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The effect of commercial herbicide and bioherbicide of Ketapang (*Terminalia catappa* L.) on germination and chlorophyll content of cayenne pepper (*Capsicum frutescens* L.)

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ABSTRACT. Herbicides that have less impact on non-target crops are better suited for weed control. The use of a commercial glyphosate-based herbicide (Roundup®) is a farmer's choice for controlling these weeds. The negative effects of glyphosate residues on cultivated crops, environment and human health have led farmers to switch to use bioherbicides. Ketapang (*Terminalia catappa* L.) leaves have been studied having the potential as bioherbicide but their effects on non-target plant have been less studied. This study aimed to examine the effects of Roundup® and Ketapang leaf extract on germination and chlorophyll content of cayenne pepper as non-target plant. This experimental study was conducted using completely randomized design (CRD) with 3 replications. Cayenne pepper was sown for 14 days. Parameters observed were germination, morphology, weight, and chlorophyll content of cayenne pepper sprouts. The data were analyzed using SPSS ver. 16 and tested with One-Way ANOVA or Kruskal Wallis tests at *p*-value of 0.05 depended on parametric assumption tests. There were significant differences between the Roundup® solution treatment with the control on all parameters (germination parameters, weight, and chlorophyll content of cayenne pepper sprouts) ($p<0.05$). The effect was getting worse seen in cayenne pepper seeds given high doses of Roundup® solution. On the contrary, there were no significant differences between the treatments of the aqueous extract of Ketapang leaves with the control on all parameters ($p>0.05$), except the vigor index. There were morphological changes in the radicle and root of cayenne pepper sprouts exposed to them. Rotted radicles were highly visible in Roundup®-exposed sprouts.

Keywords: Bioherbicide; cayenne pepper; chlorophyll content; germination parameters; Ketapang leaf extract

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INTRODUCTION

Cayenne pepper (*Capsicum frutescens* L.; Solanaceae) is one of the most important vegetable commodities in Indonesian foods and has been widely cultivated in Indonesia. It produces a spicy taste in foods. One of the challenges in caring for cultivated plants (including cayenne pepper) is the presence of weeds. Some weeds that grow around cayenne pepper include: *Spilanthes acmella* L., *Emilia sonchifolia* (L.), *Amaranthus spinosus*, *Cleome rutidosperma*, *Cyperus rotundus*, *Dactyloctenium aegyptium* (L.) Willd., *Eleusine indica* (L.) Gaertn., *Euphorbia hirta* L., *Phyllanthus amarus* Schumach. & Thonn, *Portulaca oleracea* L., and *Laportea interrupta* (L.) Chew. (Lestari & Christie, 2021). There are many studies revealing crop losses because of them (Gharde *et al*., 2018; Korav *et al*., 2018). The competition between weeds and cultivated plants for nutrients, carbon dioxide, sunlight, space, soil moisture, and other growth factors causes growth inhibition of the plants (Korav *et al*., 2018). The weeds can also act as host for pests that can attack cultivated plants (Hasan *et al*., 2021; Umer *et al*., 2022). Sabri & Ramadhani (2018) reported there were 22 species of weeds that grew around the chili farm, of which 3 species were hosts for thrips (pest), such as *Galinsoga parviflora*, *Rorippa indica*, and *Physialis angulate*. Often farmers control the weeds by direct weeding or using chemical herbicides. Topramezone and triclopyr (Brosnan & Breeden, 2013) as well as paraquat and glyphosate (Tzvetkova *et al*., 2019) are chemical herbicides which have been studied having effectiveness in inhibiting weed growth and we have known for a long period there are many bad effects of them.

Herbicide with less impact on non-target plants (including cayenne pepper) is a good choice for weed control. The use of commercial herbicides based on glyphosate such as Roundup® is still the farmer's choice to deal with these weeds in addition to direct weeding. Glyphosate is an organophosphorus herbicide that is systematic, non-selective, and has been used in agriculture on an industrial scale (Al-Rajab & Hakami, 2014). Although it has effectiveness in massive weed control, its application has some adverse effects on non-target plants such as lettuce and radish (Tzvetkova *et al*., 2019). In addition, excess glyphosate in soil can affect the activity of soil microorganisms which play an important role in maintaining soil fertility (Haney *et al*., 2000). Glyphosate residues left on vegetable commodities also have serious impacts on human health such as causing cytotoxic and genotoxic effects, inflammation, and affecting the immune system (Peillex & Pelletier, 2020).

The adverse effects of glyphosate residues on cultivated crops, environment and human health have led farmers to switch to use bioherbicides to achieve sustainable agriculture (Hasan *et al*., 2021). Bioherbicide may be developed from plant extract containing allelochemicals which are eco-friendly and safe for human health. The compounds in some plants such as phenolics, terpenoids, alkaloids, and their derivatives are allelopathic to other plants. Allelopathy is a biological phenomenon in which plants produce certain secondary metabolites that can inhibit the growth and survival of other plants (Cheng & Cheng, 2015). The allelochemicals typically have short-lived environmental persistence and low toxicity, and they often do multiple modes of action, which mitigate the risks of herbicide resistance (Hasan *et al*., 2021). Therefore, the use of bioherbicide is an eco-friendly weed control technique.

Ketapang (*Terminalia catappa* L.) leaves have been studied having the potential as bioherbicides (Khairunnisa *et al*., 2018; Nurhalina *et al*., 2021; Riskitavani & Purwani, 2013) although they have not been extensively studied. As with synthetic herbicides, the allelopathic effect is not only received by weed but also received by non-target plants. It depends on the plant resistance level. The use of herbicides in weed management in cayenne pepper is thought to interfere with its growth. Germination is an important early stage for plant life while chlorophyll content is important in plant photosynthesis. Therefore, this study aimed to examine the effects of commercial herbicide (Roundup®) and bioherbicide from Ketapang leaf extract on germination and chlorophyll content of cayenne pepper. By interfering with normal cell function and the release of harmful compounds from herbicide agents, this study is expected to become one of the reference techniques for regulating suppressed weed populations.

