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SYNERGY: COCOA MUCILAGE WITH ACETIC ACID IN THE CONTROL OF MOSS (RIGODIUM IMPLEXUM) ON CRIOLLO COCOA PLANTATIONS

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Abstract: In agronomy, the use of organic herbicides allows the farmer to avoid all kinds of risks of environmental contamination, in addition to the reuse of a raw material that is not used economically, therefore, this research identifies new alternatives to eliminate mosses that are attached to stems and branches of criollo cocoa.

It is proposed to take advantage of the Creole cocoa saliva that through a fermentation process that will act as a dispersant to control moss (Rigodium implexum), with the use of this organic solution an improvement will be made in the management of non-synthetic herbicides and avoid a environmental risk. The study area has a territorial approximation of 200 hectares of Creole cocoa crops, in addition, samples of Creole cocoa mucilage were obtained for the preparation of organic solutions and later to be applied within the study area.

The experimental part consisted of 5 treatments with 3 repetitions, each treatment has concentrations in percentages of cocoa mucilage, acetic acid, distilled water T1(15:10:75), T2(40:10:50), T3(65:10 :25), T4(90:10:0), T5(100:0:0), considering the identification of the plants with more mosses, this process was experimented on 15 criollo cocoa plants in branches identified by a certain area to obtain the percentage of moss removed by the area of moss occupied.

An ANOVA analysis was carried out in the SPSS statistical program, giving treatment 4 as effective, accepting the alternative hypothesis, however, due to its high concentration of organic herbicide mucilage, it is not considered optimal for inhibiting moss, because it destroys cocoa flowers. the low cocoa production becoming a disadvantage, which concluded that treatment 3 is more optimal for the control of moss (rigodium implexum), allowing to mitigate the use of this organic residue that is available to farmers.

Keywords: contamination, cocoa mucilage, fermentation, concentrations, organic herbicide

INTRODUCTION

Cocoa cultivation is substantial in several countries, such as the Ivory Coast, Ghana and Indonesia, which are the main producers worldwide; It is also important to mention that, "Ecuador is a country recognized worldwide for its production of fine aroma cocoa" (Santacruz & Medrano, 2021).

"In Ecuador, various types of cocoa are produced with different characteristics according to the location of the crops, where the climate, altitude, type of soil intervene, among other factors. Among the most cultivated and most renowned types of cocoa we have CCN51 cocoa and fine aroma cocoa" (Arteaga Estrella, 2013). "The production of the Ecuadorian cocoa sector represents 70% of world production, strengthening the economies of developing countries." (Colazo & Garay, 2015).

On the other hand, according to the literature, the misuse of chemical products in agriculture has affected soil fertility and people's health. (Coloma Coloma, Alulema Cuesta, Spain Escobar, & Gualliche Serdan, 2017, p. 15) As well as the generation of by-products from cocoa without adequate treatment can cause diseases and damage when discarded in crops.

"Cocoa mucilage has the ability to break down into liquid substances, this allows it to be used as a natural herbicide, since it contains polyphenols, flavonoids and alkaloids with a high effect on bacteria, fungi and weeds." (Kalvatchev, Garzaro, & Guerra Cedezo, 1998). However, due to the lack of knowledge of its properties, it has been discarded without any control, affecting crops and the environment.

Finally, the analysis of cocoa cultivation highlights the importance and challenges

associated with its production, the generation of by-products without proper treatment, and the misuse of chemical products in agriculture. Highlighting its effectiveness to be used as a natural herbicide. (Mora Fallas, Goëau, Joly, Bonnet, & Mata Montero, 2020)

METHODOLOGY

STUDY POPULATION AND SAMPLE

The study area has a territorial approximation of 200 hectares of Criollo cocoa crops, located in a sector of the Guayas province.

CHARACTERIZATION OF THE COCOA EXUDATE

The characterization of the cocoa mucilage will be developed in the laboratories of ``universidad tecnica de Machala`` and in other analysis laboratories outside the institution, among the most representative analytical studies in the research the following are detailed.

