Sitinjak 2021

by Sitinjak 2021

Submission date: 12-Sep-2021 10:58AM (UTC+0700)

Submission ID: 1646203108

File name: Script_Ramarev.pdf (487.03K)

Word count: 4841

Character count: 25340

Potential Mixture of (*Theobroma cacao* L.) Cocoa 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. It is herbicides with natural ingredients will be an alternative technique in controlling a wide spectrum of weeds in oil palm plantations. This study determined the potential mixture of cocoa pulp water with glyphosate in controlling weed in oil palm plantations. This research used experimental method of randomized block design of factorial pattern with 3 repetitions. Fermented (L1) and non-fermented cocoa pulp water with concentrations of, 75 ml (K1), 150 ml (K2), and 300 ml (K3), each added 1ml/L glyphosate (G1). The control was 2 ml glyphosate (G2), so that the present study has 8 treatments. Data were analyzed by analysis of variance (ANOVA), then continued by using Tukey statistic test with significant level 95% to know the real difference between the treatments. The results showed that the combination of unfermented cocoa pulp water with glyphosate had 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. The combination administration of 75 ml of unfermented cocoa pulp water with 1 ml of glyphosate (K1G1) was more effective in controlling weeds in oil palm plantations at week 4 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, weed, oil palm plantation.

Introduction

Oil palm is one of the plantation crops in Indonesia which has a bright future. Palm oil is a mainstay export commodity in Indonesia, the domestic market share is quite large and the export market is always open¹. Oil palm plantations are faced with the presence of plant-disturbing organisms as a limiting factor. One of the plant pests that often interfere with oil palm cultivation is weeds.² Weeds are plants whose growth is not favored by farmers/plantations because it can increase pests and diseases in plants, can reduce the nutrients needed by plants, as well as to reduce crop yield. Losses caused by weeds exceeds the loss of all categories of agricultural pests. Under water stress conditions, weeds can reduce yields by more than 50% through moisture competition alone.³ Manually, the farmers are clearing 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 Then 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 areas is generally by chemical means by using herbicide compounds. The use of herbicides in small quantities can control weeds in a large area and in a very short time, the fastest about 2 - 3 weeks. The types of herbicides that are often used in oil palm plantations are glyphosate, paraquat, and glufosinate-ammonium. This herbicide treatment (glufosinate-ammonium, paraquat, glyphosate) in oil palm plantations, is effective in weed control of the total weed mixture 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, is applied postgrowth, and is systemic. 10 Herbicides with active ingredients 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 glyphosate herbicide is effective and economical in controlling broadleaf and grass weeds in oil palm plantation areas 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 potentially herbicides in oil palm plantations, glyphosate can also be used as a source of carbon by a mixture of 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. According to Putri and Guntoro (2017) that the use of herbicides is an effective and efficient way to control weeds in oil palm plantations. However, if it is excessive, it will be harmful to the environment, 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 herbicide treatment. Glyphosate displays high levels of resistance to 12 and 144 fold. ¹⁹ Thus the herbicide can reduce production, even harm to human health. ²⁰ The administration of glyphosate, paraquat, atrazine, and 2,4-D amine in the soil exert considerable change 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 that lives 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 need to be considered. The continuous use of chemical herbicides can result in the development of weed populations that are tolerant of 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. So the herbicide eliminates susceptible species, and the proportion of resistant plants increases until the weed population can no longer be adequately controlled with the herbicide. 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 improve crop health, production and quality. According to Ekhator *et al.* (2018) 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. Likewise, the herbicide mixture glyphosate and indaziflam. However, it is likely to be more effective when applied with low-dose herbicide mixing 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) is often wasted during the harvest of cocoa fruit. Cocoa pulp contains glucose levels between 12-15% and very 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%. According to Acheampong *et al.* (2013) the application of fermented cocoa pulp liquids can control host plants of non-vascular epiphytes (significant lethal spifits) that harm cacao plants. Thus, it is possible that a combination of glyphosate herbicide with cocoa pulp water has the potential to control weeds in oil palm plantations without disrupting the surrounding ecosystems.