MATERIALS AND METHODS

This experimental research was conducted at Laboratory of Science, Faculty of Science and Technology, Universitas Islam Negeri Walisongo Semarang. This research used a completely randomized design (CRD) with three replications.

Preparation of Roundup® 486 SL. The commercial herbicide used is a synthetic herbicide purchased from online market place. A total of 10 mL of Roundup® liquid was dissolved into 100 mL of distilled water. In this study, the solution mixture was equivalent to a concentration of 100% (v/v) (pH 5). Furthermore, the solution was diluted with distilled water to obtain solutions with various concentrations (25%, 50%, 75% -v/v). The extract concentration of 0% consisted of only distilled water.

Preparation of aqueous extract of Ketapang leaves. Ketapang leaf extract was prepared using the maceration method with water as solvent and then dilution was made (Gindri *et al.,* 2020). The healthy leaves of Ketapang were collected in the morning, taken from the yard of Universitas Islam Negeri Walisongo Semarang, then washed under running tap water to remove dust and other contaminants on the leaf surface. The clean leaves were dried at room temperature (30°C) for two days in the shade. The dried leaves were cut into smaller pieces and then redried in the oven (40°C) for two days. The dried leaves were mashed using an electric blender to get coarse powder. The powders (20 g) were macerated in 200 ml of distilled water and then left for three days. The solution mixture was filtered and the filtrate was considered to have a concentration of 100% (pH 5). Furthermore, the extract was diluted with distilled water to obtain extracts with various concentrations (25%, 50%, 70%-v/v). The 0% extract concentration consisted of distilled water.

Application of Roundup® solution and aqueous extract of Ketapang leaves on cayenne pepper seeds. Cayenne pepper seeds were surface sterilized using 0.05% $KMNO_4$ for 3 min, then rinsed with water 3 times. A total of 30 sterile cayenne pepper seeds were germinated in a petri dish (diameter 90 mm) on Whatman paper No. 1 and 2 pieces of tissue under normal conditions (day temperature (25-32°C) and night temperature (25-28°C) for 14 days. Each petri dish was treated by pouring 5 ml of Roundup® solution or Ketapang leaf aqueous extract with a predetermined concentration. To maintain the humidity of the filter paper, 10 ml of tap water was watered. Starting from the 6^{th} day, 5 ml of tap aqueous was watered every day.

Measurement of germination parameters. Germination parameters measured in this study consisted of percentage of germination, germination index, vigor index, and wet weight of sprouts. Germination indicator was characterized by the appearance of a radicle from the seed. Germination parameters were calculated using the following formula (Gupta, 1993).

$$\text{Germination percentage (\%)} = \frac{\text{number of germinated seeds}}{\text{total number of seeds}}$$

$$\text{Germination index} = \frac{\text{The number of seeds that germinate on the day}}{\text{number of day}}$$

$$\text{Vigor index} = \text{Germination percentage (\%)} \times \text{sprout length (cm)}$$

Measurement of chlorophyll content. Fresh leaves (0.1 g) were crushed and then added with methanol p.a. (10 ml) or ratio (0.1:10). Next, the extract was filtered using Whatman paper. Then, the filtrate was put into a cuvette. The optical density (OD) values of chlorophyll filtrate at wavelengths (λ) 652.4 nm and 665.2 nm were read using a spectrophotometer (Genesis UV-Scanning Thermo-Scientific). Chlorophyll content was calculated using the equation (Lichtenthaler & Wellburn, 1983).

Chlorophyll a = $(16.72 \times \lambda\ 665.2 - 9.16 \times \lambda\ 652.4)$ mg/l
Chlorophyll b = $(34.09 \times \lambda\ 652.4 - 15.28 \times \lambda\ 665.2)$ mg/l
Total chlorophyll = $(24.93 \times \lambda\ 652.4 + 1.44 \times \lambda\ 665.2)$ mg/l

Data analysis. All data obtained were analyzed using SPSS ver. 16, to obtain the mean and standard deviation. The data from each parameter obtained were tested for parametric assumption tests, i.e., normality (Shapiro-Wilk's test) and homogeneity (Levene's test). If both assumption tests were met, the One-Way ANOVA test was carried out for the data at *p*-value of 0.05 and continued Duncan's test. If both assumption tests were not met, Kruskal Wallis tests was carried out for the data at *p*-value of 0.05.

RESULTS AND DISCUSSION

The effect of Roundup® to cayenne pepper seed germination. The commercial herbicide used in this study was a synthetic herbicide from Monsanto 486 SL and a glyphosate-based herbicide that the most used herbicide in agriculture worldwide (Martins-Gomes *et al*., 2022; Novotny, 2022). Roundup® is golden yellow solution and it is formulated in such a way and can dissolve in water easily (Aritonang, 2021). The active ingredient of Roundup® is isopropyl amine glyphosate 486 g/L (equivalent to glyphosate 360 g/l)(Novotny, 2022). It works using biosorb technology which is directly applied by spraying it on the leaf surface of unwanted weeds. Glyphosate also can enter to transport tissue in the root through symplastic and apoplastic pathways and affects plant metabolism (Saunders & Pezeshki, 2015). This is a way how glyphosate residue left in soil can be absorb by non-target plant. In this study, Roundup® solutions with various concentrations were watered on the surface of the cayenne pepper seeds. It was assumed that Roundup® affected germination of cayenne pepper seeds through the radicles.

