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Comportamiento de diferentes malezas de *Leptochloa virgata* L. frente a la aplicación de herbicidas inhibidores de ACCasa en el cultivo de arroz (*Oryza sativa*)

Behavior of different weeds of *Leptochloa virgata* L. against the application of ACCasa inhibitor herbicides in rice (*Oryza sativa*)

Liliana Romero[1,a], Pablo Vargas[2,b,*], Javier del Cioppo[2,c], Jhicela Moya[3,d]

[1] Universidad de Guayaquil, Guayaquil, Ecuador.
[2] Universidad Agraria del Ecuador, Guayaquil, Ecuador.
[2] Ministerio de Trabajo, Quito, Ecuador.

[a] Ing., liliananaromero@ug.edu.ec, https://orcid.org/0009-0002-5325-4963
[b] Mg., pvargas@uagraria.edu.ec, https://orcid.org/0000-0001-6815-0425
[c] Dr., jdelcioppo@uagraria.edu.ec, https://orcid.org/0000-0001-5689-5884
[d] Mg., jhicela_moya@trabajo.gob.ec, https://orcid.org/0009-0004-8991-303X

* Autor de Correspondencia: *Tel. +59 3989882926*

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Resumen

El objetivo fue evaluar el comportamiento de diferentes malezas de *Leptochloa virgata* L. frente a la aplicación de herbicidas inhibidores de ACCasa en el cultivo de arroz (*Oryza sativa*). Para ello, se colectaron 10 poblaciones de *Leptochloa virgata* L, y se confrontaron con varias dosis de los herbicidas cyhalophop-butil y fenoxaprop-p-etil. Las poblaciones 1 y 10 que correspondieron a las localidades de Samborondón y Cristóbal Colón, se confrontaron con siete dosis de los dos herbicidas en estudio las cuales fueron: 1/8x, 1/4x, 1/2x, 1x, 2x, 4x y 8x. El índice de resistencia con el herbicida cyhalophop de acuerdo a la escala del ALAM a los 7 días después de la aplicación, fue alto con las poblaciones 1, 2, 6 y 10, mientras que con fenoxaprop fueron las poblaciones 1, 2, 7 y 10 las que mostraron resistencia a este producto; sin embargo, se seleccionaron las poblaciones 1 y 10 debido a que en condiciones de campo presentan problemas de control y se compararon con una población testigo. Las poblaciones 1 y 10 mostraron resistencia tanto a los 7 como a los 14 días después de la aplicación de ambos productos. La mayor toxicidad la provocó la dosis de 8x con ambos herbicidas, al igual que sobre la altura de planta, peso de materia fresca, seca y total. La DL50 de ambos herbicidas en la población 1 fue alta (220.53 pppm i.a).

Palabras claves: inhibidor ACCasa; herbicidas; índice de resistencia; inhibidor; Leptochloa; arroz.

Abstract

The objective was to evaluate the behavior of different *Leptochloa virgata* L. weeds against the application of ACCase inhibitor herbicides in rice (*Oryza sativa*). For this purpose, 10 populations of *Leptochloa virgata* L. were collected and confronted with various doses of the herbicides cyhalophop-butyl and fenoxaprop-p-ethyl. Populations 1 and 10, which corresponded to the localities of Samborondón and Cristóbal Colón, were tested with seven doses of the two herbicides under study: 1/8x, 1/4x, 1/2x, 1x, 2x, 4x and 8x. The resistance index with the herbicide cyhalophop according to the ALAM scale 7 days after application was high with populations 1, 2, 6 and 10, while with fenoxaprop it was populations 1, 2, 7 and 10 that showed resistance to this product; however, populations 1 and 10 were selected because under field conditions they present control problems and were compared with a control population. Populations 1 and 10 showed resistance both 7 and 14 days after application of both products. The highest toxicity was caused by the 8x dose with both herbicides, as well as on plant height, fresh, dry and total matter weight. The LD50 of both herbicides in population 1 was high (220.53 pppm a.i.).

Keywords: ACCasa inhibitor, herbicides, resistance index, inhibitor, Leptochloa, rice.

1. INTRODUCTION

Rice is an important crop for ensuring food availability worldwide; however, rice production is constrained by the presence of weeds in different cropping systems, which represents a major challenge for producers and consumers around the world (Shrestha *et al*., 2020). In rice production, several operations are carried out such as preparation of the ground and practices which aim to control the weeds, because during a certain period of growth, it can be affected in a drastic for them, so management measures are very important in this process (Chauhan, 2017). In the case of harmful species that can affect through competition for water, light, nutrients, space, with *Leptochloa virgata* L, standing out belonging to the Poaceae family (Labrada *et al*., 1996).

Weed-crop interaction affects growth and interferes with rice production capacity; is conditioned by biotic, abiotic and management factors that determine the degree or level of this effect (Liu *et al*., 2023). Interference refers to the detriment caused by one plant on another; it includes competition, allelopathy and parasitism (Pratap *et al*., 2023). Competition occurs when there is competition for a growth factor that is available in limited quantities (Dass *et al*., 2017). Competition causes direct yield losses because it deprives the crop of water, minerals, space, air, solar radiation, etc., thus compromising the economic success of the crop (ADAMA, 2021; Sing *et al*., 2007).

Weeds of the genus Leptochloa can easily spread and contaminate agricultural production areas because their mature seeds are easily released; on the other hand, the excessive use of pesticides and herbicides has caused these weeds to become resistant to them (Li *et al*., 2012). The most important weed species of the genus Echinochloa are: *Leptochloa filiformis*, *Leptochloa chinensis*, *Leptochloa chloridiformis*, *Leptochloa uninervia*, *Leptochloa panicea* and *Leptochloa virgata*, which can reduce crop yields due to direct competition for nutrients, water, light and space (Peña, 2022)

According to Vivas & Astudillo (2010), *Leptochloa virgata L;* in addition to causing damage to the crop, is a host to the rice feller bug (*Oebalus spp*), one of the most common pests in the important since when it got feeding on the panicle, the grains can remain partial or completely empty or break at the time of grinding and therefore losses in the yield and quality of the grain. The most common weeds in production sowing under irrigation and dry land in the country, are the genera Echinochloa, Leptochloa and the species *Oryza sativa*; on the other hand, it stands out with a high incidence of the species *L. virgata* and *L. uninervia* (INIAP, 2007).