PH DETERMINATION

To determine the pH of a solution, the potentiometer method is used, it consists of placing the sample, in this case of cocoa mucilage, in a 250 ml beaker, and then introducing the digital pH-meter electrode to read the pH. (Tuárez García, Erazo Solórzano, Macías Salazar, & Torres Navarrete, 2021, pp. 5-11)

DETERMINATION OF TOTAL ACIDITY

The INEN 013 standard establishes the method for determining the titratable acidity by titrating a diluted sample of cocoa mucilage, for which the following reagents are used: 0.1N sodium hydroxide (NaOH), phenolphthalein as indicator, and distilled water. (Pelaez & Oswaldo, 2016)

Tare the solution with the help of a 250 ml Erlenmeyer flask, an aliquot of 50 ml and add 3 drops of 0.1% phenolphthalein indicator in ethanol, add CO2-free distilled water, proceed to homogenize the solution and titrate with standardized solution of NaOH with a concentration of 0.1 N until the indicator turns pale pink.

TOTAL NITROGEN

The quantification of total nitrogen was carried out by virtue of the Micro Kjeldahl method, the information was provided according to the process carried out in the NEMALAB laboratory.

Total nitrogen is made up of two chemical compounds such as ammoniacal nitrogen and organic nitrogen, and it can also take another form such as nitrates, nitrites or ammonium. The determination is based on the kjeldhal method, which is an old and effective method developed by the Danish chemist Johan Kjeldahl in 1883, this method is characterized by being the most used to evaluate the total nitrogen content in a variety of samples. (Espinosa Lloréns, León Hernández, & Rodriguez Petit, 2013)

TOTAL PHOSPHORUS

Total phosphorus determination was carried out using the moisture digestion method (UV), the information provided on the analysis results was provided by the NEMALAB laboratory. “In the determination of total phosphorus, two important steps are included in the analysis methods, such as the conversion of phosphorus to other chemical forms in diluted orthophosphates, acid hydrolysis and oxidative digestion” (Belizario Quispe, Capacoila Coila, Huaquisto Ramos, Cornejo Olarte, & Chui Betancur, 2019)

TOTAL POTASSIUM

The determination of total potassium was carried out by means of the Humidity digestion method (Atomic Absorption), the information of the potassium analysis was provided by the NEMALAB laboratory.

The determination of potassium is carried out using the atomic emission photometry method, where a flame photometer is used in the experimentation. In turn, it has a diversity of jobs such as food and groundwater. (Perea, Ramírez, & Villamizar, 2011, pp. 35-42)

PROCESS FOR OBTAINING ORGANIC HERBICIDE

In figure 1, you can see the extraction process of the cocoa mucilage and the preparation of the organic herbicide, to which acetic acid and distilled water will be added for the preparation of the different treatments that will be applied to the cocoa plants.

PROCESS DESCRIPTION

Reception of raw material: cocoa is obtained directly from the study farm, using the necessary tools, we proceeded to harvest around two bags of fine aroma cocoa to carry out a fermentation process of the mucilage obtained and after 13 days 24 were harvested. ears more to carry out a treatment with fresh mucilage rich in sugars.

Selection of cocoa: select from the cocoa pods that are in suitable conditions for the extraction of cocoa mucilage. Any ear that was in deplorable condition was removed from the other ears that are of good quality.

Cob weighing: weighing all the fruit in order to calculate the total net weight, the weight of the shell and the pulp of the mucilage is carried out using an artisanal scale to then apply a material balance of all the cocoa pods used.

Cocoa pulping: make cuts in the cocoa testa, both at one end and in the middle of the fruit using a previously washed small machete and proceed to devein the cocoa seeds in a plastic container (bucket) with the vein of the cocoa to carry out the fermentation of the mucilage.

Filtering and pressing: filter with a clean, previously washed fine cloth to prevent the mucilage from coming out with impurities in the liquid, a bucket is needed to store the mucilage that was obtained through filtering and, in turn, carry out a small pressing with latex gloves for long periods of time to get more mucilage exudate.

Drained and mucilage extract: drain the pulp and leave it to settle from one day to the next until all the mucilage possible is obtained during the draining time.

Fermented: ferment the cocoa mucilage for a prolonged period of 13 days at room temperature. This process involves fermentative microorganisms that carry out the fermentation process for a set period of 13 to 15 days. In this process, the % acidity during the fermentative days was 2.4333 and a Ph of 3.85.

Storage: the mucilage is stored in a clean place without the presence of ants due to the large amount of sugars it contains. The container to be used for the storage of the organic substance obtained is a clean bucket with a lid so that a fermentation process can take place anaerobically.

Organic herbicide: it is obtained through the maceration of cocoa mucilage for a set time of days at room temperature with a % acidity of 2.4333 and a pH of 3.85.