This result showed that the cayenne pepper seeds after treated with Roundup® solution and the control started germination on day-4 (Fig. 1a). The seeds exposed to Roundup® solution with a concentration of 75% showed a higher germination percentage (26.67%) than the control (14.44%) while the seeds exposed to Roundup® solution with concentrations of 50% and 25% showed low germination percentages of 2.22% and 7.78%, respectively. On day-5, day-6, and day-7, there was a quite high increase in the germination percentage in the control. On day-7, the germination percentage of the control had reached 94.44%. In contrary, a small increase of the germination percentage was observed on cayenne pepper seeds exposed to the Roundup® solution at concentrations of 25%, 50%, 75%, and 100%. Among the four concentrations, cayenne pepper seeds exposed to 75% Roundup® solution showed the highest germination percentage, i.e., 57.78% compared to concentrations of 25%, 50%, and 100% (43.33%, 17.78%, and 41.11%, respectively). Both the results of the Kruskal Wallis and One-Way ANOVA showed that the treatments of Roundup® solutions of various concentrations (25 %, 50 %, 75%, and 100 %-v/v)) indicated significant effect on cayenne pepper seed germination from the day-4 of germination to the day-14 germination ($p<0.05$). Cayenne pepper seeds exposed to Roundup® solution took a longer time than the control to achieve maximum germination percentage (Fig. 1a). On day-14, the lowest germination percentage had shown by 50% Roundup® solutions treatment (36.67%).

The Roundup® exposure at serial concentrations on day-7 and day-14 significantly affected germination index of cayenne pepper seeds. There was a significant difference on the cayenne pepper germination index between the Roundup® solution treatments and the control based on Kruskal Wallis test ($p<0.05$)(Table 1). The lowest germination index was shown by cayenne pepper seeds exposed to 50% Roundup® solution. Germination percentage and sprout length (shoot+ root) determine the vigor index. This study showed that Roundup® exposure significantly affected the vigor index of cayenne pepper. There was a significant difference on the cayenne pepper vigor index between the Roundup® solution treatments and control based One-Way ANOVA test ($p<0.05$) (Table 2). Based on Duncan test, the highest vigor index was showed by the control and the lowest was showed by 50% Roundup® solution treatment, followed by concentrations of 100%, 75%, and 25%.

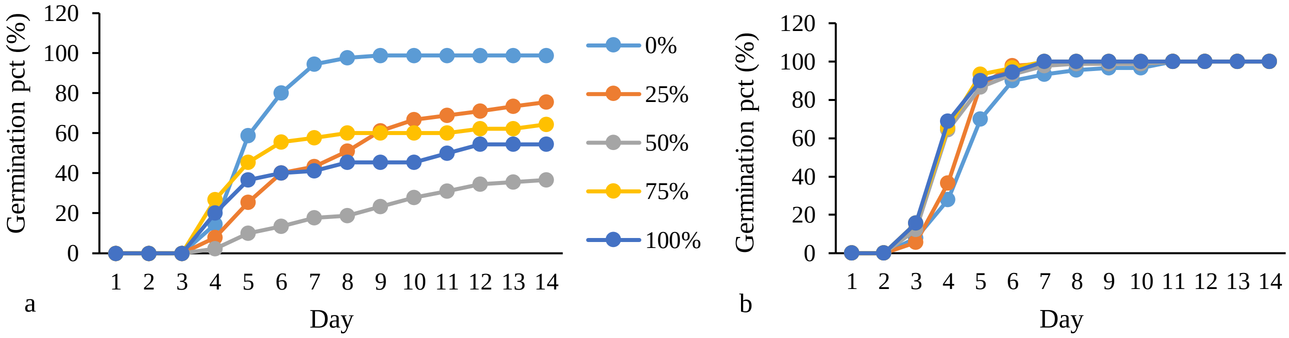

Fig. 1. Germination percentage of cayenne pepper seeds with treatments: a. Roundup® solution; b. aqueous extract of Ketapang leaves

The inhibited germination of cayenne pepper seeds indicated that the plant included non-target plant which was intolerant to Roundup® exposure. These results were in accordance with Mondal *et al*. (2017) who reported germination inhibition of *Pisum sativum* (pea) after treated by glyphosate in which the germination was reduced to 55% and 40% after treated with 3 and 4 mg/l of Roundup® on three days of germination. Roundup® exposure reduced sprout length of cayenne pepper. Comparison of sprout length between cayenne pepper sprouts with Roundup® treatment and control can be visualized in Fig. 3a. Mondal *et al*. (2017) also reported that application of Roundup® (glyphosate) reduced root and shoot length of pea sprouts during 14 days of germination.

Table 1. Germination index of cayenne pepper seeds with Roundup® solution and aqueous extract treatment of Ketapang leaves

Treatments	Germination index Roundup® solution		Aqueous extract of Ketapang leaves	
	Day-7	Day-14	Day-7	Day-14
0%	13.49 ± 0.55	7.06 ± 0.14	13.33 ± 0.96	14.29 ± 0.00
25%	6.19 ± 0.28	5.40 ± 1.13	14.13 ± 0.28	14.29 ± 0.00
50%	2.54 ± 1.20	2.62 ± 0.85	13.97 ± 0.55	14.29 ± 0.00
75%	8.25 ± 1.53	4.60 ± 0.54	14.29 ± 0.00	14.29 ± 0.00
100%	5.87 ± 1.67	3.88 ± 0.27	14.29 ± 0.00	14.29 ± 0.00
P-value	0.015 (*)	0.011 (*)	0.279 (ns)	-

Note: (*) indicates the value of p<0.05 based on the Kruskal Wallis test, n=3; ns indicated not significant