Several studies indicate that using herbicides has become a tool powerful and increasingly common, becoming a practice of overconfidence for producers, because of its efficiency, with increasingly attractive costs, no longer side other control methods (Peng *et al*., 2021; Onwuchekwa-Henry *et al*., 2023; Chauhan & Opeña, 2012). The prolonged and indiscriminate use of agrochemicals causes resistance to one or more of the compounds that comprise them, making it difficult to control and manage pests, diseases and weeds (Li *et al*., 2019). The mixture of herbicides can cause problems, such as the selection of biotypes resistant to these inputs; ongoing field level reports of lack of efficiency or loss of sensitivity to graminicides used in rice cultivation in these species of weeds it is necessary to determine the presence of individuals with resistance characteristics (Hulme, 2023; Peterson *et al*., 2018).

Resistance is understood as the ability of certain biotypes to remain unaffected and reproduce, even after being exposed to a label dose of a given herbicide that normally controls them. This characteristic is inherited to their progeny, which means that plants originating from their seeds will also resist treatments to these same herbicides (Jin *et al*., 2022; Chen *et al*., 2023). In the development of herbicide resistance, it must be considered, on the one hand, that herbicides can act on a specific site; or those of multiple action, such as growth regulator herbicides that can affect more than one site (Ziau-Ul-Haq *et al*., 2019). On the other hand, it should also be taken into account that herbicides have a mechanism of action (affecting amino acid synthesis, lipid synthesis, or chlorophyll synthesis, among others) that refers to the site of action. Globally, there are approximately 300 active ingredients and 28 mechanisms of action; however, in rice cultivation the number of molecules is limited (Paredes *et al*., 2021). In this context, it was considered important to know the behavior of different weeds of *Leptochloa virgata* L. against the application of ACC asa inhibitor herbicides in rice cultivation.

2. MATERIALS AND METHODS

2.1. Location

The present research has been developed at the field and greenhouse, in the Weed Section of the DNPV of the Experimental South Coast "Dr. Enrique Ampuero Pareja" station, of the

National Autonomous Institute for Agricultural Research (INIAP), located in Yaguachi town, Province of Guayas. Western length and at 17 meters above sea level, with an annual rainfall of 1154.3 mm, average annual temperature 26.5 ºC and 86.2 % of average annual relative humidity (INAMHI, 2022).

2.2. Materials

Flower pots, test tubes, tape measure, pressurized back spray, plastic covers, and paper, stove, analytical balance, glass jars, herbicides, gloves, mask, mixing bucket, 2L plastic bottle, substrate, sprouting drawers, potted plants 750 ml, field notebook, office supplies.

2.3. Treatments

Initially, the ten weed's samples from the species *Leptochloa virgata L.* were collected which were subjected to a pre-selectioned by applying a dose to identify individuals who are tolerant and/or resistant to the cyhalophop and fenoxaprop herbicides.

The products to be applied and their characteristics are described as follows:

- Butyl (R) – 2 – [-4-(4-cyano-2-fluorophenoxy) phenoxy] propionate (Cyhalophop –Butil/Klomer), with the concetration whose formulation is based on 180 g of ia pc; and the dose 1.25 L/Ha.
- Phenoxy]propionate or ethyl (R) -2-[4-6 chlorobenzoxazol-22yloxy) pehoxy]propionate (Fenoxaprop -P-Etil/Furore), with the concetration whose formulation is based on 45 g of ia pc; and the dose 1.00 L/Ha

The treatments to select populations of *Leptochloa virgata L*, resistant to the herbicides under study comprised the combination of these plus a control, which never received applications of the herbicides mentioned above, which constituted in 22 treatments.

Once the resistant and susceptible population had been identified, two of them were selected to which the dose-response experiment was performed, by means of the which resistance indices (IR) were established.

Descriptive statistics were used for the screening experiment and in experiment two. A Completely Random Design (DCA) was used, in which the treatments were distributed in a 2x3x7 factorial arrangement, formed by two herbicides, three populations of *Leptochloa virgate* L weed biotypes and seven doses of the two herbicides, in which a control population that never received product for its management is included.

In experiment 1, weekly visual observations were made selecting the populations with variable damage index ranging from low susceptible to highly susceptible. In experiment 2, for the comparison of means, the Duncan test was used at 5% probability for the population and herbicide factors. For the comparison of the doses, regression analysis was applied, and the resistance index based on probits to determine the LD50.

Table 1. *Combinations of herbicides and selected samples from the screening of Weeds used in this research, under the Dose-Response system INIAP. EELS, 2014*

Weeds Samples (P)*	Treatments	Dose** PC L/ha-1	Herbicide 1 cyhalophop	Herbicide 2 fenoxaprop
P1	1	1/8x	0.16	0.13
	2	1/4x	0.31	0.25
Samborondón	3	1/2x	0.63	0.50
	4	1x	1.25	1.00
km 6	5	2x	2.50	2.00
	6	4x	5.00	4.00
Vía Daule	7	8x	10.00	8.00
	1	1/8x	0.16	0.13
	2	1/4x	0.31	0.25
P10	3	1/2x	0.63	0.50
	4	1x	1.25	1.00
	5	2x	2.50	2.00
Cristóbal Colón	6	4x	5.00	4.00
	7	8x	10.00	8.00
	1	1/8x	0.16	0.13
PT	2	1/4x	0.31	0.25
Testigo	3	1/2x	0.63	0.50
	4	1x	1.25	1.00
Km 26	5	2x	2.50	2.00
	6	4x	5.00	4.00
Virgen de Fátima	7	8x	10.00	8.00

Notes: *Sample 1 (Samborondón-km 6 vía Daule). Sample 10 (Cristóbal Colón) y Sample sample control INIAP-EELS). **From commercial dosage (1x).

