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Investigating the Effect of Using Glyphosate on Microbial Soil in Sweet Corn Cultivation.

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A B S T R A C T

Glyphosate affects the activity of particular microbial soil. Depending on the soil type and concentration, Glyphosate will have different effects. The study determined the effect of Glyphosate on the microbial population and the effect of its different volumes on β-Glucosidase activity on Ultisol used in corn cultivation. The study used a completely randomized design (CRD), two factors with three replicates. The first factor was liming. The second element was Glyphosate at a dosage of 0, 5, 6, 7 L/ha. The study was conducted at the Department of Greenhouse Agriculture, Andalas University, from July to November 2022. The results showed that although there was no interaction and statistically significant between treatments for the total bacteria population. However, the treatment used lime and low volumes of Glyphosate gave the total bacteria population high density. β-Glucosidase, an enzyme produced from a specific type of bacteria in soil, decreased activity while combining lime treatment and Glyphosate, especially from the dosage at 6 L/ha, and had a statistically significant interaction between lime factor and herbicide after the second spray ($p<0.05$). In conclusion, Glyphosate and lime can alter and reduce microbial soil activity and number, particularly at high 6 and 7 L/ha volumes.

1. INTRODUCTION

1.1. Research Background

The increasing use of herbicides can lead to secondary effects, the potential effects of these chemicals on the biological processes of soil and non-target organisms. The process of biological and biochemistry in the soil is of the most crucial importance to the function of the ecosystem. Soil bacteria promote organic metabolism, release nutrients and decompose xenobamel [1]. Bacteria perform many essential ecosystem functions in soil, including improving soil structure and gathering nutrients, recycling nutrients, and recycling water [2]. Some biological parameters have been used to evaluate the quality and health of the soil when being affected by agricultural chemicals. Among them, the activity of microorganisms is an effective index compared to other parameters because they can immediately react to environmental changes [3].

Glyphosate is a widely used herbicide in the world. The annual evaluation report in Indonesia includes types containing the active ingredient Glyphosate, accounting for 73% of the total herbicides circulating. Glyphosate can control weeds in agriculture, including corn cultivation [4]. Glyphosate is a polarized compound known for its ability to adsorb Fe and Al oxides and clay minerals [5]. Enzyme activity is a sensitive parameter, often used to observe the impact of pollutants on soil microorganisms [6]. The number of microorganisms is one of the fundamental characteristics of ecological studies and may be related to parameters describing the activity of microorganisms and soil health [7]. β-glucosidase is a popular and dominant enzyme produced from a specific type of bacteria in soil . It is named after the link that it hydrolyses [8]. This enzyme plays a vital role in the soil because it participates in the catalyst of the hydrolysis and biodegradable process of many types of β-Glucosidase in the biological bodies in the ecosystem [9]. Its ultimate product is glucose, an essential source of C energy for the life of bacteria in the soil [6]. β-glucosidase is susceptible to change of pH value and soil management. [10] have reported that β-glucosidase ís sensitivity to pH changes. This feature can be used as an excellent biochemical index to measure ecological changes due to soil acidification related to enzyme activity. Moreover, under the influence of 2,4-D and Glyphosate, there is little or no effect on the activity of β-glucosidase in the soybean root area [11]. However, plants' soil and root areas different planting may react differently to other environmental conditions.

Therefore, this study was focused on Ultisol - the dominant acidic soil in Indonesia.

1.2. Research Objective

This study aimed to investigate the effects of Glyphosate on the activity of β-glucosidase and the total bacteria population in Ultisol for sweet corn cultivation.

2. MATERIALS AND METHODS

2.1. Preparation of soil

The study was conducted for four months in a research greenhouse at the Andalas University. Ultisol samples were taken 20 cm deep at the experimental site of Andalas University. Experimental polybags were sown with sweet corn and set up in a completely randomized design, two factors with three repetitions. A repetition contained four polybags (0.3 m × 0.7 m) per treatment for three. The first factor was: Glyphosate contains 486 g/L glyphosate-isopropyl ammonium. The second factor was liming, with half of the collected soil incubated with lime in a polybag for 14 days before starting the experiment. And the application rate for lime was 6.23 tons/ha dolomite $Mg(CaCO_3)_2$. The application rate for the Glyphosate treatment was 0, 5, 6, and 7 L/ha. Herbicide treatments were applied at two points: 2 weeks before planting and each time at 1-week intervals to ensure all weed seeds were killed. Using a hand-held sprayer, the herbicide was sprayed twice, with half the volume for each spraying time.