According to Gomes *et al*. (2017a), Roundup® as well as glyphosate reduced germination of non-glyphosate resistant soybean seed by increased and accumulated H_2O_2 concentrations in the embryos although the activity of some reactive oxygen species (ROS) scavenging enzymes such as APX and CAT increased in exposed seeds. They hypothesized that these enzymes failed to reduce accumulation of hydrogen peroxide (H_2O_2) concentrations then increased H_2O_2, caused oxidative bursts and decreasing seed germination through deterioration of cell structures and some components such as fatty acids, proteins, and DNA. Application of Roundup® also could reduce germination of *Dimorphandra wilsonii* seed by decreasing respiration rate of seeds (Gomes, da Silva Cruz, *et al*. 2017). Roundup® affected the activity of enzymes associated with the mitochondrial electron transport chain (ETC), with complex III as the exact target (Gomes *et al*., 2017b). In other previous study, although application of Roundup® did not affect final germination percentage of maize seeds, its application could delay germination of maize seeds (Gomes *et al*., 2019b). This happened because the application of Roundup® on maize seed could stimulate antioxidant enzymes then reduced the concentration of H_2O_2. Gomes *et al*. (2019a) reported low germination of sorghum seeds after exposure to glyphosate. They explained that the low germination percentage was not related to the accumulation of H_2O_2 in the seeds, but that the negative effect was related to the disruption of the electron transport chain in mitochondria. Glyphosate also interfered with the de novo synthesis of gibberellin which was important in stimulating germination (Gomes *et al*., 2019a).

Table 2. Vigor index of cayenne pepper seeds with aqueous extract treatment of Ketapang leaves and Roundup® solution

Treatments	Vigor index (day-14) Roundup® solution	Aqueous extract of Ketapang leaves
0%	6.03 ± 0.45 c	6.55 ± 0.38 d
25%	1.00 ± 0.25 b	3.39 ± 0.69 c
50%	0.10 ± 0.04 a	2.21 ± 0.45 ab
75%	0.21 ± 0.01 a	2.78 ± 0.40 bc
100%	0.11 ± 0.03 a	1.82 ± 0.05 a
P-value	0.00 (*)	0.00 (*)

Note: (*) indicates the value of p<0.05 based on the One-Way ANOVA test, n=3

Glyphosate included as an inhibitor in which it could interfere with the shikimate pathway in plants thereby inhibiting the production of aromatic amino acids which are the basis for several plant metabolites (Fuchs *et al*., 2021; Van Bruggen *et al*., 2021). The synthesis of aromatic amino acids was impaired due to inhibition of the enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) (Gomes *et al*., 2014). This enzyme was important for the shikimic acid pathway to produce chorismite, an intermediate precursor molecule for the aromatic amino acid such as phenylalanine, tyrosine and tryptophan and for various secondary metabolites (Saunders & Pezeshki, 2015). Gomes *et al*. (2017a) reported that Roundup® or glyphosate caused shikimate accumulation in embryo of non-glyphosate resistant soybean seeds. This indicated that Roundup® or glyphosate inhibited the EPSPs enzyme and led to shikimate accumulation. Shikimate accumulation could be biomarker for glyphosate sensitivity in plant (Zulet-Gonzalez *et al*., 2023). Based on the results, it was possible that germination inhibition of cayenne pepper seeds happened because of the explained ways affecting plant metabolism.

The effect of aqueous extract of Ketapang leaves to cayenne pepper seed germination. The adverse effects of glyphosate residues on cultivated crops (non-targeted plant), environment and even on human health have led farmers to switch to use bioherbicides. Bioherbicides can come from plants that contain phytotoxic allelochemicals or come from certain disease-carrying microbes that can suppress weed populations (Hasan *et al*., 2021; Duke *et al*., 2022). The use of bioherbicides has shown great promise in inhibiting the germination of weed seeds. Bioherbicides attack the weeds by causing phytotoxicity through their allelochemicals. These toxic compounds can disrupt the integrity of cell membranes and important biochemical processes in weeds. The phytotoxic impact of weeds can be reflected in a decrease in growth parameters such as short roots, reduced plant height, and reduced wet or dry weight of plants. Decreased growth can be caused by disturbances in nutrient absorption, protein synthesis and growth hormone synthesis. Compounds contained in bioherbicides contribute to plant stress due to increased production of reactive oxygen species (ROS) and abnormal antioxidant production (Hasan *et al*., 2021).

Ketapang has been studied as having potential as a bioherbicide although it has not been extensively studied. Syukran *et al*. (2022) reported that 70% aqueous extract of Ketapang leaves could inhibit germination of Sembung Rambat (*Mikania micrantha*) seeds while methanol and ethanol extracts of the plant with small concentration i.e., 30% could inhibit the germination of *Mikania micrantha* seeds. Likewise, other parameters such as root length and plumule of the sprouts showed reduction after treated by those extracts. The inhibition effect of ethanol extract of Ketapang leaves also has been studied on other weed i.e., Grinting grass weed (*Cynodon dactylon* (L.) Pers.) (Nurhalina *et al*., 2021). Mahardika *et al*. (2016) also reported that methanol extract of Ketapang leaves with a minimum concentration of 50% was able to inhibit the germination of the other weed i.e., Putri malu (*Mimosa pudica* L.). According to their analysis, the germination inhibition of Putri malu was due to inhibition of the action of some hormones which played a role in seed germination which was also stimulated by the allelochemicals in the Ketapang leaf extract.