ORGANIC HERBICIDE DOSAGES

5 treatments were developed, these treatments as an experimental part in the application of organic solutions (cocoa mucilage and acetic acid) will be carried out in different dosages as indicated in Table 1.

The elaboration of the following organic solutions was carried out in 5 compositions in

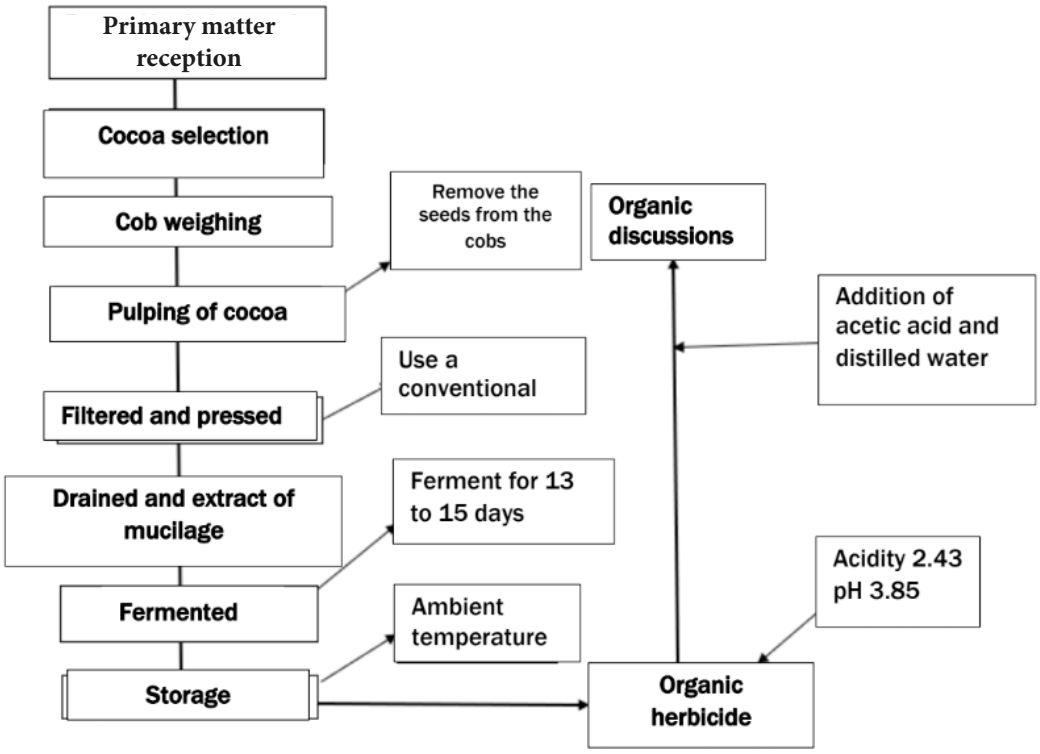

Figure 1. Process diagram for the production of organic herbicide.

Source: : Own elaboration.

Organic solutions	
Treatments	**Solutions**
T1: 15% cocoa mucilage - 10% acetic acid - 75% distilled water.	**240ml: 10ml:7 50ml**
T2: 40% cocoa mucilage - 10% acid - 50% distilled water.	**490ml: 10mI: 400ml**
T3: 65% cocoa mucilage - 10% acid - 25% distilled water.	**740ml: 10ml: 250ml**
T4: 90% cocoa mucilage - 10% acetic acid - 0% distilled water.	**900ml: 10ml: Omi**
T5: 100% control (fresh cocoa mucilage)	**IOOOml**

Table 1. Preparation of organic solutions

Source: Own elaboration

Formula parameters	
Aliquot	50
M.M. acetic acid	60 g/mol
concentration of NaOH	0,1 M

Table 2. Acidity parameters.

Source: Own elaboration

relation to 1L with the solutions of mucilage, acetic acid and distilled water, in which, it was carried out in percentage portions of each solution, to obtain the mixture of an organic herbicide.

To determine the % acidity of the cocoa slime, it was carried out by titration volumetric analysis.

The parameters of the formula detailed in the following Table represent the procedure to calculate the percentage of acidity of the cocoa mucilage, for which a 50ml aliquot was required, the milliequivalent of acetic acid and the amount of NaOH used to reach the point neutralization of this solution.