2.4. Collection of the population of *Leptochloa virgata L.*

Seeds were collected from ten populations of *Leptochloa*; coming from different localities in the rice-growing areas of the Guayas province, in which there have been reported low efficiency of herbicides. In addition, seeds from plants collected in places where the herbicides under study have not been applied which will serve as a susceptible control.

2.5. Hotbed

With each of the weed populations collected in the three zones, they were seedbed, for this, germinating drawers were used that contained field soil rice cooker, in them the seeds were placed for their germination and subsequent study with the two herbicides cyhalophop and fenoxaprop. When the seedlings had two leaves true pots containing a mixture of soil, sand and peat were transplanted in a 2: 1: 1 ratio; the capacity of the pots was 750 ml, in which planted five seedlings of the species under study, during the development of the investigation carried out the following tasks.

2.6. Irrigation

This irrigation was done daily, preventing the plants from stressing due to lack of water.

2.7. Herbicide application

Herbicides were applied with a pressurized CO_2 pump equipped with a Teejet 8002 nozzle which was calibrated for a water consumption of 200 L/ha. The pH of the water and it was regulated with Cosmoagua, prior to mixing the two products to evaluate.

2.8. Variables studied

After the application of the herbicides, the following variables were evaluated:

2.8.1. Toxicity

Toxic indices of the plants were evaluated through visual observations taking into counts symptoms such as chlorosis, necrosis and growth failure, at the 7 and 14 days after application of the product, using the scale proposed by ALAM1/, as detailed below: 0 = no damage; 1-3 = light damage; 4-6 = moderate damage; 7-9 = heavy damage; 10 death.

2.8.2. Plant height

The initial height was evaluated at the time of the applications and then at 7 and 14 days, taking from the neck of the plant to the node of the most prominent leaf and expressed in centimeters.

2.8.3. Total fresh matter

At 14 days, after treating the plants, they were carefully removed from the pot, proceeding to wash with a continuous jet to clean its root part leaving it without soil residues, avoiding mechanical damage and loss of mass, subsequently proceeded to weigh the total fresh matter that includes its root part and aerial part was expressed in grams (g).

2.8.4. Total dry matter

Immediately afterwards these fresh plants were placed in paper covers with their respective identification and they were dried in an oven at 70 ºC, for 72 hours (weight constant), and after this time they were removed, their weight was determined from both the aerial as root, expressed in grams (g).

2.8.5. Resistance index

Then the values were recorded and the resistance index between different populations. For this, the following formula was applied:

$$IR = \frac{DL\ 50\ m}{DL\ 50\ t}$$

In what: LD 50 m = Average lethal dose that reduces 50% of the growth of the plant under study; LD 50 t = Average lethal dose that reduces 50% of the growth of the control plant.

Plant height was recorded in centimeters in populations P1, P10 and Control (T), calculating the percentage of inhibition compared to the lowest dose applied (1 / 8x Dose commercial). The inhibition values were transformed into probit values and estimated the linear regression against the logarithm of the doses in parts per million. Starting from each regression equation the LD50 was calculated for each population and ingredient active. The Resistance Index (IR) was calculated using the quotient between the LD50 of each population versus the Control population.

3. RESULTS AND DISCUSSION

3.1. Effect of ACCasa inhibitory herbicides on different samples of Leptochloa virgata

Initially a screening was done and the results obtained 7 days after the application of cyhalophop, it was observed that among the 11 weed's samples collected from different locations plus the sample control described above, two presented high susceptibility, with values of 8 and 7.33 on the ALAM scale, while

samples1, 2, 3, 6, 7, 9 and 10 showed slight damage, while the control moderate damage.
At 14 days after the application of cyahalophop, it was observed that the weed's samples 4 and 11 indicated a susceptibility index to this product, while samples 2, 6 and 7 showed resistance to it, the control also showed medium susceptibility.

Also, shows that plants exposed to fenoxaprop herbicide 7 days after of the application, treatments 4 and 5 showed high susceptibility with 8 and 7 on the ALAM scale, while treatments 1, 7 and 10 had slight damage, indicating resistance to this product, the control was moderately damaged.

Fenoxaprop treatment 14 days after application, it was observed that samples 4, 5 and 11 showed susceptibility, while samples 1, 2 and 3 were resistant, the control was susceptible. All the information is show in the Figure 1.

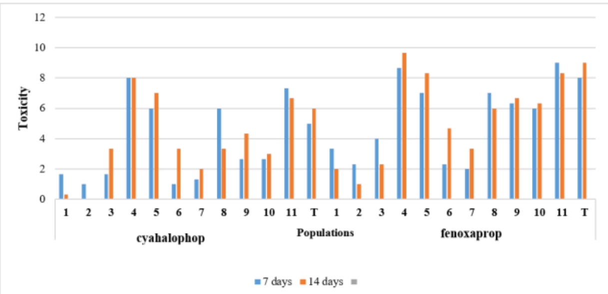

Figure 1. Screening of *Leptochloa virgata* treatments, exposed to commercially recommended doses of cyhalofop and fenoxaprop for 7 and 14 days after application. INIAP, EELS, 2014.

3.2. Determination of the resistance index (IR) of the selected samples of the first experiment (P1, P10) plus the control to the herbicides cyhalophop and fenoxaprop.

From the screening experiment, the weed's samples 1 and 10 were selected that correspond to the towns of Samborondón km 6 via Daule and Cristóbal Colón in its order, because of field observations in these sectors they have shown economic problems in rice crops.

3.2.1. Simple effects

Weed's samples: Simple treatment effects in the P1 weed crops showed moderate toxicity at 7 days (6.85) and necrotic tissue (8.07) at 14 days. In the with regard to plant height, at 7 and 14 days, the P10 weed's sample presented highest, reaching 12.39 cm in the last evaluation, equally in the weight of the fresh leaf material (FFPM), total fresh material (TFPM), leaf dry matter (LDPS) and total dry matter (TDS), the P10 weed's sample presented the highest values (Table 2).