Erida *et al*. (2020) have studied above the metabolite compounds contained in methanol extract of green Ketapang leaves by GC-MS. These compounds consisted of primary metabolites such as n-hexadecenoic acid (fatty acid) (8,20%) and 9,12,15-octadecanoic acid,2,3-dihydroxypropyl ester, (Z, Z, Z)- (fatty acid) (3.25%). Meanwhile, the secondary metabolites contained in the methanol extract of green Ketapang leaves consisted of lupeol (triterpenoid)(25.84%), stigmasterol, 22.23-dihydro-(steroid) (15.43%), alpha-amyrin (triterpenoids)(9.81%), beta-amyrin (triterpenoids)(7.90%), germanicol (triterpenoids)(5.67%), and 3,7,11,15-tetramethyl-2-hexadecen-1-o(triterpenoid) (3.26%)(Erida *et al*., 2020). The allelopathic effect of Ketapang leaves may be caused by those metabolites contained in Ketapang leaves.

The use of herbicides which do not harm non-target plants or cultivated plants is certainly the best choice for eradicating weeds without reducing crop yields of cultivated plants (non-target plants). Bioherbicide is eco-friendly, safe for non-target organisms, practically easy to use, and not involved in risks related to human and animal health. In this study, aqueous extracts of Ketapang leaves were tested on non-target plant seeds i.e., cayenne pepper to determine whether Ketapang extract affected germination and chlorophyll content of non-target plants (cayenne pepper).

These results showed that cayenne pepper seeds treated with aqueous extract of Ketapang leaves performed early germination on day-3 (Fig. 1b). On day-5, the germination percentages of the seeds with aqueous extract of Ketapang leaves treatments were higher than the control. This trend continued until day-9. On day-10 to day-14, the germination percentage of all treatments and control showed a maximum value of 100% (Fig. 1b). The germination speed was relatively the same between the treatments and the control. There was a significant difference on the germination percentage only on day-4 between the Ketapang extract treatments and the control based on One-Way ANOVA test ($p<0.05$). Meanwhile, on day-5 to day-9, the was no significant difference on the germination percentage between the Ketapang extract treatments and the control ($p>0.05$).

Germination index between the treatments and the control on day-7 of germination showed not significant different ($p>0.05$) based on Kruskal Wallis test (Table 1). Likewise on the 14^{th} day, the germination index between the treatments showed clearly no difference. Unlike Roundup®, apparently, the application of aqueous extract of Ketapang leaves did not affect cayenne pepper seed germination. In contrary, only vigor index was significantly affected by aqueous extract of Ketapang leaves based on the One-Way ANOVA test ($p<0.05$) (Table 2). Based Duncan test, the highest vigor index was indicated by the control and the lowest was indicated by the 100% aqueous extract of Ketapang leaf treatment. This is the only inhibitory effect shown by aqueous extract of Ketapang leaves. In other words, the extract also affected and reduced the sprout length. Mahardika *et al*. (2016) also reported inhibition of sprout growth of *M. pudica* expressed by 0 cm in sprout length after treated with methanol extract of Ketapang leaves with concentration of 50% and 75%. We assumed that there were allelochemicals in Ketapang leaves that caused reduced cell division. As explained by Li *et al*. (2010), phenolic allelochemicals could inhibit cell division and root elongation and change ultra-cell structure which led to interfere with the normal growth and development of plant.

The effect of Roundup® to cayenne pepper sprout morphology. On the day-5 of germination, it was observed that there were morphological changes in the radicle of the seeds, especially color changes to be dark brown to black at the tip of the seed radicles exposed to 75% and 100% Roundup® solution (Fig. 2a-e). Meanwhile, the other seeds exposed to 25% and 50% Roundup® solutions were still in good condition as in the control. On day-10, the dark brown radicles were seen on all Roundup® solution treatments (except the control). On 75% and 100% concentration, the tips of the radicles were getting black and rotten and begins to dry out. In contrary to the control, it was seen that the cayenne pepper sprouts grew well and the first two healthy green leaves appeared (Fig. 2f-j).