Materials and Methods

This research was conducted from November to December 2018, in the area of old oil palm plantation in PTP II Binjai, latitude 3°45′35″ N and longitude 98°23′35″ E. The materials used are: Glifosat (Roundup 360 SL), cocoa pulp water, and clean water. Tools used are handsprayer, measuring instruments, digital scales, straps, stationery, pipette. The research design used was randomized block design of factorial pattern. The first factor is cocoa pulp water consisting of 2 groups: Group I is unfermented cocoa pulp water and group II is natural fermented cocoa pulp water (L1). The second factor is the concentration of cocoa pulp water consisting of 3 levels ie: 75 ml (K1), 150 ml (K2), and 300 ml (K3). The control is 2 ml glyphosate (G2) (concentration according to recommendation). Each level of cocoa pulp water was mixed with 1 ml glyphosate (G1), so that resulting in 8 treatments (G2, G1, K1G1, K2G1, K3G1, L1K1G1, L1K2G1, L1K3G1) with 3 replications. Observations are done every week until week 4. The parameters observed were percentage mortality of weed vegetation. The result data was analyzed by analysis of variance (ANOVA), and continued with Tukey test at 5% significant level to know the difference between treatment, using 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 plantation 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 has the potential to kill weeds of 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 15.44% after one week of application.

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

Number	Treatment	16	k to-		
	_	I	II	Ш	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	L1K3(7)	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%.

In the second week after the application, giving of 2 ml glyphosate (G2) was significantly different from all treatments. This treatment can 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 to reach 86.22%, and increased to 87.94% at week 4. This treatment was significantly different for almost all treatments (G1, K3G1, L1K1G1, L1K2G1, and L1K3G1), but not significantly different from the treatment of K1G1 even 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 can 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.

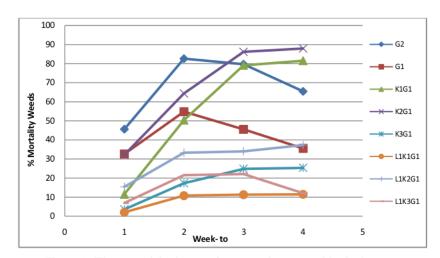


Figure 1: The potential mixture of cocoa pulp water with glyphosate in controlling weeds in oil palm plantations.

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 from 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 in **Figure 2A**.



Figure 2: Weed Conditions after Spraying 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.

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 (standard below 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. 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 the environment, so that the toxic effects of the mixture become maximally deadly for weeds, but safe for 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 maximum weeds, can also increase selectivity between plants and weed species which are the main targets by bioherbicide. According to Budu et al. (2014) herbicide mixtures provide good control at much lower doses than doses used in a single application. Then 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 (bioherbisida).²⁸ Structural modification of natural compounds can often increase its 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. So that 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 potential maximal 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 highly effective, yet highly toxic and environmentally safe, non-selective herbicides that translocate mainly to the 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 plant 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 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 way of action of glyphosate is localized to the shikimic pathway of aromatic amino acid biosynthesis, the pathway that connects primary and secondary metabolism. 32 The way it action 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 inhibits the EPSPS enzyme in the shikimate path, 30,33 which synthesizes the aromatic amino acids: phenylalanine, tyrosine, and tryptophan and many other compounds used in secondary metabolic pathways.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 become obstructed. This causes the metabolic activity in weed plants will stop, so that the weeds lack primary and secondary metabolites, including aromatic amino acids. The result will be the death of weeds.

In this study, also found that almost all of the treatment of glyphosate mixtures with unfermented cocoa pulp water has a higher potential than the treatment of glyphosate mixture with the naturally feremented cocoa pulp water in weed control in oil palm plantations. Unfermented cocoa pulp water (especially newly obtained from cocoa bean) contains very high gas, bad odor, and whitish yellow. While the natural fermented cocoa pulp water has a clear colour, the odour and gas pressure disappear (vissually seen). 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 Because in the process of natural fermentation, which work as decomposers is 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. cereviseae* yeast

obtained higher ethanol levels.²⁶ Anyoh et al. (2010) also reported that fermentation conducted with controlled yeast, gave higher ethanol yields compared with 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 glyphosateresistant plants (GR). According to Johal and Huber (2009) the pattern 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

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 fermented one.

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