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Potential of the Mixture of Cocoa (*Theobroma cacao* L.) Pulp Water with Glyphosate in Controlling Weeds in Oil Palm Plantations

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ABSTRACT

Weeds are an important issue in oil palm cultivation, and weed control systems are an important factor in increasing oil palm growth and production. Mixing herbicides with natural ingredients will be an alternative technique in controlling a wide spectrum of weeds in oil palm plantations. This study aims to determine the best concentration of mixing cocoa pulp water with the herbicide glyphosate in weed control in oil palm plantations. This research used experimental method of randomized block factorial design pattern with 3 repetitions. Of the fermented (L1) and non-fermented cocoa pulp water with concentrations of 75 mL (K1), 150 mL (K2), and 300 mL (K3), each of which was added with 1 mL/L glyphosate (G1). The control was 2 mL glyphosate (G2), so that the present study had 8 treatments. Data were analyzed by analysis of variance (ANOVA), followed by Tukey's post-hoc test with significant level of 95% to know the real difference between treatments. The results show that the combination of unfermented cocoa pulp water with glyphosate has a significant effect on weed death in oil palm plantations. The combination treatment of 150 mL of cocoa pulp water without fermentation with 1 mL of glyphosate (K2G1) has the highest potential to kill weeds up to 87.94% (34.41% higher than the 2 mL glyphosate treatment) at the 4th week after application. Mixing unfermented cocoa pulp water with glyphosate (lower than recommended concentration) is effective in controlling weeds in oil palm plantations.

***Keywords*:** Cacao pulp water, Glyphosate, Weeds, Oil palm plantations.

Introduction

Oil palm is one of the plantation crops in Indonesia with great economic prospect. Palm oil is a mainstay export commodity in Indonesia, the domestic market share is quite large, and the export market is always open.[1] Oil palm plantations are faced with the presence of plant-disturbing organisms as a limiting factor. One of the plant pests that often interferes with oil palm cultivations is weeds.[2] Weeds are plants whose growth is not favored by farmers/plantations because it can increase pests and diseases in plants, reduce the nutrients needed by plants, as well as reduce crop yields. Losses caused by weeds exceed losses of all categories of agricultural pests.[3] Manually, farmers clear the weeds by hoeing or buckling, even by burning. However, the mechanical method still has limitations in weed control in oil palm plantations because it is influenced by several factors, such as labour shortages, global climate change, inadequate infrastructure, and increased input costs.[4] Furthermore, water infiltration and base saturation tend to be higher in mechanical weeding.[5] Manual weed control can also increase soil erosion, especially on plantation slopes.[6]

One way to control weeds in oil palm plantation is generally by chemical means using herbicides. The use of herbicides in small quantities can control weeds in a large area and in a very short time, the fastest of which is about 2 - 3 weeks.[7] The types of herbicides that are often used in oil palm plantations are glyphosate, paraquat, and glufosinate-ammonium.

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This herbicide treatment (glufosinate-ammonium, paraquat, glyphosate) in oil palm plantations is effective in weed control of the total weed population for 8 weeks after application.[8] However, the herbicide glyphosate is being used more frequently, largely because of its effectiveness, safety for non-target species and the environment, and the development of glyphosate-tolerant plantation crops.[9] Glyphosate has a broad control spectrum, it is applied post-growth, and is systemic.[10] Herbicides with active ingredients of isopropylamine glyphosate can suppress weed growth and do not cause phytotoxicity symptoms in oil palm plants.[11] Then a mixture of the herbicide glyphosate with methyl metsulfuron can suppress weed growth and is effective in controlling weeds in oil palm plantations.[12] Several studies have proven that the application of herbicide glyphosate is effective and economical in controlling broadleaf and grass weeds in oil palm plantations without having a negative impact on the growth of the surrounding oil palm plants and can increase growth and production.[2,13] The application of herbicides at the recommended level does not result in residual effects on the ecological components surrounding oil palm plantations (significantly harmless to the development of palm oil production or populations of fungal and bacterial microorganisms in the soil).[14] In addition to potential herbicides in oil palm plantations, glyphosate can also be used as a source of carbon by some bacteria, such as *Stenotrophomonas maltophilia* and *Providencia alcalifaciens*.[15]