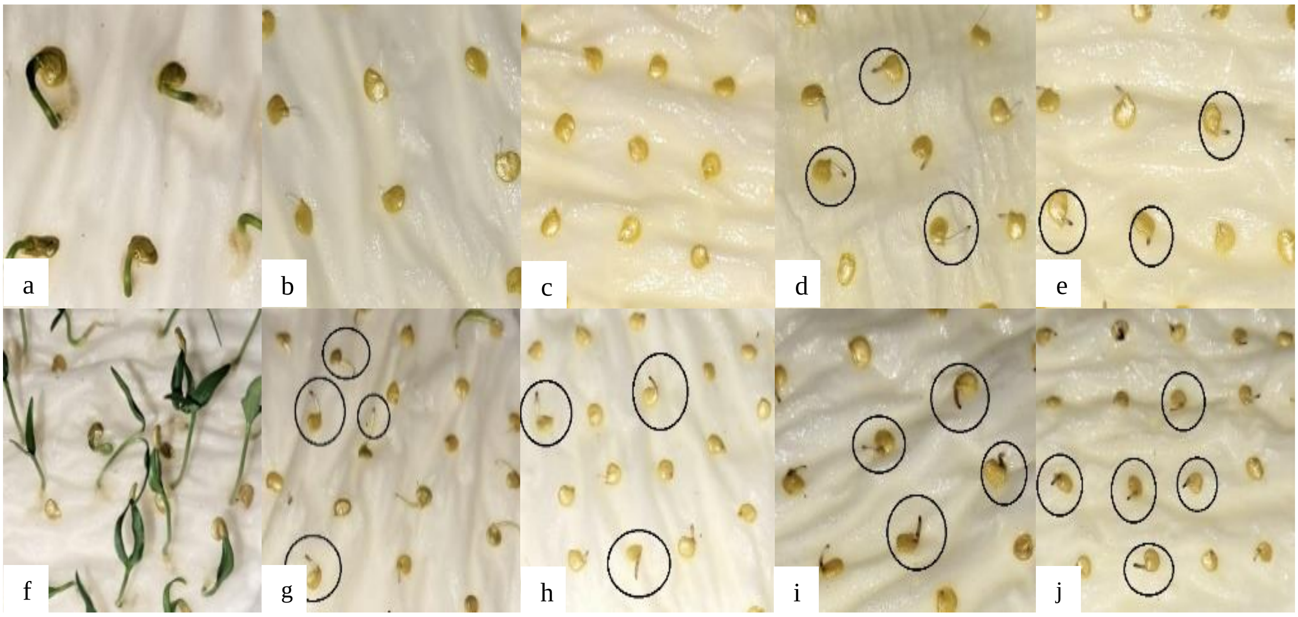

Fig. 2. The morphological appearance of cayenne pepper sprouts treated with Roundup® solution on day-5: a. 0%; b. 25%; c. 50%; d. 75%; e. 100%; on day-10: f. 0%; g. 25%; h. 50%; i. 75%; j. 100%. Black circle marks indicated brown-black radicles

On day-14 of germination, the difference between cayenne pepper sprouts exposed to Roundup® solution and control was clearly visible from the size of the sprouts and leaf color (Fig. 3a). The short and pale light green leaves were observed on 25% Roundup® solution treatment (Fig. 3b). Chlorosis was seen in cayenne pepper sprouts exposed to 25% Roundup® as reported by Silva *et al*. (2014) and

Mondal *et al*. (2017) that chlorosis was one of toxicity symptoms from Roundup®, particularly at higher concentrations. This indicated that cayenne pepper sprouts were sensitive to Roundup®. Meanwhile, the cayenne pepper seeds exposed to 50%, 75%, and 100% Roundup® solution still failed to grow. Roundup® or glyphosate reduced root length because it interfered with auxin synthesis thereby preventing cell division (Gomes, Richardi, *et al*., 2019; Tzvetkova *et al*., 2019). Disturbances in respiratory chain caused by Roundup® could stimulate stress oxidative in mitochondria (Strilbyska *et al*., 2022). Glyphosate could modulate and affect plant hormonal homeostasis and affected performance of non-target plant (Fuchs *et al*., 2022). Physiological disturbances in plant resulted in poor morphological appearance.

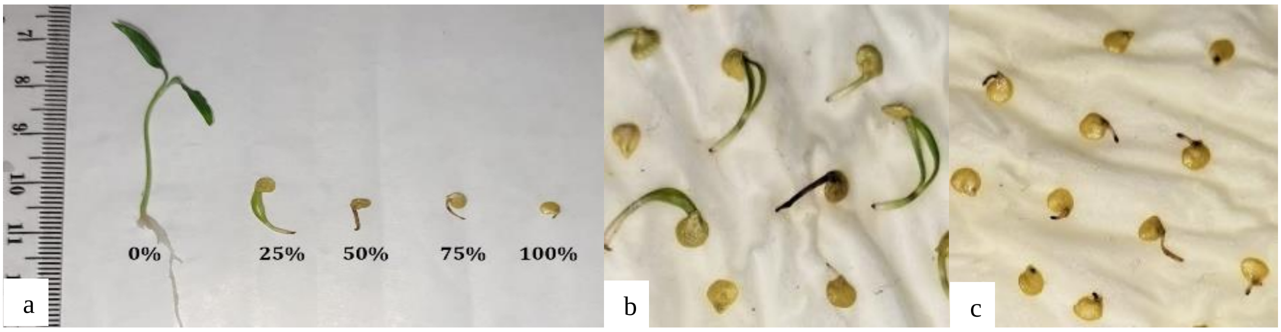

Fig. 3. The morphological appearance of cayenne pepper sprouts treated with Roundup® solution on day-14: a. each sprout after application of Roundup® solution; comparison of cayenne pepper sprouts on Roundup® treatments: b. 25%; c. 50%

The effect of aqueous extract of Ketapang leaves to cayenne pepper sprout morphology. The Other results showed that cayenne pepper seeds treated with aqueous extract of Ketapang leaves normally germinated with the morphology appearance as the control, on the day-5 of germination (Fig. 4a-e). On the day-10, it appeared that the roots of cayenne pepper sprouts treated with 50%, 75% and 100% aqueous extract of Ketapang leaves showed a short brown color because they had absorbed the extract (Fig. 4f-j). Meanwhile, the leaf cayenne pepper sprouts started to grow and was dark green in color. On the day-14 of germination, the morphological appearance of cayenne pepper sprouts treated with 50%, 75%, 100% aqueous extract of Ketapang leaves showed shorter brown roots than that of the control (Fig. 4k). According to Cheng & Cheng (2015) the shape and structure of plant cells are affected by allelochemicals. Short brown sprout roots indicated an allelopathic effect of Ketapang leaf extract.

Table 3. Wet weight of cayenne pepper seeds with aqueous extract treatment of Ketapang leaves and Roundup® solution

Treatments	Wet weight (g) (day-14) Roundup® solution	Aqueous extract of Ketapang leaves
0%	0.90 ± 0.05	0.79 ± 0.22
25%	0.08 ± 0.02	0.70 ± 0.28
50%	0	0.44 ± 0.05
75%	0	0.37 ± 0.01
100%	0	0.54 ± 0.13
P-value	0.008 (*)	0.066 (ns)

Note: (*) indicates the value of $p<0.05$ based on the Kruskal Wallis test, n=3; ; ns indicated not significant

The effect of Roundup® to wet weight of cayenne pepper sprouts. The wet weight data of cayenne pepper sprouts were measured on the 14th day of germination. Because parametric assumption tests were not met, Kruskal Wallis test was carried out for the data of wet weight of cayenne pepper sprouts treated with Roundup® solution at *p*-value of 0.05. The result showed that there was significant difference in wet weight between the treatment and control ($p<0.05$) (Table 3). The wet weight was not measured in the 50%, 75%, and 100% Roundup® treatments because of shoot absence. Application of Roundup® could stimulate overproduction of ROS and oxidative damage to membrane lipids (Gomes *et al*. 2017a), then could lead to death cell. Thus, the reduced weight of sprouts became one of the symptoms of Roundup® toxicity.