Bulk soil samples were collected before sowing, three days after each herbicide application, and three days after each fertilizer application (at 7, 15, and 40 days after sowing). Four replicate soil cores were collected from each treatment plot into 5-10 cm depths near the root and mixed to form one composite soil sample per polybag. Soil samples were sieved (≤ 2 mm) by a nylon sieve to remove stones, large pieces of plant material, and soil animals. Portions of the samples were kept moist in the dark at 4^0C to analyze.

2.2. Analytical methods

Enzyme activity determination: The activity of β-glucosidase was assessed using p-nitrophenol (pNP)-linked substrates based on the colorimetric determination. Briefly, pNP-based enzyme activities were calculated and exhibited under the µmoles of substrate consumed per g dry weight per hour (µmol $pNP.g^{-1}.h^{-1}$). Analysis of β-glucosidase used acetate buffer pH 5.0. Before the samples were incubation, a substrate was added to each sample. Then, the sample tube was incubated at 25^0C, in the dark room for two hours. After incubation, 1N NaOH was added to stop the reaction. Light absorbance was measured using a spectrophotometer at 410 nm. The enzyme activity was calculated by equation:

Calculation : $[(A-B)x\ 5\ x\ 1{,}7\ x\ C]/\ (1\ x\ 1{,}5)$
$= \mu g\ pNP.\ g^{-1}\ .h^{-1}$ (1)

A : Sample concentration
B : Control concentration
C : Dilution factor
D : Water content of soil

Total bacterial population determination: Add 1 gram of soil to a 9 mL sterile distilled water in a test tube and place on a vortex (100 rpm) at room temperature for 1 min. The solution after mixing well was dilluted eight times (10^{-8}, The recommended dilution is 50 to 300 colonies detected per petri dish. Preparation of culture medium: Put 15ml of nutrient agar medium in each petri dish. Inoculate the solution into the medium: Pipette 0.1 ml at different dilutions (10^{-7}, 10^{-8}) and incubated for 48h at 37^0C. After two days, count the number of growing colonies on the medium at different dilutions. Total bacterial population was calculated by equation:

Calculation : CFU/g = (A x DF)/W (2)
A : average CFU count at best dilution
DF : dilution
W : weight of 1 g of soil analyzed

2.3. Data processing

Analysis of variance (ANOVA) was used to test the differences in β-glucosidase activity amd Total bacteria. Significant differences between the treatments were estimated by using the Tukey test at $P< 0.05$.

3. RESULT AND DISCUSSION

3.1. Variation of pH, total bacteria population, and β-glucosidase

Soil pH is considered a key variable in soil because it controls many chemical processes. It specifically affects the availability of phytonutrients by controlling the chemical forms of the nutrient [12]. Adding dolomite to the soil changed the pH from 3.54 to 4.75 after 14 days (Table 1, [13]). Soil pH analysis on the treatments supplemented with lime at the observation time was more significant than those without lime addition. It showed that the increase in pH was due to liming and adding organic matter. Lime is a widely used material to improve soil fertility. The lime application could directly provide many cations essential for crop production as part of the components present in the lime feedstock, such as Ca^{2+} and Mg^{2+} [14]. Furthermore, liming could also affect both metabolism and nutrient uptake by plants through its indirect effects on soil microbial activity. Sudden changes in fundamental ecological factors such as temperature, aeration, structure, and pH is able to affect the activity of enzymes in the soil [15].

Table 1: Characteristics of soil after incubation with lime

	Before incubation	After incubation
pH KCl (1:2)	3.54	4.75
Total bacteria (CFU/g)	8.36	8.25
β-glucosidase (µmol pNP. $g^{-1}.h^{-1}$)	0.43	0.29

Specifically, in this study, when the soil pH was increased, the total bacteria population and the activity of β-glucosidase decreased (Table 1). Indeed, as previously reported by [10] β-glucosidase is very sensitive to changes in pH. The Total bacteria population and the activities of this enzyme were observed at five different time points of sweet corn plant growth stages before and after sowing.

