicide doses of 0.5 $L.ha^{-1}$ to 1 $L.ha^{-1}$ showed a significant decrease in chlorophyll content compared with no administration of the herbicide of glyphosate.

Figure 6. The effect of the herbicides of 2,4-D and glyphosate on the chlorophyll content of *C. asiatica*

Table 12. The average carotenoid content (µmol) of *C. asiatica* on the administration of the herbicide of 2,4-D

Type of herbicide	Dose of herbicide				
	0 L/ha	**0.25 L/ha**	**0.5 L/ha**	**0.75 L/ha**	**1 L/ha**
2,4-D	0.517^{b}	0.603^{ab}	0.657^{a}	0.664^{a}	0.662^{a}

Table 13. The average carotenoid content (µmol) of *C. asiatica* on the administration of the herbicide of glyphosate

Type of herbicide	Dose of herbicide				
	0 L/ha	**0.25 L/ha**	**0.5 L/ha**	**0.75 L/ha**	**1 L/ha**
Glyphosate	0.667^{a}	0.657^{a}	0.614^{b}	0.592^{b}	0.588^{b}

Note: Numbers followed by the same letter in the same row or column indicate no significant difference in the DMRT (Duncan's Multiple Range Test) at a 5% level

Figure 7 shows that increasing the dose of 2,4-D caused the carotenoid content of the *C. asiatica* to be high. This may be due to the number of carotenoids in balance with the amount of chlorophyll. When the amount of chlorophyll is high (Figure 6), the number of carotenoids is also high and vice versa. High carotenoid content may be a form of self-defense. Salisbury and Ross (1995) opinion that carotenoids are isoprenoid polyene compounds that are lipophilic or insoluble in water, easily isomerized and oxidized, absorb light, reduce singlet oxygen, block free radical reactions, and can bind to hydrophobic surfaces. The increase in plant stress levels due to herbicide doses will also increase the number of plant carotenoids.

The glyphosate herbicide (Figure 7) generally resulted in lower carotenoid content formed compared to the herbicide of 2,4-D. This is due to damage to the roots causing a decrease in root activity in absorbing nutrients, so photosynthesis is disrupted. Both of these are caused by damage to the cell structure. The damage to the cell structure is preceded by damage to the cell membrane, followed by damage to cell organelles, such as chloroplasts, mitochondria, and the nucleus. The damage to each membrane also precedes these organelles, making its structure unclear (Einhelling 1995). Carotenoids are present in the plastid membrane and have a double membrane. One of the most important types of plastids is the chloroplast. If the chloroplast is damaged, the biosynthesis of carotenoids may be inhibited.

The biosynthesis of carotenoids begins with the formation of prenyl pyrophosphate in plant plastids, which are the precursors of carotenoid biosynthesis. Prenyl pyrophosphate is formed by prenyl transferase, which forms dimethylallyl pyrophosphate (IPP). Then, it was synthesized by geranyl-geranyl pyrophosphate (GGPP). The condensation of 2 GGPP molecules forms a pyrophosphate prephytoen as an intermediate (phytoene synthesis). Phytoene is formed by removing the pyrophosphate group. Furthermore, the conversion of phytoene into lycopene forms a variety of carotenoids (Hirschberg et al. 1997).

Based on the results of the *t*-test (Appendix IV), it can be seen that there was no significant difference in the carotenoid content of *C. asiatica* in the administration of the herbicides of 2,4-D and glyphosate. This means that *C. asiatica* had the same response to variations in the types and doses of herbicides.

Respiration rate

Plants carry out the process of photosynthesis and respiration. Respiration is a process of dismantling (catabolism or dissimilation) of stored chemical energy (carbohydrate organic substances resulting from photosynthesis) to carry out life processes such as the formation of organic substances, activities in absorption (osmosis), accumulation of salts, protoplasm drainage, cell division and other activities (Dwijoseputro 1993).

Analysis of variance showed that the application of the herbicide of 2,4-D did not significantly affect the respiration rate of each plant. Meanwhile, the administration of glyphosate affected the respiration rate significantly. The average respiration rates of the herbicides of 2,4-D and glyphosate are presented in Tables 14 and 15.