3.2.2. Herbicides

At 7 days after the application of herbicides it was found greater toxicity with the cyhalophop molecule with 5.05 ALAM scale, while at 14 days it reached a slightly higher value (6.38) with moderate to strong damage fenoxaprop both herbicides. In PMFF, PMFT, PMSF and PMST, with the herbicide cyhalopop the values were statistically superior compared to what was obtained within the treatments with fenoxaprop (Table 2).

Herbicide Dose: Herbicide doses 8x at 7 and 14 days after application caused strong damage according to the ALAM scale with 8.17 and 9.33 degrees in its order. The height of initial plant (0 days) with lower values were the doses of 2x and 8x with 5.40 and 5.48 cm, respectively; at 7 and 14 days, the weight of total fresh leaf material, weight air dry matter and total dry matter was higher in the low-doses herbicides and lower values at higher doses (Table 2).

3.2.3. Interaction effects

a. Toxicity index at 7 and 14 (daa) days after application

According to the analysis of variance, the highly significant values corresponded to herbicide samples and dosages including P x H; P x D and H x D along with the triple P x H x D interaction, was 4.94, with a coefficient of variation of 12.44 % (Table 1).

The interaction between the weed's sample and herbicides, a toxic 7 on the ALAM scale (severe damage) with fenoxaprop in weed's sample 1 whose origin was from Samborondón town km 6 via Daule, while with cyhalophop was found a slight degree of toxicity; with weed crops 10 coming from the Cristobal Colón town enclosure with both herbicides the damage was slight and with the control (T) whose origin was of Virgen de Fátima km 26 showed moderate damage (Figure 2).

The analysis of variance presented highly significant values for the samples and the Dose, including the double interactions P x H, P x D, H x D together with the triple P x H x D interaction. The overall average for this variable was 6.30, with a coefficient of variation of 9.72%.

The interaction between weed's samples of *Leptochloa spp*. and herbicides, were found to have a toxicity of 9 (severe damage) with the herbicide fenoxaprop in weed's sample 1, while that with cyhalophop a lesser effect was observed, with no damage with weed's sample 1, going to slight damage with the weed's sample 10 and strong toxicity with the control (Figure 2).

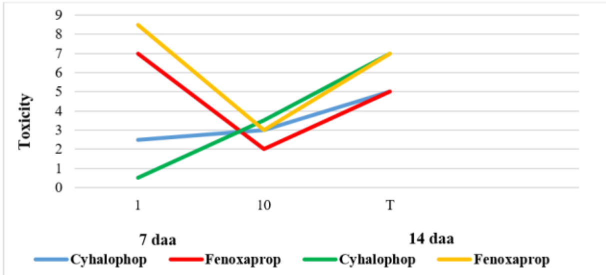

Figure 2. Weeds crops interaction by herbicide (P x H) at 7 and 14 daa by effect of three weed crops with two herbicides. INIAP. EELS, 2014.

With the herbicide doses of 1/8x in the samples 10 and T (control), a slight toxicity index; sample 10 remains on this scale with 1/4 x y 1/2x, is increased with the 8x dose. Weed's sample 1 with the doses of 1/8x, 1/4x and 1/2x, the damage was moderate. The doses are increased in weed's sample 1 the damage was greatest, in the T (control) with 2 x, 4 x and 8 x L/ha there was plant death (Figure 3).

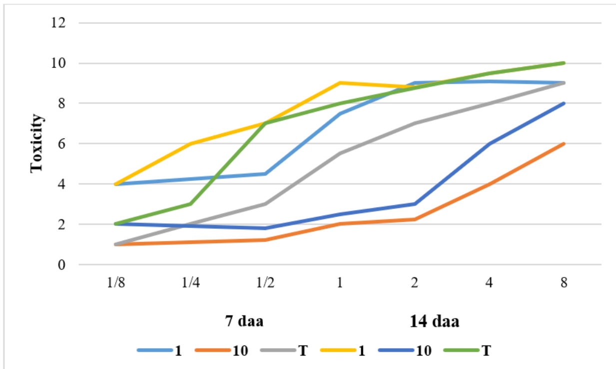

Figure 3. Sample interaction by herbicides (DxP) at 7daa and 14 daa, by effect of three samples with seven herbicide dosages. INIAP, EELS, 2014.

In the interaction of weeed's samples by dose (P x D) it was observed that weed's sample 1 could control from the 1x dose, so that showed high damage, while, the weed's sample 10 had a slight damage. With a dose higher than the recommended one, all weed's samples of *Leptochloa* showed damage (Figure 3).

Interaction between herbicides by dose (H x D), trends were similar, having a slight damage with both herbicides in the doses of 1/8x, 1/4x and 1/2x and these increased as the doses were increased, the damage to the weeds was higher with doses of 4 x to 8 x L/ha (Figure 4A).