EXPERIMENTAL DESIGN

The experimentation process that will be evaluated will be 15 cocoa plants, which have 3 experimental units comprised of 5 treatments and 3 repetitions. The proposed experimental design will help to evaluate the behaviors of one or more variables in the experimentation, therefore, an ANOVA analysis will be carried out to identify the best applied treatment.

RESULTS

DETERMINATION OF ACIDITY AND PH

Treatment T5 presented the highest acidity, requiring an amount of 83.8 ml of NaOH during titration using the volumetric titration method. On the other hand, treatment T1 used the least amount of reagent, with a consumption of 25 ml of NaOH.

> Titratable acidity is characterized by the interaction between a base and an acid substance with the support of an indicator. This method of analysis is unique and is based on the addition of a determined volume of the substance in question, followed by the determination of the required volume of a base solution (NaOH) to reach the point of neutralization, normally indicated by the use of phenolphthalein. (Fabro, and others, 2006, pp. 859-861)

In addition, a pH-meter was used to determine the pH of each solution. Treatment T5 registered the most acid pH, with a value of 3.52, while treatment T2 showed the lowest pH, indicating a less acid solution, with a value of 4.06.

BOX PLOT OF MOSS REMOVED

The results of the elimination of moss in the cocoa branches show that the treatments T3, T4 and T5 were highly effective. These treatments involved the application of organic solutions made from cocoa mucilage and acetic acid, together with the technique of coating using plastic covers on the treated area. It is noteworthy that treatments T4 and T5 had a significant impact on moss removal, with percentages of dead moss of 49.11% and 36.25%, respectively. However, it is important to take into account that these treatments also affected the cocoa flower present on the branches covered with Rigodium implexum moss, which has an indirect impact on the production of cocoa fruits on the plant "generally this product is considered as an organic residue not used in the agricultural sector "(Vallejo Torres, et al., 2016)

> They state that the waste generated in the cocoa sector, such as cocoa mucilage, is used as a friendly alternative to replace synthetic chemicals in agriculture, being applied as herbicides, pesticides and fungicides, promoting the use of the mucilage generated by the cocoa fruit. (Moreno, Morán, Quijije, & Ochoa, 2021)

Tosif, affirms that the mucilages of seeds and fruits provide an excellent activity against Gram-positive pathogens and Gram-negative bacteria, the mechanism of action carried out by the mucilage is when it comes into contact with the cell membranes and infiltrates inside the cell, causing damage. in DNA, protein

Treatment	Experimental unit	Repetitions	Total of plants
T1	**3**	**3**	
T2	**3**	**3**	
T3	**3**	**3**	
T4	**3**	**3**	
T5	**3**	**3**	
TOTAL	15	15	15

Table 3. Experimental treatments

Source: Own elaboration

Treatments	Expenditure(ml NaOH Consumed)	%Acidity	Ph
T1	**25,00**	**0,8333**	**3,98**
T2	**36,50**	**1,2167**	**4,06**
T3	**55,30**	**1,8433**	**4,00**
T4	**73,00**	**2,4333**	**3,85**
T5	**83,80**	**2,7933**	**3,52**

Table 4. Determination of acidity and Ph of the applied treatments

Source: Own elaboration.