Table 14 shows that the herbicide treatment of 2,4-D showed the highest average respiration rate at a dose of 1 $L.ha^{-1}$. These results were not different from that of *C. asiatica* without herbicide. The administration of the herbicide doses was 0.25 $L.ha^{-1}$, 0.5 $L.ha^{-1,}$ and 0.75 $L.ha^{-1}$. The administration of the glyphosate herbicide (Table 15) at a dose of 1 $L.ha^{-1}$ showed the highest results and was significantly different from the control plant, doses of 0.25 $L.ha^{-1}$, 0.5 $L.ha^{-1,}$ and 75 $L.ha^{-1}$.

Table 14. The average respiration rate (ppm/l/min) on the administration of the herbicide of 2,4-D

Type of herbicide	Dose of herbicide				
	0 L/ha	**0.25 L/ha**	**0.5 L/ha**	**0.75 L/ha**	**1 L/ha**
2,4-D	46.00	21.00	27.00	31.00	50.00

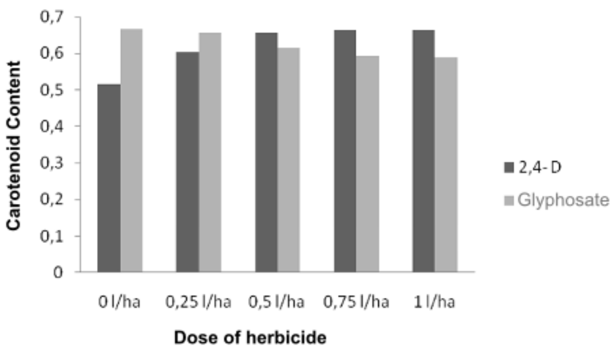

Figure 7. The effect of herbicides of 2,4-D and glyphosate on carotenoid content of *C. asiatica*

Table 15. The average respiration rate (ppm/l/min) on the administration of the herbicide of glyphosate

Type of herbicide	Dose of herbicide				
	0 L/ha	0.25 L/ha	0.5 L/ha	0.75 L/ha	1 L/ha
Glyphosate	43.00[b]	21.00[c]	21.00[c]	26.00b[c]	75.00[a]

Note: Numbers followed by the same letter in the same row or column indicate no significant difference in the DMRT (Duncan's Multiple Range Test) at a 5% level

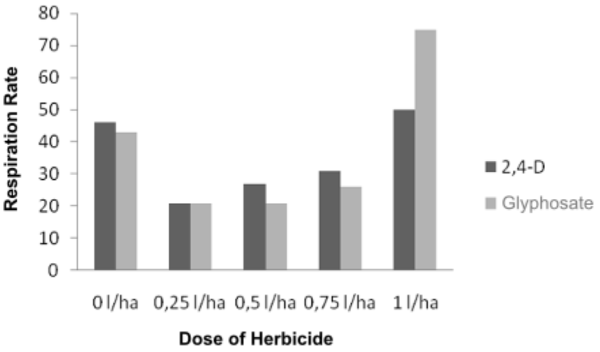

Figure 8. The effect of the herbicides of 2,4-D and glyphosate on the respiration rate of *C. asiatica*

The graph of the average rate of respiration of *C. asiatica* (Figure 8) shows that the use of the herbicide of 2,4-D shows that the respiration rate of *C. asiatica* is higher. This is because 2,4-D affects the mitochondrial membrane as a site for oxidative phosphorylation. Electrons penetrate the membrane so that there is no energy accumulation in the form of ATP (Moenandir 1990). The increase in respiration rate is considered to be due to the prevention of ATP synthesis by 2,4-D (non-combining group) so that it can stimulate respiration in phosphate-deficient media, supporting ATP hydrolysis. This follows the opinion of Nurjanah (2003) that herbicides with the active ingredient 2,4-D can inhibit weed growth by accelerating respiration. Increased respiration can result in starch being constantly overhauled to produce energy in self-defense. When starch is unavailable, energy is not produced for self-defense, and eventually, the plant dies. The increase in respiration rate aims to maintain the assimilate gradient in the form of glucose or starch (Moenandir 1990). The herbicide of 2,4-D causes damage to the phloem in the leaves. This results in the accumulation of assimilation in the leaves, so it is necessary to balance the assimilate gradient so that it is not excessive. Plants increase the rate of respiration with the aim that assimilation can be decomposed.