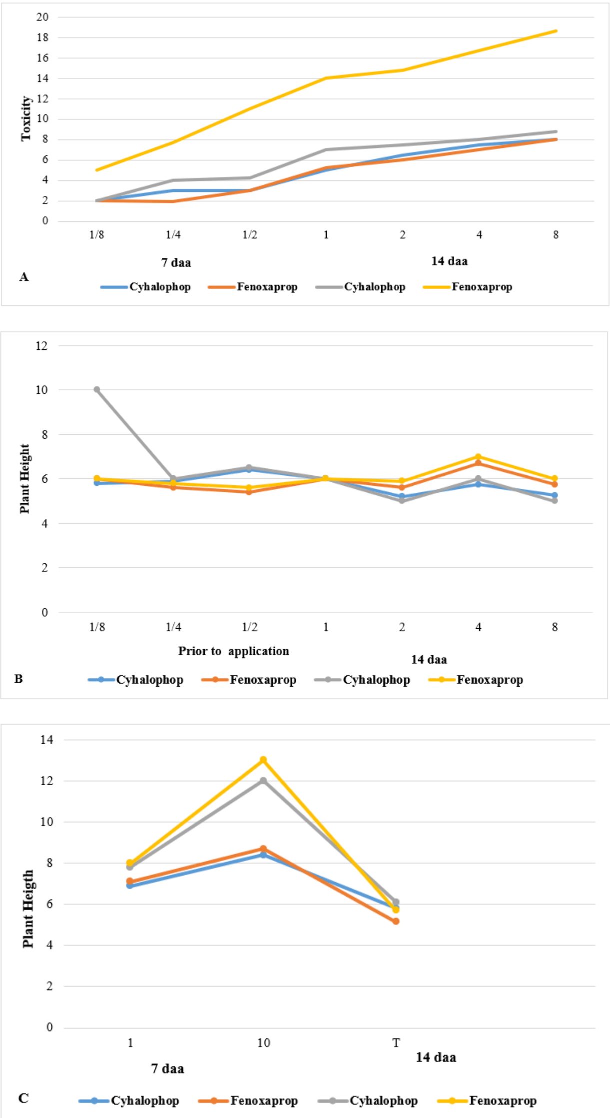

Figure 4. a) Interaction of (H x D) herbicides by dose at 7 and 14 daa, by effect of the application of two herbicides with seven doses in toxicity; b) Interaction of (H x D) Herbicides by dose over initial plant height, and 14 daa, by effect of application of two herbicides with seven doses; c) Interaction between sample by herbicides (P x H) over plant height at 7daa and 14 daa.

The behavior of herbicide interaction by dose (H x D), the trends were similar, observing a slight damage in both herbicides with the doses 1/8x and 1/4x, L/ha. With the higher doses of 1x of both herbicides there was strong damage and with 8x L/ha practically the death of plants occurred (Figure 4A).

b. Initial plant height (prior to application) and 14 day

According to the analysis of variance, the significant values corresponded to herbicide doses and H x D interaction, highly significant values were found for sample and triple interaction (P x H x D), herbicides, P x H and P x D were not significant. The general average of this variable was 5.82 cm, with a coefficient of variation of 20.24%.

The interaction of herbicides by dose (H x D), their behavior in initial height of *Leptochloa virgata* plants were practically the same with doses of 1/8x with both herbicides, later with the dose of 1/4x and 1/2x the height was increased with cyalophop with a 1x L/ha were equal and finally with the doses of 2x, 4x and 8x had the same tendency, however, at these doses the herbicide fenoxaprop presented the highest initial plant height values (Figure 4B).

In the herbicides' interaction by dose, the plant height was within the interval from 5.15 to 6.69 cm, with both herbicides within all the studied doses, with except for the herbicide cyhalophop which with the dose of 1/8 x, presented the largest average plant height (Figure 4B).

c. Plant height 7 and 14 days after application

The interpretation of the analysis of variance showed significant value for interaction P x H, also highly significant values were found from the weed's samples and doses including P x D interactions and the triple interaction (P x H x D), while the herbicides and doses; H x D interaction did not significance. The general average of this variable were 7.08 cm, with a coefficient variation of 19.41%.

In the interaction of *Leptochloa virgata* samples by herbicides, a similar trend with the two herbicides in plant height, with the sample 10 observed greater plant height, followed by sample 1 and the lowest was for the weed control sample (Figure 4C).

According to the analysis of variance, the significant values corresponded to interactions P x H, H x D and to the triple interaction (P x H x D); as highly significant values corresponded to sample, dose and interaction (P x D); while herbicides did not manifest any significance. The average of overall length of this variable was 8.70 cm, with a coefficient of variation of 18.86%.

In the interaction of *Leptochloa virgata* samples by herbicides, at 14 days after application of the product a similar trend is observed with both herbicides in plant height, with the weed's sample 10 it was observed greater plant height, followed by sample 1 and the lowest was for the weed control sample (Figure 4C).

In the interaction of weed's samples of *Leptochloa virgata* by doses of herbicides, it observes that with sample 10 it presents values of plant height higher than the sample 1 and the control in herbicide doses that are within the range of 1/8x at 2x L/h-1 a, with 8x Liters /ha-1 the height of the plant decreased, it is to emphasize that with the control sample the plant height was lower with all levels of herbicides (Figure 5).

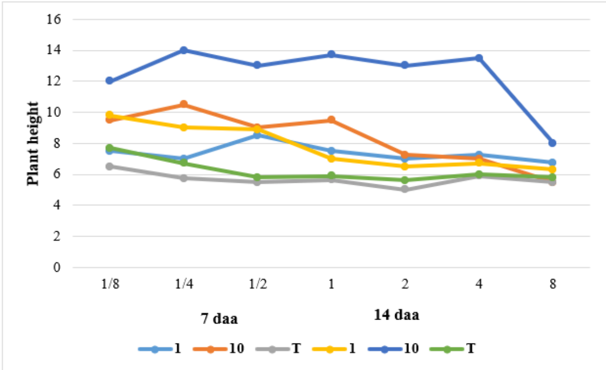

Figure 5. Sample interaction by dose (P x D) over plant height 7 and 14 days after application. INIAP. EELS, 2014.

According to the interaction of *Leptochloa virgata* samples by herbicide dose, the observes that with weed's sample 10 it presents values of plant height higher than weed's samples 1 and the control in herbicide doses that are within the range of 1/8x to 2x L/ha with 8x L/ha the height of the plant decreases, it is to emphasize that with in the control sample, the altitude was lower with all doses of herbicides (Figure 5).

3.3. Weight of Fresh leaf matter (WFLM); Total Fresh Material Weight (TFMW); Leaf dry matter weight (LDMW) y Total dry matter weight (TDMW)

According to the analysis of variance, the highly significant values corresponded to weed's samples, herbicides, dosage with double interactions P x D, H x D and the interaction triple P x H x D, while the P x H interaction wasn't significant. The overall's average weight of this variable was 0.37 g, with a coefficient of variation of 46.14%.

In the sample-by-dose interaction, WFLM had reached the highest value with the weed's Sample 10 in all herbicide doses, then followed by weed's sample 1, where the highest of plant reached the dose of 1/8x and from there had began to decrease the weight until the dose of 1x L/ha-1 and practically stabilized the weight with 2x, 4x and 8x L/ha being the lowest averages with this sample. The weed control sample were the one that had reached the lowest average of all the weed's sample, this one presented average of fresh matter higher doses at lower doses and from 1/2x to 8x L/ha of herbicide the values of this variable were low (Figure 6).