3.2. Total bacteria population

The statistical analysis results in Figure 1 showed that the interaction between lime and herbicide use was insignificant ($p>0.05$) in the total bacteria population. However, individual

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factors produced statistical differences at some stages, such as after the first, second herbicide, and first fertilizer applications ($p<0.05$). After the first and second sprays, the total bacteria population decreased gradually with the increase of glyphosate concentration compared to the control treatment (Figure 1a, 1b). The total bacteria population declined during the second herbicide application; the treated treatments were consistently lower than the control treatments. At the same time, the total bacteria population was also significantly different between the soils with lime added at 9.87 log(CFU/g) and no lime added at 9.42 log(CFU/g) ($p<0.05$).

By the time of the second Glyphosate and the first fertilizer application, the bacterial population showed signs of increasing again. Although the total bacteria population increased, in treatment 7 L/ha without lime at 9.37 log(CFU/g), the total bacteria population was still the lowest compared with all other treatments (Figure 1b, 1c). At the time of soil collection after the second and third fertilization, the total bacteria population in the soil continuously decreased, and there was no statistical difference. The average Total bacterial population at these two-time points was 9.62 log(CFU/g) and 9.03 log(CFU/g), respectively (Figure 1d, 1e).

The other reported that Roundup herbicides negatively affect fungal growth in vitro and reduce beneficial bacteria colonization in roots [16]. Most studies recommended the concentration of this herbicide at 50 mg/kg. Roundup causes negligible effects on microbial community structure objects. Similar to the study using the herbicides 2,4-DEE, Butachlor, Pretilachlor, and Pyrazosulfuron ethyl at different concentrations for their impacts on all heterozygous bacteria, fungi, and actinomycetes in the laboratory population reduction of all bacteria [17]. In this research, this effect was more substantial with increasing herbicide concentrations. Moreover, the bacterial population was restored within 30 days after the population treatment was not significantly different from the control treatments.

In this research, lime treatment gave sound effects compared to the treatment without lime. This result was the same as [18] reported that treating forest soil with lime and ash resulted in a pH change from about 4 to 7, increasing bacterial growth fivefold. Another study that included 19 different soils from various soil-use areas, the pH values ranging from 4 to 8, showed increased bacterial growth with high pH, more than quadrupled between pH 4 and 8 [18]. The decline in microbial growth when soil pH declines might be the independent physiological limitations of the pH of the individual decomposition groups; a low concentration of H^+ ions restricts the growth of fungi, and a high concentration of H^+ ions determines the growth of bacteria [16]. One possible mechanism for the negative correlation between bacterial growth and fungal growth along the Horsfield acid band, consistent with previous findings, was that low pH is physiologically damaging to bacteria, reducing bacterial competition and thus favoring fungal growth [19].