However, inappropriate use of herbicides will have a negative impact on the growth and production of plants, as well as on the surrounding environment.[16] According to Putri and Guntoro (2017), the use of herbicides is an effective and efficient way to control weeds in oil palm plantations.[17] However, if it is excessive, it will be harmful to the environment, and it can even cause adverse impacts on agricultural areas and water sources through the process of washing and infiltration of water in addition to causing herbicide resistance to weeds such as goosegrass (*Eleusine indica* (L.) Gaertn.).[18] *Eleusine indica* grass populations evolved resistance to herbicidal treatment. Glyphosate displays high levels of resistance up to 12 and 144 fold.[19]

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Thus the herbicide can reduce production, and may even harm human health.[20] The administration of glyphosate, paraquat, atrazine, and 2,4-D amine in the soil exerts considerable changes in the growth and development of soil microorganisms. The toxic effects of some herbicides are felt immediately after application, while the herbicidal treatment such as paraquat has a direct effect on most microorganisms. The bacterial population may increase sharply and then decrease sharply. The pattern of change may vary as a result of differences in the exposure period, the concentration of the active ingredient in the formulation, the exposure time, and the environmental factors.[21]

Herbicide applications have become an integral part of agricultural productivity around the world because its benefits have been abundant for years. However, its toxic impact on non-target soil microorganisms that play a role in reducing organic matter, nitrogen recycling, nutrition and decomposition needs to be considered. The continuous use of chemical herbicides can result in the development of weed populations that is tolerant to herbicides.[21] A single control method will not provide adequate long-term weed management, often resulting in the development of resistance. The use of herbicide mixtures and herbicide rotation can reduce the risk of evolution of resistance in weeds.[22] A population of weeds that is initially susceptible to herbicides but contains a small proportion of resistant biotypes can gradually develop into a resistant population. Therefore, the herbicide eliminates susceptible species, and the proportion of resistant plants increases until the weed population can no longer be adequately controlled with the herbicides. Weed populations that are resistant to herbicides can be minimized by using a mixture of herbicides with various modes of action and integrated pest management (IPM).[23] Chemical control has been widely used as a weed management tool in oil palm plants. However, there is still the possibility of introducing some herbicides, especially pre-grown and pre-mixed herbicides, for effective weed control. Enhanced and supplementary tank mixes with appropriate herbicide combinations should be developed to delay the evolution of herbicide resistance, and at the same time to improve crop health, production, and quality.[4] According to Ekhator *et al*. (2018), there are abundant broad spectrum weed species in oil palm cropping systems; therefore, a mixture of herbicides will be needed in controlling these weeds. Glyphosate added with metsulfurone is effective and efficient in broad spectrum weed control in young palms compared to fluroxypr, glufosinate and triclopyr. This herbicide mixture is very efficacious and also maintains weed control for up to 12 weeks after treatment.[24]

The combined application of glyphosate and metsulfuron-methyl is effective in controlling both broadleaf and grasses in oil palm plantations.[7] Likewise, the herbicide mixture of glyphosate and indaziflam.[25] However, it is likely to be more effective when applied with low-dose herbicide mixed with bioherbicides from natural ingredients such as cocoa pulp water. Cocoa pulp water is the water contained in the cocoa pulp (the white part that covers the cocoa beans) often wasted during the harvest of cocoa fruit. Cocoa pulp contains glucose levels between 12-15% and it has a great potential to be used as bioethanol feedstock. The alcohol content contained in cocoa pulp fluid can be increased through fermentation by addition of *S. cereviseae* yeast up to 5.93%.[26] According to Acheampong *et al*. (2013), the application of fermented cocoa pulp liquid can control host plants of non-vascular epiphytes (significant lethal spifits) that harm cacao plants.[27] This indicates that there is a possibility that mixing cocoa pulp water with the herbicide glyphosate has the potential to control weeds in oil palm plantations without disturbing the surrounding ecosystem. In addition, until now there has been no research showing that cocoa pulp water has the potential to control weeds in oil palm plantations. Thus, it is very important to conduct this study with the aim of finding the best concentration of mixing cocoa pulp water with the herbicide glyphosate in weed control in oil palm plantations.