The analysis of variance of the variable (TFMW), presented highly significant values samples, herbicides, doses also double interactions such as P x D, H x D and the triple P x H x D interaction except for the P x H interaction which was not significant. The overall average of this variable was 0.58 g, with a coefficient of variation of 43.77%.

In the weed's sample-by-dose (P x D) interaction, total fresh matter reached the highest value with weed's sample 10 with all doses of herbicides, with weed's sample 1 the highest height of the plant reached it with the dose of 1/8x and from there as the herbicide doses the values of this variable decreased, being lower from of the dose of 1x L/ha up to 8x L/ha. The sample weed control sample were the one that reached the lowest average of all the samples, this one presented averages of fresh matter higher total doses at low doses and from 1x up to 8x L/ha-1 of herbicide the values of this variable were low (Figure 6).

In the LDMW, and according to the analysis of variance the highly significant values corresponded to weed's samples, doses and the double interactions P x D, H x D together with the triple P x H x D interaction, while herbicides and the P x H interaction were not significant.

The average of this variable was 0.07 grams with a coefficient of variation of 39.42%.

Leptochloa virgata sample 10 has the highest PMSF, followed by weed's sample 1 of this weed and finally the control, which has a dry matter weight leaf practically similar with the doses of 4x and 8x L/ha. In all the samples of as the herbicide doses were increased, a decrease was observed in the plants (Figure 6).

In the vaiable TDMW, according to the analysis of variance presented significant value for the herbicides, also found highly significant values for weed's samples, doses and the double interactions P x D, H x D, as well as the triple interaction P x H x D, the P x H interaction showed no significance. The overall average for this variable was 0.09 g with a coefficient of variation of 39.66%.

The highest weight of total dry matter is obtained with the sample 10 of *Leptochloa* followed by weed's sample 1 of this weed and finally the control, which has practically the same behavior in PMST, with doses of 4x and 8x L/ha. At all plant samples were observed to decrease as the herbicide dosage (Figure 6).

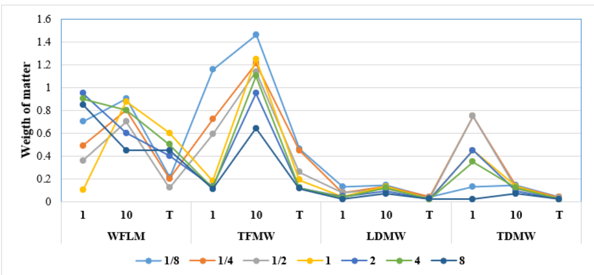

Figure 6*.* Interaction of Weight of Fresh leaf matter (WFLM); Total Fresh Material Weight (TFMW); Leaf dry matter weight (LDMW) y Total dry matter weight (TDMW) in (g) and three samples of *Leptochloa virgata* with seven doses of herbicides. INIAP. EELS, 2014.

In the variable WFLM, the interaction of herbicides by dose (H x D), showed similar trends, without however, these two had higher fresh leaf material averages with the lower doses of herbicides and decreased these values as the doses of herbicides increased (Figure 7).

According to the interaction of herbicides by dose (H x D), in the analysis of TFMW, the tendency of both herbicides was to decrease the total fresh matter content as increase the doses of these, the behavior of these two herbicides was different coinciding their values statistically with the doses of 1/8x, 1x and 2x L/ha (Figure 7).

The analysis of LDMW, in the interaction of herbicides by dose (H x D), it is observed that as increase herbicide doses lower PMSF values; the herbicide cyhalophop tends not to affect the weight of dry matter from doses of 1/8x to 1x L/ha-1without seizure from 2x L/ha the dry leaf matter decreases considerably (Figure 7).

In the interaction between herbicides and doses of the same (TDMW), it is observed that although both herbicides have varying degrees of response to total dry matter weight, the trend is decreasing in both, that is, as the doses of herbicides lowers the PMST, decreasing significantly when 2x doses are used, 4x and 8x L/h (Figure 7).

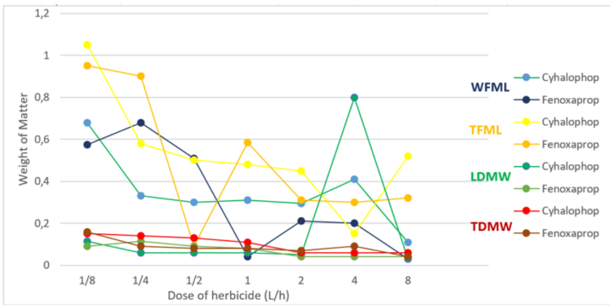

Figure 7. Interaction of Weight of Fresh leaf matter (WFLM); Total Fresh Material Weight (TFMW); Leaf dry matter weight (LDMW) y Total dry matter weight (TDMW) in (g), and seven doses of two herbicide INIAP. EELS, 2014.

3.4. Resistance index

For cyhalophop, it was observed that in the P10 sample there was no inhibition in height of the plant up to the dose 2x, in the case of P1 there was effect from the dose 1x, and in Witnessed inhibition was evident from the 1/4x dose. With fenoxaprop, at P1, the inhibition started with the 1x dose, while the P10, was from the 8x dose, as for the Control weed’s sample (WT) damage was evident with the 1/4x dose (Table 3).

For each herbicide, the Lethal Dose 50 was obtained, these were obtained in part per million active ingredients (ppm), including in the witness. This leads to a poor adjustment in the regression equations obtained with the exception of the P1 sample with cyhalophop (0.88). Although a resistance index (0.89) could be calculated for of P10 with cyhalphop, this apparent low value is contradicted when we see the DL50 (6.20E+06 ppm), since also in the Witness sample the DL50 was high (Table 4).