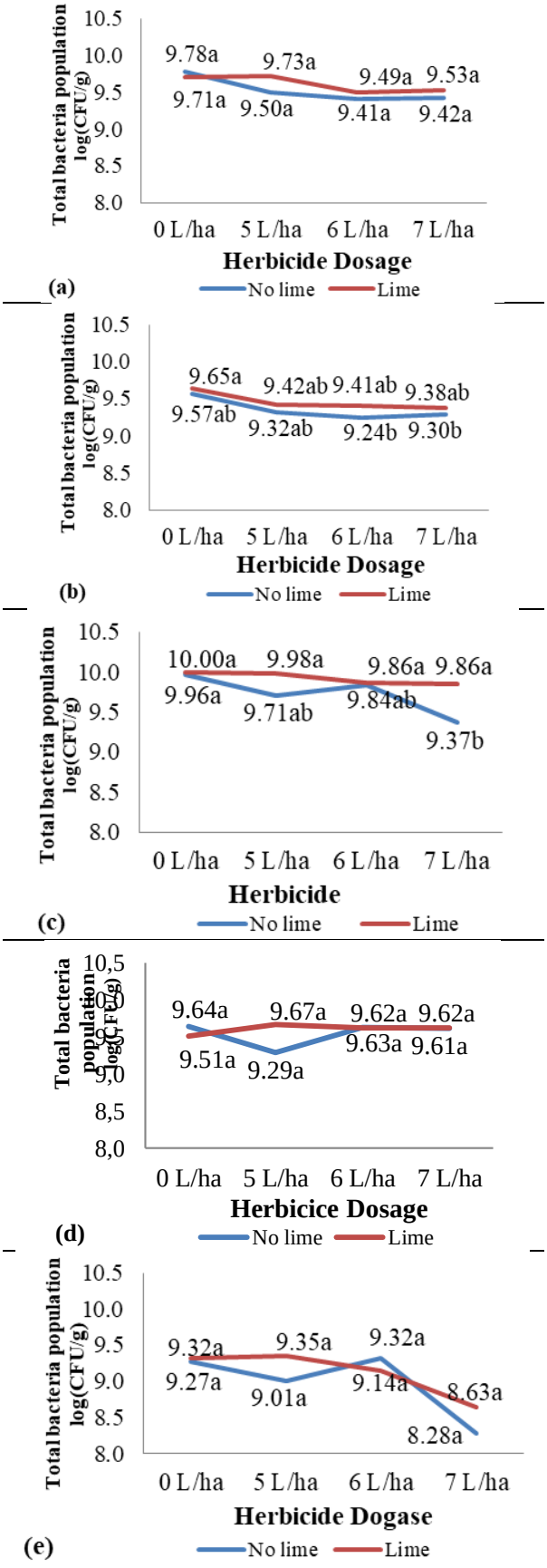

Fig. 1. Total bacteria population content in soil through treatment stages. The means followed by a similar letter are not significantly different ($P< 0.05$). a: After the first spray, b: After the second spray, c: After the first fertilizer, d: After the second fertilizer, e: After the third fertilizer.

In this study, the trends observed in bacterial populations were inversely proportional to herbicide volume and low pH. The bacterial population was significantly reduced in the high-volume Glyphosate sprayed treatments. There was a significant difference in the short time ($p<0.05$) only after the first and second sprays of Glyphosate. Different from the report of [20] reported a temporary increase in fungal shoots and an effect on culturable bacteria after adding Glyphosate (50 mg/kg). Culturing rapeseed with herbicides significantly increased the growth of the analyzed groups of bacteria in the soil [21]. However, most of these reports used the lower volumes of Glyphosate than this study. One of the reasons for the increase in bacteria is Glyphosate's ability to survive as an enhanced nutrient source. Glyphosate provided nutrients for bacterial growth, as evidenced by a significant increase in bacterial numbers but at low concentrations. In addition, in another study, Glyphosate treatment (500 mg/L) increased the bacterial population in the soil, and continuous application of Glyphosate over a long period adapted the bacteria to the toxic effects Glyphosate [3].

Moreover, they can use it as a source of nutrition. This conclusion is similar to [22], who found that microorganisms can make better use of Glyphosate after repeated application of Glyphosate. Furthermore, the effect of Glyphosate on bacterial populations is dose-dependent and highly temporal, and it can use a source of nutrients created by the decomposition of Glyphosate [3].

3.3. β-glucosidase activity

Based on the statistical analysis results in Figure 2, it could only be seen that the β-glucosidase activity three days after the second application of Glyphosate had a statistically significant interaction between Glyphosate and lime ($p<0.05$). Significant statistical test differences existed between increasing dose of herbicide under lime conditions; the activity of this enzyme is reduced. After the first herbicide spray, the results in Figure 2a showed no difference between treatments ($p<0.05$); however, the lowest herbicide of 5 L/ha gave the highest β-glucosidase results compared to the volume of 0 and 6 L/ha three days after spraying. Compared with the control treatment, the 5 L/ha treatment had the highest enzyme activity among the four treatments.

After the second herbicide spray, Figure 2b showed that the high-dose herbicide treatment had the lower enzyme activity than the untreated and low-dose herbicide no lime. The highest was still in the treatment without using herbicides and lime at 1.04 µmol $pNP.g^{-1}.h^{-1}$; the lowest used was 7 L/ha and lime at 0.49 µmol $pNP.g^{-1}.h^{-1}$. This result suggested that Glyphosate stimulated β-glucosidase activity, but the β-glucosidase concentration decreased markedly with increasing Glyphosate concentration under lime (Figure 7b).