Materials and Methods

This research was conducted from November to December 2018, in an old area of oil palm plantations in PTP II Binjai, latitude $3^0 45' 35''$ N and longitude $98^0 23' 35''$ E. The materials used were: glyphosate (Roundup 360 SL), cocoa pulp water, and clean water. Tools used were handsprayer, measuring instruments, digital scales, straps, stationery, and pipette. Cocoa pulp water was obtained by collecting cocoa pods that have been peeled from the skin, then put in a plastic bag. The plastic bag was then hung, so that the cocoa pulp water will drip out of the plastic bag. The research design used was randomized block factorial design pattern. The first factor was cocoa pulp water consisting of 2 groups: group I was the unfermented cocoa pulp water, and group II was the natural fermented cocoa pulp water (L1). The second factor was the concentration of cocoa pulp water consisting of 3 levels, i.e.: 75 mL (K1), 150 mL (K2), and 300 mL (K3). The control was 2 mL glyphosate (G2) (concentration according to recommendation). Each level of cocoa pulp water was mixed with 1 mL glyphosate (G1), so that it resulted in 8 treatments (G2, G1, K1G1, K2G1, K3G1, L1K1G1, L1K2G1, L1K3G1) with 3 replications. Observations were done every week until week 4. The parameters observed were percentage mortality of weed vegetation.

Statistical analysis

The results of data were analyzed by analysis of variance (ANOVA) followed by Tukey's post-hoc test at 5% significance level to know the difference between treatments, using the statistical program software: SAS 9.1.3.

Results and Discussion

Potential of cocoa pulp water with glyphosate on weed growth

The types of weeds found in the oil palm plantation areas studied were *Achyranthes aspera* L, *Ageratum conyzoides, Alysicarpur vaginalis, Asytasia intrusa, Cyperus kylingia, Echinocloa colonum, Mimosa pudica, Paspalum conjugatum*. Cocoa pulp water mixture with 1 mL glyphosate had an effect in controlling weeds in oil palm plantations from week 1 to week 4. In the first week, the administration of 1 mL (G1) and 2 mL glyphosate (G2) was not significantly different from the application of a mixture of 150 mL of unfermented cocoa pulp water with 1 mL glyphosate (K2G1), which had the potential to kill weeds by 32.180%. The K2G1 treatment was significantly different from all treatments of a mixture of 1 mL glyphosate with unfermented cocoa pulp water (K1G1 and K3G1) and naturally fermented (L1K1G1 and L1K3G1), except for the treatment of L1K2G1, which potentially killed weeds by 15.44% after one week of application.

In the second week after application, application of 2 mL glyphosate (G2) was significantly different from all treatments. This treatment could reach the highest potential up to 82.603% to kill weeds. However, in the third week after the application, of all treatments observed, K2G1 treatment had the highest potential to kill weeds in oil palm plantations up to 86.22%, and increased to 87.94% at week 4. This treatment was significantly different from almost all treatments (G1, K3G1, L1K1G1, L1K2G1, and L1K3G1), but not significantly different from the treatment with K1G1, and with the treatment of 2 mL glyphosate (G2). This can be seen more clearly in Table 1. Thus, the application of a 75 mL mixture of unfermented cocoa pulp water with 1 mL of glyphosate (K1G1) at week 4 was significantly effective in controlling weeds in oil palm plantations, which could kill weeds at around 81.56%. This is even more clearly seen in Figure 1, that the potential of K1G1 treatment in killing weeds is almost close to the potential of giving K2G1 treatment in the 2nd to 4th week after application. However, when viewed from the high potential of lethal weeds, the application of a mixture of 150 mL of unfermented cocoa pulp water with 1 mL of glyphosate (K2G1) has the highest potential of killing weeds around 87.94% (7.82% higher than the application of K1G1 treatment and 34.41% higher than control). The potential of K2G1 treatment to kill weeds can be seen more clearly in Figure 2B. The potential of this treatment is even more obvious when compared to weed conditions at week 1 after application as shown in Figure 2A.

This shows that besides the concentration of glyphosate and cocoa pulp water, the duration of fermentation can also affect the potential of bioherbicides in controlling weeds in oil palm plantations. In this study, a mixture of 1 mL of glyphosate (below standard concentration) with 75-150 mL of unfermented cocoa pulp water has the potential to control weeds in oil palm plantations after 3-4 weeks of application.