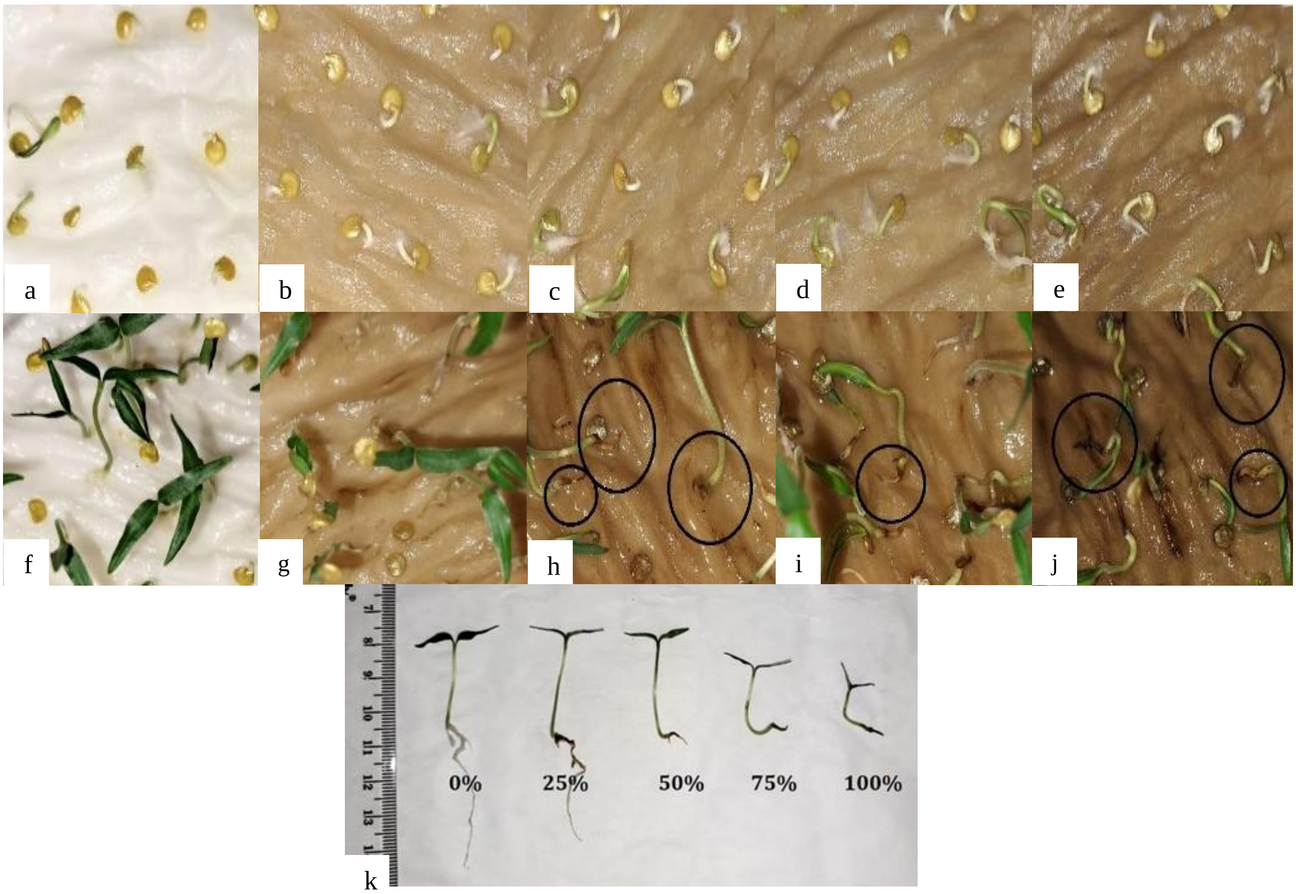

Fig. 4. The morphological appearance of cayenne pepper sprouts treated with aqueous extract of Ketapang leaves on day-5: a. 0%; b. 25%; c. 50%; d. 75%; e. 100%; on day-10: f. 0%; g. 25%; h. 50%; i. 75%; j. 100%; k. on day-14. Black circle marks indicated short and brown roots

The effect of aqueous extract of Ketapang leaves to wet weight of cayenne pepper sprouts. Because parametric assumption tests were met, the One-Way ANOVA test was carried out for wet weight of cayenne pepper sprouts treated with aqueous extract of Ketapang leaves. The results showed that there was no significant difference in wet weight between the treatment and control ($p>0.05$) (Table 3). However, the wet weight of cayenne pepper sprouts treated with Ketapang leaf extract with a concentration of 75% showed the lowest value among all treatments. These results contrasted with the decreased wet and dry weight of Teki grass after being given Ketapang leaf extract (Riskitavani & Purwani, 2013).

The effect of Roundup® to chlorophyll content. This result showed that the application of Roundup® significantly reduced chlorophyll content of cayenne pepper. There were significant differences in chlorophyll-a, chlorophyll-b and total chlorophyll contents between the cayenne pepper seeds with the Roundup® treatments and the control based on The Kruskal Wallis test ($p<0.05$) (Table 4). The chlorophyll content in 25% Roundup® solution treatment was lower than that in control. In accordance with the observation of the sprout morphology, the sprouts exposed to 25% Roundup® were pale greenish yellow while the control sprouts were fresh green (Fig. 3). Meanwhile, the chlorophyll content was not measured in the 50%, 75%, and 100% Roundup® solution treatments because of the absent shoots (Fig. 2). Effect of Roundup® in reducing the chlorophyll content could be explained with how glyphosate reduced the chlorophyll content.