Administration of the dose of the glyphosate herbicide (Figure 8) in general caused the rate of respiration to be higher than that of the herbicide of 2,4-D. This is because plants carry out a defense system against glyphosate's active substance by forming ATP through glycolysis. The more doses of herbicide administrated, the more the defense system will work harder, and the respiration rate will be faster. Increased respiration is caused by low energy production; 2 ATP under anaerobic conditions from each glucose molecule compared to 36 ATP produced under aerobic conditions. Because cells still need NAD^+, the glycolysis process continues under anaerobic conditions due to the inhibition of O_2 uptake by glyphosate at high doses (Moenandir 1988a,b). The respiration rate must be significantly increased to meet the minimum needs (Delita et al. 2008).

Based on the results of the *t*-test (Appendix IV), it can be seen that there was no significant difference in the respiration rate of *C. asiatica* in the administration of the herbicides of 2,4-D and glyphosate. This means that *C. asiatica* had the same response to variations in the types and doses of herbicides.

Nitrate reductase

Nitrate reductase (NR) is one of the most sensitive plant enzymes studied. NR has been studied intensively because its activity often affects the rate of protein synthesis in plants that absorb NO_3^- as the main nitrogen source. NR activity is influenced by several factors, including the rate of synthesis and the rate of an overhaul by protein-destroying enzymes. Inhibitors and activators also influence it in the cell (Salisbury and Ross 1995).

The analysis of variance showed that the treatment with the herbicides of 2,4-D and glyphosate did not significantly affect the nitrate reductase content of plants. The average levels of nitrate reductase due to treatment with the herbicides of 2,4-D and glyphosate are presented in Tables 16 and 17.

Table 16 shows that for the treatment of the herbicide of 2,4-D, the highest average nitrite reductase content was at a dose of 1 $L.ha^{-1}$. These results were not significantly different from that of *C. asiatica* without herbicides, doses of 0.25 $L.ha^{-1}$, 0.5 $L.ha^{-1,}$ and 75 $L.ha^{-1}$. Meanwhile, in administering the glyphosate herbicide (Table 17), the highest average nitrate reductase content was at a dose of 0.75 $L.ha^{-1}$. These results were not significantly different from the control plant, with doses of 0.25 $L.ha^{-1}$, 0.5 $L.ha^{-1,}$ and 1 $L.ha^{-1}$. In administering the herbicide glyphosate, the highest average nitrate reductase content was at a dose of 0.5 $L.ha^{-1}$. These results were not significantly different from the herbicide administration at doses of 0 $L.ha^{-1}$, 0.25 $L.ha^{-1}$, 0.75 $L.ha^{-1}$, and 1 $L.ha^{-1}$.

Figure 9 shows that the application of the herbicide of 2,4-D caused high nitrate reductase content formed in the *C. asiatica*. According to Planchett (2004), nitrate reductase (ANR) activity is positively correlated to stress. As a result, plants carry out a biochemical response by increasing the efficiency of using nitrate as an alternative electron acceptor by reducing nitrate to nitrite by the enzyme nitrate reductase. This is due to swelling in the root area resulting in disturbed oxygen absorption through the roots and anaerobic conditions in the area around the roots. This anaerobic condition causes the reduction of nitrate to nitrite and is the only way to replace the role of oxygen in electron transport.