Table 2. *Average toxicity in the variables plant height, fresh and dry leaf material and total, at 0, 7 and 14 dda, INIAP EELS, 2014*

	Toxicity		Plant height			Material weigth			
Factors	**7dda**	**14dda**	**0**	**7dda**	**14dda**	**Fresh leaf**	**Total fresh**	**Dry leaf**	**Total dry**
Weed Samples									
P1	6.85 a [1/]	8.07 a	6.25 a	7.13 b	7.83 b	0.26 b	0.41 b	0.06 b	0.08 b
P10	2.66 c	3.61 c	6.15 a	8.55 a	12.39 a	0.74 a	1.10 a	0.12 a	0.16 a
PT	5.32 b	7.23 b	5.06 b	5.56 c	5.08 c	0.19 c	0.24 c	0.03 c	0.04 c
Herbicides									
Cyhalophop	5.05 a[1/]	6.23 b	5.77 N.S.	7.03 N.S.	8.59 N.S.	0.40 a	0.63 a	0.07 N.S.	0.10 a
Fenoxaprop	4.83 b	6.38 a	5.87	7.14	8.81	0.34 b	0.53 b	0.07	0.09 b
Doses									
1/8x	2.00 g[1/]	2.67 g	5.94 abc	7.94 a	9.86 a	0.61 a	1.02 a	0.10 a	0.15 a
1/4x	2.50 f	3.88 f	5.78 abc	7.61 a	9.87 a	0.51 b	0.78 b	0.08 b	0.12 b
1/2x	3.02 e	5.40 e	5.87 abc	7.70 a	9.06 b	0.39 c	0.65 c	0.08 bc	0.11 cb
1x	5.21 d	6.95 d	5.98 ab	7.54 a	8.93 b	0.34 dc	0.53 d	0.07 cd	0.09 c
2x	6.36 c	7.55 c	5.40 c	6.40 b	8.07 c	0.25 fe	0.39 e	0.05 ef	0.07 d
4x	7.33 b	8.36 b	6.28 a	6.62 b	8.65 cb	0.32 de	0.42 ed	0.06ed	0.08 d
8x	8.17 a	9.33 a	5.48 bc	5.75 c	6.43 d	0.19 f	0.28 f	0.04 f	0.05 e
Promedio	4.94	6.3	5.82	7.08	8.7	0.37	0.58	0.07	0.09
C.V. (%)	12.44	9.72	20.24	19.41	18.86	46.14	43.77	39.42	39.66

1/ Values indicated by the same letter do not differ statistically from each other (Duncan 5% probability); N. S. Not Significant.

Table 3. *Plant height and percentage of inhibition recorded in three samples of Leptochloa virgata versus seven doses of the herbicides cyhalophop and fenoxaprop. INIAP. EELS, 2014*

Cyhalophop				Fenoxaprop			
Weed's Sample	**Doses L/ha^{-1}**	**Height (cm)**	**Inhibition (%)**	**Weed's Sample**	**Doses L/ha^{-1}**	**Height (cm)**	**Inhibition (%)**
P1	1/8x	9.21	0.00	P1	1/8x	9.86	0.00
P1	1/4x	8.86	3.88	P1	1/4x	8.24	0.00
P1	1/2x	10.79	0.00	P1	1/2x	8.07	0.00
P1	1x	7.10	22.95	P1	1x	6.96	12.88
P1	2x	6.30	31.63	P1	2x	5.86	26.65
P1	4x	5.94	35.50	P1	4x	7.79	0.00
P1	8x	4.80	47.91	P1	8x	7.90	1.07
P10	1/8x	11.91	0.00	P10	1/8x	13.03	0.00
P10	1/4x	14.66	0.00	P10	1/4x	13.63	0.00
P10	1/2x	13.17	0.00	P10	1/2x	12.96	0.00
P10	1x	13.66	0.00	P10	1x	13.94	0.00
P10	2x	11.63	2.40	P10	2x	13.51	0.00
P10	4x	10.86	8.87	P10	4x	14.91	0.00
P10	8x	7,87	33.97	P10	8x	6.64	16.82
T	1/8x	7.99	0.00	T	1/8x	7.04	0.00
T	1/4x	6.80	14.85	T	1/4x	6.64	16.82
T	1/2x	5.59	30.05	T	1/2x	5.50	31.13
T	1x	6.19	22.54	T	1x	5.46	31.66
T	2x	5.81	27.19	T	2x	4.06	49.19
T	4x	6.40	19.86	T	4x	5.31	33.45
T	8x	5.29	33.81	**T**	8x	5.13	35.78

Table 4. *Lethal Dose 50 (LD50) and Resistance Index (IR) against ingredients active cyhalophop and fenoxaprop in three samples of Leptochloa virgate INIAP. EELS, 2014*

Active ingredient	Sample	Equation	R2	DL 50	IR
	P1	y = 1.1605x - 2.2804	0.88	220.53	
Cyhalophop	P10	y = 0.6866x - 0.3364	0.58	6.20E+06	0.89
	T	y = 0.5889x + 0.9698	0.54	6.98E+06	
	P1	y = 0.0776x + 2.8859	0.01	2.E+27	
Fenoxaprop	P10	y = 0.3587x + 1.5099	0.38	5.37E+09	
	T	y = 0.7508x + 0.8327	0.60	0.60 3.55E+05	

3.5. Screening experiment

The preliminary study carried out with the species *Leptochloa virgata* with two products graminicides (cyhalophop and fenoxaprop) on the biotypes of the eleven locations province of Guayas, showed that samples 4 and 11 were the most susceptible to cyhalophop 7 days after application. While fenoxaprop showed medium chlorosis as observed in samples 4, 5 and 11; subsequently, 14 days after application of cyhalophop, *L. virgata* biotypes 4, 5, 8 and 11 showed a high chlorotic index, i.e., greater susceptibility to this molecule. On the other hand, the application made with fenoxaprop at 14 days indicated that samples 4, 5 and 11 showed a high index of susceptibility, and according to what has been observed in these locations, they constitute minor problems in rice fields. Vargas & Fleck (1999), indicated that there are genetic differences between cultivars of two biotypes of the same species for tolerance to herbicides of the chemical family aryloxyphenoxypropionate, and highlighted the presence of different degrees of response in white oats, black oats and raigras; this shows that the genetic composition of a species or cultivar can determine different degrees of tolerance or susceptibility to herbicides. Zita (2012), indicates that in dose response trials there is resistance in samples *Phalaris minor* to ACCase inhibitors; while Lopez-Ovejero *et al* (2006), mention that ALS herbicide inhibitors have a high number of cases of resistance. It is worth mentioning that the high efficiency, residual activity, ecological adaptability of resistant biotypes and mutations, which can confer resistance to one or more herbicides from this group.

