

Use of pine extract (*Pinus patula*) for weed control in agave cultivation (*Agave tequilana* Weber var. Azul)

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ABSTRACT

Objective: To evaluate the pine extract with two solvents (water and ethanol), as a pre-emergence weed control in agave crop in the town of San Miguel el Alto, Jalisco.

Design/methodology/approach: The experimental design was in randomized blocks, with six treatments and four replicates, using two synthetic herbicides and two pine extracts (aqueous and ethanolic), during the rainfed period from July to September 2023. Plots were 30 m² with 10 agaves. Applications were made pre-emergence and twice more per month at a concentration of 25%. The variables recorded for weeds were number of specimens, species and visual coverage; for the crop, number of detached leaves, plant height, leaf width and length. The Shannon index, % control, EC and pH were obtained per treatment.

Results: The pine extract with both solvents showed phytotoxicity (chlorosis and stunting), and similar control to that observed with synthetic herbicides was not observed. The type of weed leaf determines the efficacy of the herbicide used, as it does for the extracts. Although the desired significant differences between treatments were not found, changes in weed populations were observed, suggesting that the extracts should be further evaluated.



Limitations on study/implications: N/A

Findings/conclusions:

- First report on the phytotoxic effect of *Pinus patula* extract.
- Pine extract with both solvents causes phytotoxic symptoms (stunting and chlorosis).
- The ideal application rate must be determined.
- *Digitaria ciliaris*, *Paspalum lividum* and *Chloris virgata* are reported as species tolerant or resistant to glyphosate and glufosinate ammonium.

Keywords: Allelopathy, plant extracts, *Pinus patula*, weed control.

INTRODUCTION

Weeds are a problem in crops, since they decrease the yield when they do not receive timely attention, causing important losses; they are one of the biotic factors with greatest impact in agriculture. The use of synthetic agrochemicals is the main tool for producers, even if they cause damage to the environment and to the population's health (Scavo and Mauromicale, 2020). In this regard, glyphosate residues have been detected in foods such as soy and maize, and these residues reach the end consumer causing harm to health, the environment and wildlife (CONACYT, 2020; Suppa *et al.*, 2020; Ordoñez-Beltrán *et al.*, 2019; Serpa *et al.*, 2015). In Jalisco, agave cultivation has designation of origin, so it is one of the most important crops, in addition to recently having gained terrain, with 133,763.52 ha planted by the end of 2023 (SIAP, 2024). This crop does not have selective herbicides and herbicides evaluated in other crops, to which agave turns out to be tolerant, are used. An herbicide of common use in this crop is glufosinate ammonium, which is efficient although quite residual; for example, when agave is harvested and maize is to be planted, it cannot be established because of the toxicity of the herbicide applied. Then, the producer must seek options that allow planting maize. An efficient control method is manual, but it must be applied two to three times during the rainy season, which is costly; the optimal option would be integrated control (Dominschek *et al.*, 2021). Derived from this, there is a requirement to find less costly strategies by observing the behavior of nature. Plant extracts have been reported since 1970 with inhibitory activity on plant germination and growth (Belz, 2007; Cruz-Ortiz and Flores-Méndez, 2021). These are options for weed control, since their metabolites (natural chemical substances) could alter the cell structure and cause biodegradation or the inhibition of germination. In the market, "organic" products for weed control have been found, and a constant factor is pine. Therefore, the objective of this study is to evaluate the pine extract with two solvents (water and ethanol), as pre-emergence control on weeds in the agave crop in the locality of San Miguel el Alto, Jalisco. In addition, there are reports in the literature that pine essence (*Pinus patula* L.) can inhibit the germination of *Sinapis arvensis* L., *Lolium rigidum* (Gaudin) Weiss ex Nyman, *Phalaris canariensis* L. and *Trifolium campestre* Schreb (Amri *et al.*, 2011), and 93% of inhibition in weed germination was obtained with the aqueous extract of *Pinus caribbean* Morelet, without reporting the species (Jiménez Ferrer *et al.*, 2006). On the other hand, the pine extract had significant results in the germinative inhibition of *Arthraxon quartinianus* (A. Rich.) Nash (Portuguez-García *et al.*, 2020).