Table 1: The potential of the mixture of cocoa pulp water with glyphosate in controlling weeds in oil palm plantations

Number	Treatment	Week to-			
		I	II	III	IV
1	G2 (control)	45.627 a	82.603 a	79.710 a	65.427 a
2	G1	32.620 a	54.840 b	45.563 b	35.570 b
3	K1G1	11.507 c	50.380 b	79.143 a	81.563 a
4	K2G1	32.180 ab	64.353 b	86.217 a	87.940 a
5	K3G1	3.603 c	17.323 d	24.883 c	25.373 b
6	L1K1G1	2.080 c	10.767 d	11.267 d	11.440 b
7	L1K2G1	15.443 bc	33.317 c	33.997 bc	37,287 b
8	L1K3G1	6.977 c	21.547 cd	22.150 cd	12.060 b

Note: The average followed by the same letters are not significantly different at the level of Tukey 5%.

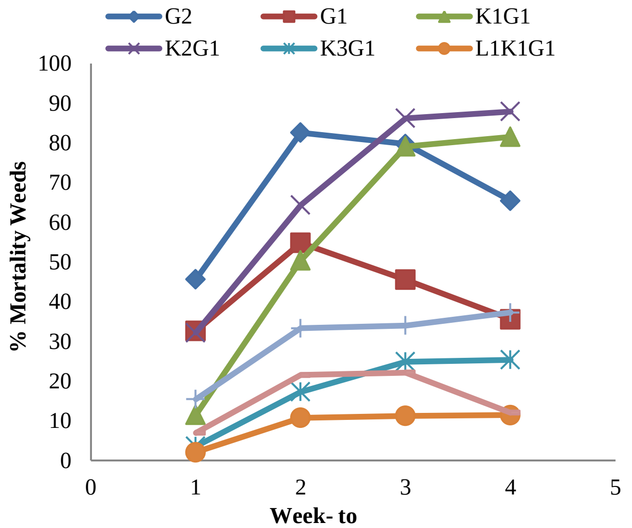

Figure 1: The potential of the mixture of cocoa pulp water with glyphosate in controlling weeds in oil palm plantations.

Natural compounds contained in 150 mL of unfermented cocoa pulp water are likely to add to the toxic effect of 1 mL of glyphosate to weeds, but reduce the toxic effects on plants and environment, so that the toxic effects of the mixture become maximally deadly for weeds, but safe for the plants and the environment. Thus, a mixture of 1 mL of glyphosate with 150 mL (optimum concentration) of unfermented cocoa pulp water, in addition to potentially deadly to weeds, can also increase selectivity between plants and weed species which are the main targets by bioherbicides. According to Budu *et al*. (2014), herbicide mixtures provide good control at much lower doses than doses used in a single application.[7] Varshney *et al*. (2012) also argue that in order to obtain better plant growth and production, better weed management techniques are needed, which require environmentally friendly products such as natural compounds produced by plants, which are good weed killers (bioherbicides).[28] Structural modification of natural compounds can often increase their activity in the target location as well as the physicochemical properties required for absorption, translocation, and adequate half-life of the environment.[29]

Then, weeds sprayed with mixed glyphosate and cocoa pulp water of different concentrations and fermentation time will stimulate the sensitivity of weed leaf cells. All primarily meristematic weed cells may be more sensitive to the concentration of K2G1 treatment after application. Therefore, this treatment is more maximal in weed control than all treatments. This treatment (K2G1) only takes 3-4 weeks of maximum potential to kill weeds in oil palm plantations. Although this treatment works more slowly than G2 treatment, but the chemical compound level is still lower (below standard), so that this treatment besides having the maximal potential to kill weeds is also safe for the growth and production of oil palm crops, as well as for the surrounding environment. According to Duke and Powles (2008), glyphosate is a broad spectrum herbicide and is highly effective, yet highly toxic and environmentally safe, non-selective herbicides that translocate mainly to metabolic sinks and turn off the meristematic tissue away from the application site, floem-mobile, and its action is quite slow in shutting down weeds, allowing herbicides to move around the plants to kill all meristems, making them effective for weeds control.[30,31] From the leaf surface, glyphosate molecules are absorbed into plant cells, which are simply translocated to growing plant meristems.[32]