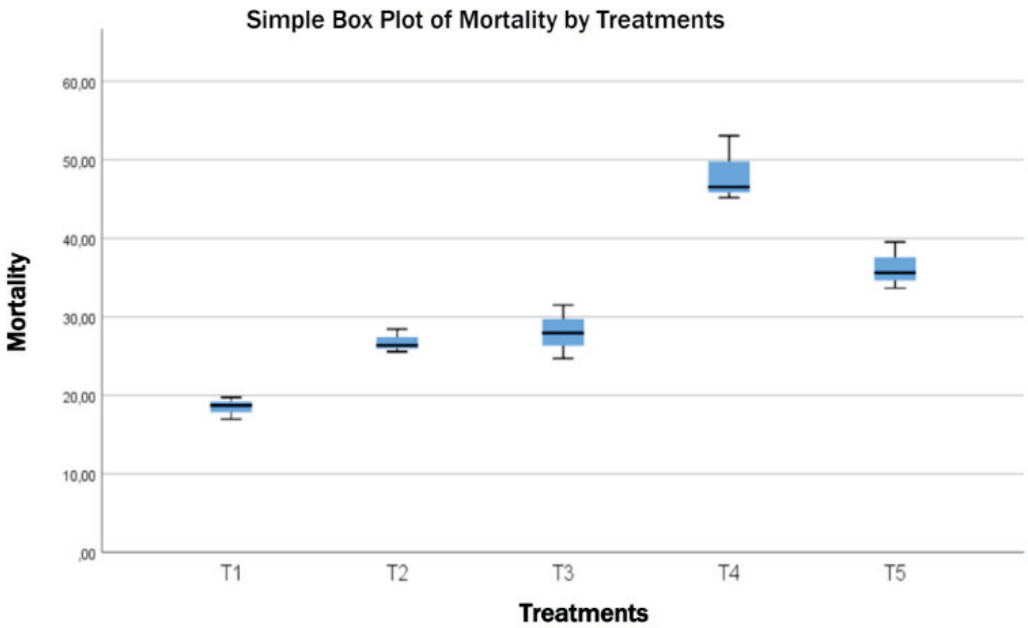

Figure 2. Percentage of moss removed on selected cocoa plants

Source: Own elaboration

degradation, cell membrane damage and mitochondrial damage. "The mucilage is useful in the development of free radicals in the cell membrane, which leads to cell porosity and death." (Tosif, and others, 2021)

ANOVA OF A SINGLE STATISTICAL FACTOR

Table 5 indicates the ANOVA analysis performed on the mortality variable in relation to the different study treatments.

> In statistical analysis, the null hypothesis (Ho) is considered when there is no relationship between the variables investigated, while the alternative hypothesis (H1) is accepted when there is a relationship between the variables. The decision to accept or reject a hypothesis is based on the established level of significance. If the significance level is greater than 0.05, the null hypothesis is accepted; otherwise, the alternative hypothesis is accepted. The F value is used to measure the relationship between variables, with a higher value indicating a stronger relationship. (Rubio Hurtado & Berlanga Silvente, 2012)

DESCRIPTIVE ANALYSIS OF THE MOSS MORTALITY VARIABLE

The following Table shows the descriptive analysis obtained in effect of the percentage of moss eliminated in the cocoa branches based on the dosages expressed in the treatments.

The results of the descriptive analysis, presented in Table 6, show that the total inhibition average of the moss is 31.56%, as reflected in Table 5. Treatments 3 and 4 show greater effectiveness in moss mortality. compared to the other treatments used. However, in treatment 4, the total elimination of both the moss and the cocoa flowers was observed, possibly due to the concentration of mucilage used (see Table 1). On the other hand, treatment 3 showed a less effective action in the inhibition of moss, which suggests that the most effective solution is found in treatment 3, with concentrations similar to other data investigated (see Table 1). Previous investigations, such as that of Coloma et al. in 2017.

> They have used a solution with 40% cocoa mucilage and 60% water, adding more water to reduce its viscosity; however, these results may vary depending on the fermentation time or weather conditions during the study. (Coloma Coloma, Alulema Cuesta, Spain Escobar, & Gualliche Serdan, 2017)

MULTIPLE COMPARISONS OF TREATMENTS WITH THE TUKEY TEST

The results of Table 7 present the Tukey multiple comparison test with a significance level of 95% for the moss death percentage variable when applying the 5 treatments, according to the Tukey analysis, a significant difference was found between treatment 1 and treatments 4 and 5.

However, no significant differences were found between treatments 2 and 3, since their percentages of applied solutions are 0.37% and 0.16% respectively. This indicates that treatment 1 is statistically equivalent to treatments 2 and 3.

The Tukey analysis of treatment 2 did not reveal significant differences between treatments 1, 3 and 5, with means of 0.37, 0.982 and 0.17 respectively. However, a significant difference was found with treatment 3, which had an applied dissolution percentage of 0.00, as shown in table 7, which suggests that treatment 2 is different from treatment 3.

In relation to treatment 3, the comparisons with treatments 1, 2 and 5 (with applied dissolution percentages of 0.86, 0.982 and 0.39 respectively) did not show significant differences, indicating that they are statistically equivalent.

However, a significant difference was found with treatment 4, which had an applied

ANOVA					
Mortality					
	Sum of squares	gl	Root mean square	F	Sig.
Between groups	1521,392	4	380,348	44,668	,000
Within groups	85,149	10	8,515		
Total	1606,542	14			

Table 5. Determination of significance level in Anova.