Table 16. The average nitrate reductase (μmol/g) on the administration of the herbicide of 2,4-D

Type of herbicide	Dose of herbicide				
	0 L/ha	**0.25 L/ha**	**0.5 L/ha**	**0.75 L/ha**	**1 L/ha**
2,4-D	28.182	17.870	28.959	74.306	59.46

Table 17. The average nitrate reductase (μmol/g) on the administration of the herbicide of glyphosate

Type of herbicide	Dose of herbicide				
	0 L/ha	**0.25 L/ha**	**0.5 L/ha**	**0.75 L/ha**	**1 L/ha**
Glyphosate	15.555	22.186	28.017	27.666	27.809

Note: Numbers followed by the same letter in the same row or column indicate no significant difference in the DMRT (Duncan's Multiple Range Test) at a 5% level

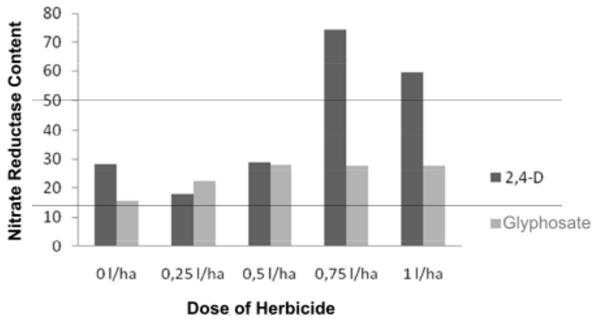

Figure 9. The effect of the herbicides of 2,4-D and glyphosate on nitrate reductase of *C.asiatica*

Glyphosate herbicide administration (Figure 9) generally resulted in lower nitrate reductase content formed compared to the herbicide of 2,4-D. Still, nitrate reductase content in glyphosate treatment of various concentrations was relatively stable compared to the control plant. This means that the glyphosate herbicide does not inhibit the formation of the enzyme nitrate reductase. According to Niswati et al. (1995), cultivation without tillage with the application of the glyphosate herbicide increases the availability of organic N and C and soil microbes. N availability in the soil will increase the nitrate reduction process that occurs in two different reactions. The first reaction is catalyzed by nitrate reductase, an enzyme that will transport two electrons from NADH or NADPH and produce nitrite. The second reaction of the whole nitrate reduction process is the conversion of nitrite to ammonium (NH_4^+) (Planchett 2004).

Nitrate reductase is an essential enzyme in the chain of nitrate reduction to ammonium which is useful in forming amino acids, proteins, and other compounds containing the element N (Levitt 1980). Various environmental and nutritional factors determine the amount of the enzyme nitrate reductase in an organism. Plants in an environment rich in nitrate will have a large amount of the enzyme nitrate. However, the amount of the enzyme will decrease if they are in an environment with a lot of ammonium ions (Linbald and Guerrero 1993).

Ammonium is the product of nitrate reductase that can catalyze the nitrate reduction process. If the amount of product continues to increase beyond the level of cell demand, the product will become a blocker. Many enzymes will be inactive until the product of the final compound is reduced in number. This mechanism is called the feedback mechanism as a fast and sensitive mechanism to avoid the excessive synthesis of a final product (Lakitan 1993).

Based on the results of the *t*-test (Appendix IV), it can be seen that there was no significant difference in the nitrate reductase content of *C. asiatica* in the administration of the herbicides of 2,4-D and glyphosate. This means that *C. asiatica* had the same response to variations in the types and doses of herbicides.

Based on the research that has been conducted, it can be seen that: (i) Administration of the glyphosate herbicide at a dose of 0.25 to 1 L/ha did not inhibit the number of leaves, dry weight, and nitrate reductase. The glyphosate dose of 0.25 to 0.75 L/ha did not inhibit the formation of gross weight, chlorophyll content, and respiration rate. The glyphosate dose of 0.25 to 0.5 L/ha did not inhibit the leaf area and carotenoid content of *C. asiatica*. (ii) The herbicide of 2,4-D up to a dose of 0.25 to 1 L/ha has not inhibited chlorophyll, respiration rate, and nitrate reductase. The glyphosate dose of 0.25 to 0.5 L/ha did not inhibit the formation of the number of leaves, leaf area, gross weight, dry weight, and carotenoid content. (iii) The effect of glyphosate herbicide showed a lower inhibitory effect than the herbicide of 2,4-D in terms of growth: number of leaves, leaf area, gross weight, and dry weight. Cultivation of *C. asiatica* is more suitable than using the herbicide glyphosate because the growth of *C. asiatica* is generally good.

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