MATERIALS AND METHODS

The study was conducted on a plot located in San Miguel el Alto, which is on the limits with the municipality of Arandas in the state of Jalisco, Mexico, belonging to the zone called Altos de Jalisco; it is located on geographical coordinates 20.897021674240175 latitude, 102.4581126284207 longitude. The climate is semi-warm, semi-humid (34.1%), and temperate sub-humid (65.9%). The mean annual temperature is 17.3 °C and the mean annual precipitation is 919 mm, with the month of July being the one with most rainfall. The predominant soil is Luvisol (34.6%) and presents reddish soils that accumulate a large amount of clay; the ones used for agriculture have moderate yields and are quite susceptible to erosion. Agriculture (61.7%) is the dominant land use in the municipality (INEGI, 2001, 2018). The experiment was established in the rainfed season (July-October 2023) and had a completely random block arrangement, where the treatments were randomized via a raffle.

Treatments

The experiment was established in a newly transplanted, one month old, agave plantation (*Agave tequilana* f.a.c. Weber), with planting density of 3,333 plants/ha. An experimental plot of 720 m² was established with six treatments and four replicates, T1: Clean control (kept clean with hoe and shovel), T2: Weedy control (without treatment), T3: Glufosinate ammonium, T4: Glyphosate, T5: Aqueous pine extract, T6: Ethanolic pine extract; this results in a total of 24 quadrants of 30 m² each (3×10 m). For the design of completely randomized blocks (James *et al.*, 2002), the plot was divided into lines of 10 agaves per treatment, leaving two agaves without any application, between treatments.

Preparation of plant extracts (ethanolic and aqueous)

The plant material (plant) that was used for this experiment was chosen because of its availability in the region and its allelopathic qualities reported for the genus and/or species (Marín-Velásquez and Barrutia-Barreto, 2020), in addition to the prior mention about its use in commercial “organic” products. Specimens of *Pinus patula* were found in the region and fresh needles were collected that were transported to the laboratory for their processing. Two types of extracts were elaborated, aqueous and ethanolic. For the elaboration of the extracts, 200 g of fresh needles were used for every liter of solution, aqueous or ethanolic; for the case of the latter, a solution of denatured ethanol at 70% was used, and for the aqueous, the ethanol was replaced for potable water, with proportion of 1:100 p/v. The needles were diced to facilitate grinding, for which a classic Oster[®] blender was used; the plant was placed and ethanolic solution was added, grinding was done for 10 seconds in three repetitions, and then the entire content of grinding was placed in a wide-mouth and amber-color recipient; it was left to rest for 15 days and shaken twice per day for 5 min. At the end of the resting period, the extract obtained was filtered with a kitchen strainer to remove the plant residues, then with a tulle fabric sieve to remove the large particles of plant tissue from the extract, and finally with number 4 Whatman[®] filter paper and a funnel to remove any small plant particle that could clog up the nozzle of the spraying pumps. The filtered extract was placed in duly labeled amber color containers.

Preparation of herbicide extracts

From the extracts obtained, a dose of 25% from each extract was used and 5.7 liters per extract were prepared to be applied on the agave plot and each of the four repetitions; to determine the amount of extract necessary, the spraying bag was previously calibrated.

Preparation of synthetic herbicides

The synthetic herbicides used were glyphosate (chemical control) (3 L ha^{-1}) and glufosinate ammonium (1.5 L ha^{-1}); the latter is the chemical regional control (which received that name because it is the one the producer uses). The doses are those recommended on the label. Glyphosate was applied with a bell to avoid damaging the agave, since it is a non-selective herbicide. For the extract, 50 L ha^{-1} were used, and its concentration was 25%. The treatments were applied with a spraying pump (SWEDISH HUSKY POWER® Electrical Fumigator with Tank Capacity of 20 L), at a pressure between 30 and 40 pounds, measuring with a manometer adapted to the pump and TeeJet® 8004 nozzle.