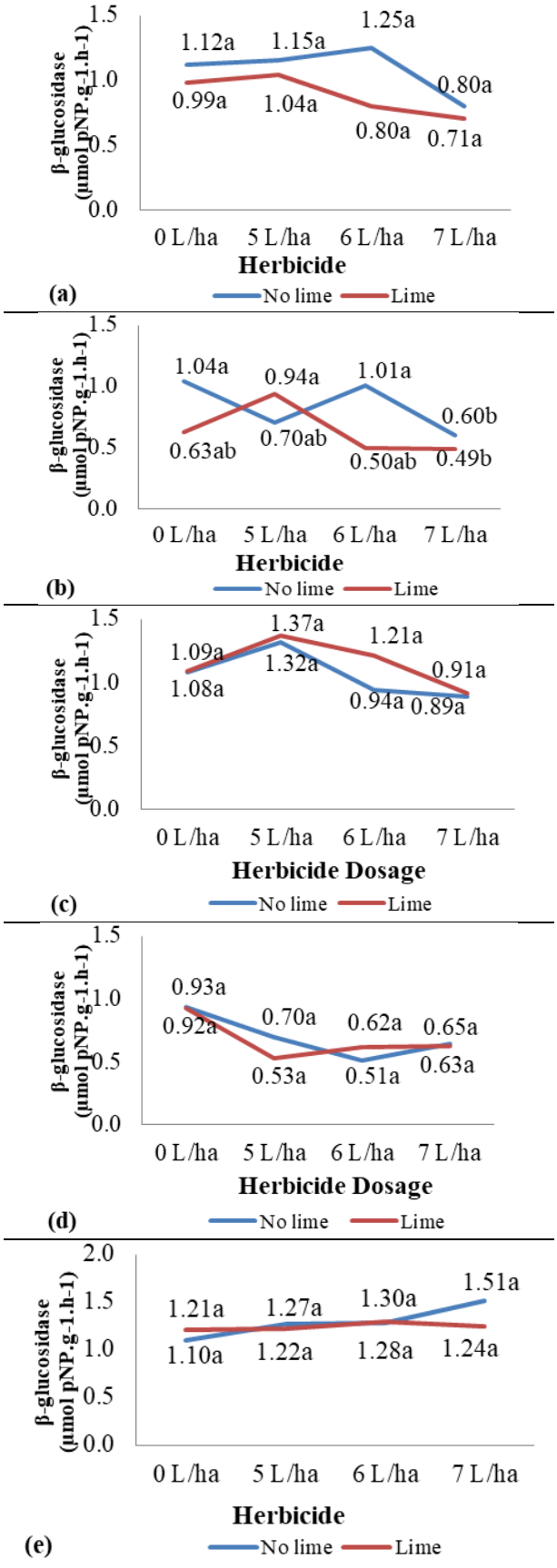

Fig. 2. β-glucosidase content in soil through treatment stages. The means followed by a similar letter are not significantly different ($P< 0.05$). a: After the first spray, b: After the second spray, c: After the first fertilizer, d: After the second fertilizer, e: After the third fertilizer.

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At the first, second, and third fertilizer applications, β-glucosidase activity tended to increase, then decrease and increase again, respectively, but there was no difference between treatments (Figure 2c, 2d, 2e; $p>0.05$). Specifically, β-glucosidase was less active in 7 L/ha than in 0, 5, and 6 L/ha (Figure 2c).

The β-glucosidase activity in the lime treatment was consistently lower than that in the non-lime treatment at all time points of monitoring. The action of β-glucosidase decreased as the soil pH increased from 4.5 to 8.5. The sensitivity of β-glucosidase to pH changes could be a reliable biochemical indicator to assess environmental changes caused by soil acidification [23]. Over time it was observed that, generally, β-glucosidase was not significantly affected by Glyphosate. The same result of the study using Glyphosate, Glufosinate, Paraquat, and Paraquat-diquat on the function and diversity of soil microorganisms, none of the herbicides significantly affected the abundance, uniformity, and composition of bacterial and archaeal communities [24]. From a functional perspective, the herbicide did not significantly affect the hydrolytic activity of fluorescein diacetate and β-glucosidase at recommended doses. Microbial activity is an important factor in the behavior of Glyphosate in soil. β-glucosidase activity in the soil increased in untreated or low-concentration of Glyphosate application and without lime treatment. This phenomenon can be explained due to an increase in the microbial population with the ability to utilize Glyphosate as a carbon or other source of nutrients. Previous studies reported such effects on several soil enzymes [25]. However, higher concentrations of Glyphosate inhibited β-glucosidase activity. And there have been studies showing that using Glyphosate in low dosages can stimulate the activity of the catalase enzyme, in contrast to the high doses that will inhibit this enzyme [21].

4. CONCLUSION

This research confirmed that Glyphosate application and pH of soil may alter with the decrease in soil microbial activity and total bacteria population. Glyphosate herbicide at high doses of 6, 7 L/ha and liming strongly reduced the activity of β-Glucosidase. The activity of the above biological indicators decreased three days after herbicide spraying and gradually stabilized between treatments about 60 days after the first spraying. According to these effects, the recommendation for the dosage of herbicide usage was lower than 5L/ha.

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