It was determined that sample 1 of the Samborondón km 6 road to Daule, showed greater resistance to the herbicides cyhalophop and fenoxaprop, being this location with high incidence in the same, also in the sample 10 of the locality Cristobal Colón town presented susceptibility to these products, presenting this site equally abundant problems with these weed biotypes, which is why they were selected for next phase of this study. On the other hand, Sosa (2014), indicates that it may be possible that inert ingredients behave similarly.

The continuous use of a product can interfere with resistance, so in this respect Li *et al*. (2019), indicates that indiscriminate applications of herbicides lead to development of many cases of resistance to these compounds for various species of Weeds.

The P10 sample in the town of Cristobal Colón showed resistance to these products, INIAP (2007), points out with respect to saline soils and that the weed *Leptochloa virgate* is currently considered to be of average harmfulness and its incidence is increasing in fields that present some level of salinity. While Moss (2002), indicates that the term quantitative variation implies a continuous response to the herbicide within the sample, which range from susceptible, partially resistant to highly resistant. This occurs due to a progressive increase in the level of resistance in the entire sample, and not to a increase in the proportion of resistant individuals.

Valverde *et al.* (2000), indicate that highly effective herbicides can impose a extremely high selection, capable of provoking the evolution of resistance in some how many generations. Such is the case with ALS inhibitors and ACCasa. Fenoxaprop-ethyl resistance (ACCasa inhibitor) was present in Costa Rica, after only four years of repeated use.

3.6. Effect of herbicide doses on three *Leptochloa virgata L biotypes* selected from the Screening

The toxicity index according to the ALAM scale at 7 days after application, was high with sample 1, while with sample 10 it was more tolerant and, in the control, (km 26) the toxicity with both cyhalophop and fenoxaprop was practically the same. An increase in toxicity was also observed after seven days due to dose effect from one liter to 8x L/ha of herbicide. Sample 1 was the most susceptible to all, with 1x L/ha of both herbicides; moderate damage was increasing the dose increases because with 8x L/ha the two herbicides caused the weed's death.

The 14 days after application, *Leptochloa* sample 1 showed low toxicity with cyhalophop, while when fenoxaprop was applied, he presented tissue weed's death. The control sample had high toxicity with both herbicides. Regarding doses, the sample 1 and control are more susceptible to toxicity at virtually all doses, increased from the dose of 1/2x L/ha up to 8x L/ha the sample of 10 was the most tolerant. As with the seven-day evaluations, the values of toxicity were incremental as the dose of both herbicides was increased causing the death of the weeds with 4x and 8xL/ha, the results differ from the exposed Espinoza (2002), whose studies indicate that resistant biotypes do not present morphological characteristics that differentiate them from susceptible plants and that survive at higher doses than required when this phenomenon exists. Resistance should not be confused with the natural tolerance that can be determined by species of weed to a herbicide or group of herbicides; one of the weeds known as vulpia has a high natural tolerance to all ACCase inhibiting herbicides.

In plant height at both 7 and 14 days, it was observed that in the control sample PT obtained lower values compared to the other two samples; the sample 10 presented the highest values when using the doses 8x L/ha. In fresh foliage and total at 7 days the values were high at lower doses of herbicides and were decreasing as the doses were increased, in this respect Ortiz *et al*. (2015), showed fresh weights greater than 20% of the untreated plants, are considered as resistant. The dry leaf and total dry matter were higher with the sample 10 with all herbicide doses compared to samples 1 and control, also their value decreased as the doses of both herbicides increased, as did behavior in value trends occurred in the total dry matter weight, being clear the effect of the application of the doses both herbicides as increase the doses.

3.7. Determination of the Average Lethal Dose (LD50)

Possible explanations for this could be a limited number of repetitions per treatment. Another possibility, although it would not be safe in this case, is that there would be some effect of hormones, as mentioned by Ortiz *et al*. (2015), i.e., low doses of herbicide could exert some stimulus in the growth of the plants, and at high doses be toxic to them, however, when the lack of inhibition is observed in most of cases, this phenomenon should be ruled out. On the other hand, Civerira (2012), mentions that the massive application of a single herbicide, caused by the planting of resistant to herbicides, can be very detrimental to agro-ecosystems. This would generate a phenomenon called selective pressure that can activate the excessive growth of herbicide-resistant weeds and decreased biodiversity, which could have a negative effect on beneficial insects and wildlife (Gage *et al*., 2019). While Probits analysis showed that sample 1 requires very high doses 220.53 ppm of cyhalophop a.i. to cause damage, this explains the problem with producers for the management of this weed.

4. CONCLUSIONES

The resistance index with the herbicide cyhalophop according to the ALAM at 7 days after application, was high with samples 1, 2, 6 and 10. Samples 1, 2, 7, and 10 showed resistance to fenoxaprop. Samples 1 and 10 were selected because under field conditions present control problems. Sample 1 of Samborondón km 6 via Daule, showed resistance moderated to the products evaluated. The sample 10 of the Cristóbal Colón town was observed to be moderately susceptible to the products of cyhalophop and fenoxaprop. In both locations *Leptochloa virgata* has a high incidence and represent a problem for the rice.

Sample 10 showed resistance at both 7 and 14 days later to both products. The highest toxicity of both herbicides was with the 8x dose. Both herbicides had an effect on plant height. The herbicide cyhalophop presented the highest fresh matter weight (HFMW), total fresh matter weight (TFMW), leaf dry matter weight (LDMW) and total dry matter weight (TDMW). Herbicide doses 8x at 7 and 14 days after application caused a strong toxicity damage.

Declaración de intereses

Ninguna.

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