Source: Own elaboration

Descriptives								
Mortality								
	N	Half	Dev. Deviation	Dev. Mistake	95% confidence interval for the mean		Minimum	Maximum
					Lower limit	Upper limit		
T1	3	18,4633	1,3939	,80476	15,0007	21,9260	16,97	19,73
T2	3	26,7767	1,4867	,85832	23,0836	30,4697	25,55	28,43
T3	3	28,0433	3,4062	1,96656	19,5819	36,5047	24,69	31,50
T4	3	48,2433	4,2256	2,43966	37,7463	58,7403	45,16	53,06
T5	3	36,2533	2,9939	1,72856	28,8160	43,6907	33,64	39,52
Total	15	31,5560	10,7122	2,76590	25,6237	37,4883	16,97	53,06

Table 6. Descriptive Analysis of Moss Mortality (Rigodium implexum)

Source: Own elaboration

Multiple comparisons						
Dependent variable: mortality						
HSD Tukey						
(I) Treatments	(J) Treatments	(I-J) Difference in measurements	Dev. Mistake	Sig.	95% confidence interval	
					Lower limit	Upper limit
T1	T2	-8,31333*	2,3826	,037	-16,1546	-,4721
	T3	-9,58000*	2,3826	,016	-17,4212	-1,7388
	T4	-29,78000*	2,3826	,000	-37,6212	-21,9388
	T5	-17,79000*	2,3826	,000	-25,6312	-9,9488
T2	T1	8,31333*	2,3826	,037	,4721	16,1546
	T3	-1,26667	2,3826	,982	-91079	6,5746
	T4	-21,46667*	2,3826	,000	-29,3079	-13,6254
	T5	-9,47667*	2,3826	,017	-17,3179	-1,6354
T3	T1	9,58000*	2,3826	,016	1,7388	17,4212
	T2	1,26667	2,3826	,982	-6,5746	9,1079
	T4	-20,20000*	2,3826	,000	-28,0412	-12,3588
	T5	-8,21000*	2,3826	,039	-16,0512	-,3688
T4	T1	29,78000*	2,3826	,000	21,9388	37,6212
	T2	21,46667*	2,3826	,000	13,6254	29,3079
	T3	20,20000*	2,3826	,000	12,3588	28,0412
	T5	11,99000*	2,3826	,004	4,1488	19,8312
T5	T1	17,79000*	2,3826	,000	9,9488	25,6312
	T2	9,47667*	2,3826	,017	1,6354	17,3179
	T3	8,21000*	2,3826	,039	,3688	16,0512
	T4	-11,99000*	2,3826	,004	-19,8312	-4,1488
The difference in measurements is significant at the 0.05 level.						

Table 7. Multiple comparisons test with Tukey of the Mortality variable

Source: self made

dissolution percentage of 0.00.

The statistics of treatment 4 show that it is different from the rest of the treatments applied to inhibit moss (Rigodium implexum), which include treatments 1, 2, 3 and 5, with averages of 0.00 and 0.04 respectively. This suggests that treatment 4 is more effective to eliminate the moss under study. However, treatments 2 and 3 in the case of treatment 5 did not show significant differences, with averages of 0.17 and 0.39 respectively. Furthermore, treatment 1 and treatment 4 are considered statistically similar to treatment 5, with an average percentage of dead moss of 0.00.

In conclusion, according to the Tukey analysis, the most effective treatment among those applied to combat this weed was treatment 4, due to its significant differences with the other treatments. It is noted that the probability of finding an effective organic herbicide could be related to the solution used in treatment 4 (see table 1).

CONCLUSIONS

The reuse of the cocoa slime has had a significant impact on the farmers of La Adelina, generating great interest in its use to develop natural herbicides. These herbicides have shown excellent results when used in cocoa crops with the presence of moss (Rigodium implexum), preventing its spread and improving farmers save on chemical products being improved in economic terms and also to help prevent possible damage caused by the indiscriminate use of chemical products.

The organic herbicide has an impact on the moss when it comes into contact with the product on the cocoa branches. Its action is based on its acidity, which causes desiccation and elimination of the moss by breaking its cell wall and destroying its membrane in cocoa plants.

Treatments 4 (T4) and 5 (T5) proved to be the most effective to control moss in cocoa plants, with mortality rates of 49.11% and 36.25%, respectively. In contrast, Treatment 3 (T3) did not negatively affect cocoa flowering and showed a mortality rate of 28.04%.

Therefore, it is considered that Treatment 3 (T3) is the most appropriate option to apply in cocoa plantations and control mosses in this specific cocoa area.

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