Glyphosate is the only herbicide that targets the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme.[30] According to Pérez *et al*. (2011), although glyphosate may eventually interfere with various biochemical processes including protein synthesis, nucleic acid synthesis, photosynthesis and respiration, but the main mode of action of glyphosate is localized to the shikimic pathway of aromatic amino acid biosynthesis, the pathway that connects primary and secondary metabolisms.[32] The way it acts is competitive inhibition of 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) enzyme, chloroplast-localization enzyme in shikimate pathway. This narrows the possibility of the workplace into 3 enzymes, which are involved in converting shikimate into chorismate. Glyphosate is the only herbicide that inhibits the EPSPS enzyme in the shikimate pathway,[30,33] which synthesizes the aromatic amino acids: phenylalanine, tyrosine, and tryptophan, and many other compounds used in secondary metabolic pathway.[33] Therefore, in this study, it is possible that the EPSPS enzyme contained in weed plants is sensitive to the combination of 1 mL of glyphosate and 75 mL of cocoa pulp water without fermentation (K1G1), but is even more sensitive to the K2G1 treatment, so the work of the enzyme becomes obstructed. This causes the metabolic activity in weed plants to stop, so that the weeds lack primary and secondary metabolites, including aromatic amino acids. The result will be the death of weeds.

All treatments with a mixture of glyphosate and unfermented cocoa pulp water were more potent in killing weeds than the treatment with a mixture of glyphosate and naturally fermented cocoa pulp water. Unfermented cocoa pulp water (especially newly obtained from cocoa bean) contains very high gas, bad odour, and whitish yellow. While the natural fermented cocoa pulp water has a clear colour, the odour and gas pressure disappear. Natural fermentation can occur with the help of catabolic microbes, breaking down complex compounds into simple compounds. As in the natural fermented cocoa mucilaginous juice, various microbes are found: yeasts, fungi, bacteria of aerobic mesophilic acid, and lactate to accelerate the fermentation process.[34] This is because in the process of natural fermentation, those which work as decomposers are mostly aerobic microbes, so that the substrate oxidization becomes perfect (fermented sugar will produce carbon dioxide and water). However, fermented cocoa pulp fluid with the addition of *S. ceriviseae* yeast obtained higher ethanol levels.[26] Anvoh *et al*. (2010) also reported that fermentation conducted with

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controlled yeast gave higher ethanol yields compared to the natural fermentation.[34] Total sugar levels dropped dramatically during the fermentation process. Fermented cocoa pulp water, its pH is increased, reducing sugars, and total pulp density decreased.[35] According to Racine *et al*. (2019), the water sucrose content of fermented cocoa pulp can decrease from >40 mg/L to undetectable at 96 hours.[36] Takrama *et al*. (2015) also reported that cocoa pulp juice fermented with the addition of yeast (especially *Saccharomyces cerevisiae* and *Issatchenkia orientalis*) provided higher ethanol production than spontaneous (natural) fermentation. The ethanol content resulting from the fermentation process is also influenced by yeast type and fermentation time.[37] Thus, in this study, natural fermented cocoa pulp water probably contained lower alcohols, higher pH, and lower reducing sugars, making it less potentially to kill weeds in oil palm plantations when compared to unfermented cocoa pulp water mixed with glyphosate. The best treatment in this study may also be to reduce the damaging effects of glyphosate on glyphosate-resistant plants (GR). According to Johal and Huber (2009), the pattern of approach to the use of glyphosate herbicide will not only reduce the tendency of GR plant diseases, but will also benefit farmers and the environment.[38]

Figure 2: Weed Conditions after being sprayed with a Mixture of 150 mL Cocoa Pulp Water and 1 mL of Glyphosate (K2G1). A) Weed Conditions after 1 Week of Application, and B) Weed Conditions after 4 Weeks of Application.

Conclusion

The herbicide mixture of glyphosate and cocoa pulp water (fermented or unfermented) with various concentrations has the potential to control weeds in oil palm plantations. Administration of 75 mL concentration of unfermented cocoa pulp water mixed with 1 mL concentration of the herbicide glyphosate (K1G1) was more effective in controlling weeds in oil palm plantations, until it was able to kill around 81.56% of weeds after the 4th week of application. Application of a mixture of the herbicide glyphosate with cocoa pulp water (as a natural ingredient) can be more effective in controlling weeds in oil palm plantations, even at concentrations lower than recommended. Unfermented cocoa pulp water has more potential as a bioherbicide than the fermented one.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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