Table 4. Chlorophyll contents of cayenne pepper sprouts with aqueous extract treatment of Ketapang leaves and Roundup® solution

Treatments	Chlorophyll contents (mg/L) (day-14)	
	Roundup® solution	Aqueous extract of Ketapang leaves
Chlorophyll-a		
0%	14.35 ± 0.47	16.35 ± 1.52
25%	1.85 ± 0.24	15.62 ± 0.79
50%	-	14.32 ± 3.10
75%	-	14.52 ± 0.78
100%	-	13.74 ± 1.70
P-value	0.008 (*)	0.38 (ns)
Chlorophyll-b		
0%	6.29 ± 0.20	6.89 ± 0.93
25%	0.97 ± 0.04	6.53 ± 0.56
50%	-	5.82 ± 1.60
75%	-	6.16 ± 0.60
100%	-	5.56 ± 0.52
P-value	0.008 (*)	0.46 (ns)
Total Chlorophyll		
0%	20.64 ± 0.86	23.24 ± 2.45
25%	2.83 ± 0.26	22.15 ± 1.35
50%	-	20.12 ± 4.70
75%	-	21.01 ± 1.96
100%	-	19.30 ± 2.20
P-value	0.008 (*)	0.44 (ns)

Note: (*) indicates the value of $p<0.05$ based on the Kruskal Wallis test, n=3

Gomes *et al*. (2016) have reported that the application of glyphosate decreased the chlorophyll content and photosynthetic rate of *Salix miyabeana* cultivar SX64 after a 12-hour exposure period. It induced ROS accumulation in *S. miyabeana* leaves through lipid peroxidation. Application of glyphosate also decreased total chlorophyll and plastoquinone concentrations of *Salix miyabeana* (willow plant leaves)(Gomes *et al*. 2017c). Kamdem *et al*. (2016) also reported that the application of Roundup® at a dose of 1 g/kg of soil could reduce the growth and chlorophyll content of pea and maize, as well as affect the nodulation of pea. Van Bruggen *et al*. (2021) also explained that glyphosate and its derivatives (amino methyl phosphonic acid/AMPA) can reduce photosynthesis. It is possible that the low chlorophyll content can be the one of causes of the low photosynthesis rate. Decreased chlorophyll-a and-b contents, the net photosynthetic rate (PN), effective quantum yield of photochemical energy conversion (yield), and the relative rate of electron transport through PSII (ETR) also were observed on cogon grass (*Imperata cylindrical* L.) after treated with different concentration levels of glyphosate (Huang *et al*. 2012). In susceptible soybean, an application of 0.28 kg/ha reduced chlorophyll content (49%), and shoot and root dry weight (50 and 57%, respectively) at 2 weeks after treatment (Reddy *et al*., 2000).

The effect of Aqueous extract of Ketapang leaves to chlorophyll content. The Kruskal Wallis test was also carried out for the chlorophyll-a and total chlorophyll contents of cayenne pepper sprouts treated with aqueous extract of Ketapang leaves while One-Way ANOVA test was carried out for the chlorophyl-b. The results showed that there were no significant differences in the chlorophyll-a, chlorophyll-b and total chlorophyll contents between the treatments and control samples ($p>0.05$) (Table 4). In contrary to the effect of Roundup® in reducing chlorophyll content, the application of aqueous extract of Ketapang leaves had no effect on chlorophyll content. The results showed that there were no differences in chlorophyll-a, chlorophyll-b, and total chlorophyll contents between the treatments (cayenne pepper seeds treated with aqueous extract of Ketapang leaves) and the control. Like germination parameters and wet weight, the application of aqueous extract of Ketapang leaves did not significantly affect the chlorophyll contents. These results were in line with Khairunnisa *et al*. (2018), that the application of ethanol extract of Ketapang leaves did not affect the chlorophyll content of teki grass weeds. These results were contrary to several studies regarding the potential of bioherbicides from certain plants that could inhibit weed growth by reducing chlorophyll levels (Hasan *et al*., 2021). Three allelochemical compounds from the phenolic group such as p-coumarin, ferulic acid, and o-hydroxyphenyl acetate have been shown to inhibit chlorophyll biosynthesis and affect the activity of chlorophyllase and Mg-dechelatase enzymes which are responsible for the degradative pathway of chlorophyll in rice leaves (*Oryza sativa* cv. TN67)(Yang *et al*., 2004).

The use of plants as bioherbicides is one of the biological controls to reduce weeds. The intended biological control at least does not produce residues that pollute the environment, does not affect non-target plants and is safer to use (Telkar *et al*., 2015; Umer *et al*., 2022). Apart from not significantly reducing germination, the application of aqueous extract of Ketapang leaves also did not affect the wet weight of the sprouts ($p>0.05$). The solvent used in this research was distilled water. The effect given might be different if it used other solvents. According to Syukran *et al*. (2022), the ethanol and methanol extracts of Ketapang leaves had a greater inhibitory effect than those of aqueous extracts. It might be explained from the flavonoid and tannin content in methanol extract of Ketapang leaves which had higher than those of water and ethanol extracts. Thus, the type of solvent determined the effectiveness of the bioherbicide from Ketapang leaves.

CONCLUSION

The exposure to Roundup® solution had a significant effect ($p<0.05$) on germination parameters and chlorophyll content of cayenne pepper sprouts, while the application the aqueous extract of Ketapang leaves had no effect on all parameters ($p>0.05$), except vigor index. There were morphological changes in the radicle and root of cayenne sprouts exposed to them.

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