Variables evaluated and statistical analysis

The applications were carried out 30 days after the crop establishment; meanwhile, the extracts were applied three times, one per month for three months; and for the evaluation of the effect of treatment on weeds, the first measurement was carried out 15 days after application (DAA) and the next two, with one month separation between them. The number of plants per treatment/repetition was recorded, and the visual coverage (%) which represents the effect of the herbicides. The percentage of control was calculated, as well as the diversity of species present; for this purpose, the quadrant method was used, with a quadrant of $50 \times 50 \text{ cm}$, thrown twice, and whatever was inside the quadrant was recorded (species and frequency of appearance) (Monroy-Reyes *et al.*, 2005), with which the Shannon index was obtained. The electrical conductivity and pH of a sample made up of soil per treatment after its application was also recorded. To evaluate the effect of weeds and treatments applied on the crop, a sample of 5 plants (agave) was recorded, per repetition and treatment. The initial number of leaves, the number of leaves detached, and those totally detached from the shoot were recorded as detached leaves. The plant height, width of the last leaf detached, and height of this same leaf were recorded; to avoid confusion, the tip of the last leaf detached was cut (Monroy-Reyes *et al.*, 2005; Pimienta-Barrios *et al.*, 2005), and plant toxicity observations were also made on the crop. These measurements were carried out once per month, for four months. In the data analysis, a logarithmic (variable number of weeds) and square root (variables of percentage of control and cover) data transformation was conducted; in the end, the assumptions were not fulfilled, and a repeated measurements analysis was used with Kruskal Wallis (Kay *et al.*, 2021). Then, a contrast comparison test was carried out with $\alpha=0.05$ (Elkin *et al.*, 2021).

RESULTS AND DISCUSSION

The treatments with extracts were applied in pre-emergence and the glufosinate and glyphosate once the weeds had emerged (post-emergence), so the first measurement of

the number of weeds was recorded at 15 DAA. Results were grouped per type of leaf in the weeds: broad leaf refers to the dicotyledons, and narrow leaf mostly to the grasses, although Cyperasea were also grouped here. The results from the statistical analysis (Kruskal Wallis) for the variable “number of weeds” between treatments shows significant differences ($p\text{-value}=1.4244^{e-07}$) (Figure 1).

A significant interaction was also found between treatment *type of leaf ($p\text{-value}=5.9778^{e-10}$), meaning that the effect from the treatment depends on the type of leaf (broad and narrow). These effects have been mentioned by the producers, because the synthetic chemical herbicides cannot manage to control all the species cataloged as weed in a plot. Because of the type of plant metabolism and the synthetic chemical used, some species are affected more than others and the extracts functioned equally, affecting the broad-leaf or narrow-leaf species ($p\text{-value}=1.5524^{e-07}$) in different ways. Therefore, different herbicides are used depending on the crop and the type and species of weeds present (Herrera-Murillo and Picado-Arroyo, 2024). Grasses are mostly difficult to control, and commercial herbicides take time to have an impact and do not completely eliminate them; this can be observed since this group (grasses/narrow leaf) was higher in number of individuals in all the treatments and their decrease is observed with time, also with the extracts, except more slowly. The aqueous and ethanolic pine extracts do not compete with the synthetic herbicides with the dose applied. The ethanolic extract affects more the emergence of broad-leaf weeds, the same as glyphosate and glufosinate ammonium (Figure 1). Regarding the effect through the three measurements (months), a significant difference was also found ($p\text{-value}=0.014799$), which was expected, because the effect of the synthetic

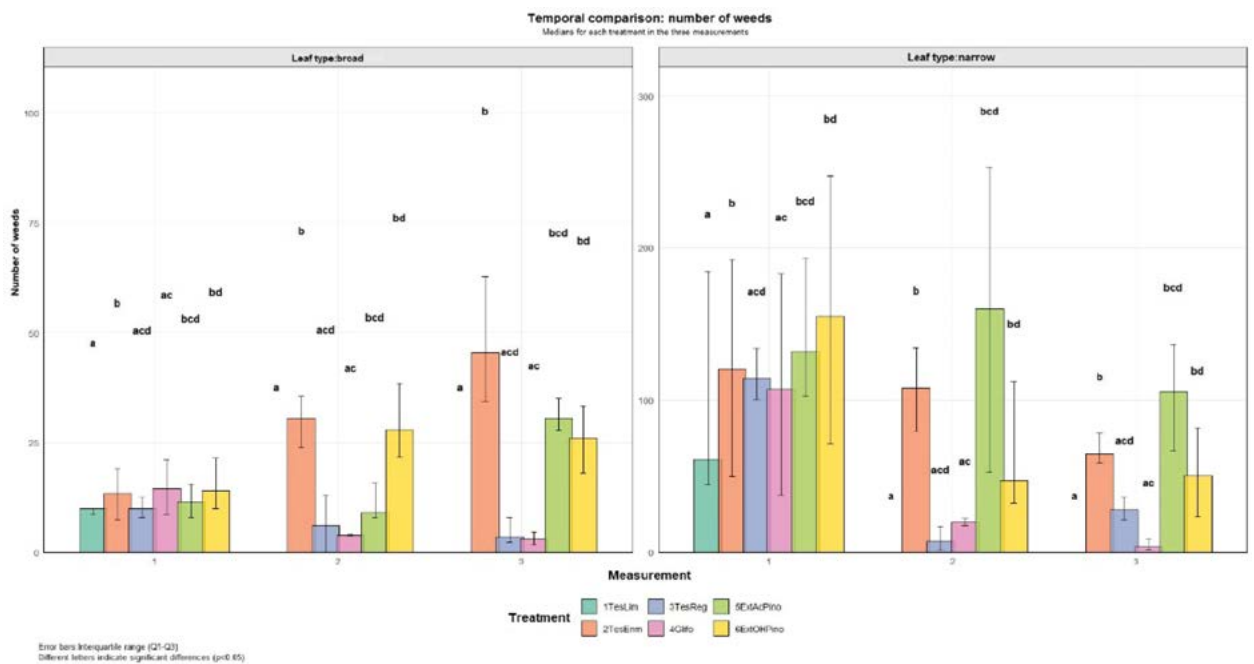


Figure 1. Means comparison of the number of weeds present in the agave crop through treatment and type of leaf, in the three measurements 1, measurement 1, 2, measurement 2 and 3, measurement 3. 1TesLim: Clean control, 2TesEnm: Weedy control, 3TesReg: Glufosinate ammonium, 4TGlifo: Glyphosate, 5ExAcPino: Aqueous pine extract, 6ExOHlPino: Alcoholic pine extract.

chemical herbicides and of the extracts is not immediate, and this is corroborated through the measurements.

Regarding Dunn’s test (Table 1), significant differences are found when the treatments are compared, so that they can be grouped into weedy control, with higher number of weeds, clean control with lower number of weeds, glyphosate with a small number of weeds, and treatments with glufosinate ammonium (regional control) and the two extracts (aqueous and ethanolic) that had an intermediate level of weeds.

This indicates that there are strong temporal effects which were observed in symptomatology recorded, such as chlorosis and stunting. However, the extracts present effectiveness which is generally variable in time (Figure 2), since they reduce the number of weeds at the end of the study. This is important because most commercial organic herbicides contain pine extract, although only effects of chlorosis, stunting and possible

Table 1. Significant values in Dunn’s Test for Treatment.

Comparison	P_value
Clean control-Weedy control	7.050 ^{e-07***}
Weedy control - Glufosinate	3.72 ^{e-02*}
Weedy control - Glif	4.99 ^{e-03**}
Clean control - ExtAcPino	3.92 ^{e-05***}
Clean control - ExtOHPino	2.32 ^{e05***}
Glif-ExtOHPino	4.89 ^{e-02*}

1TesLim: Clean control, 2TesEnm: Weedy control, 3TesReg: Glufosinate ammonium, 4TGlifo: Glyphosate, 5ExAcPino: Aqueous pine extract, 6ExOHPino: Alcoholic pine extract.

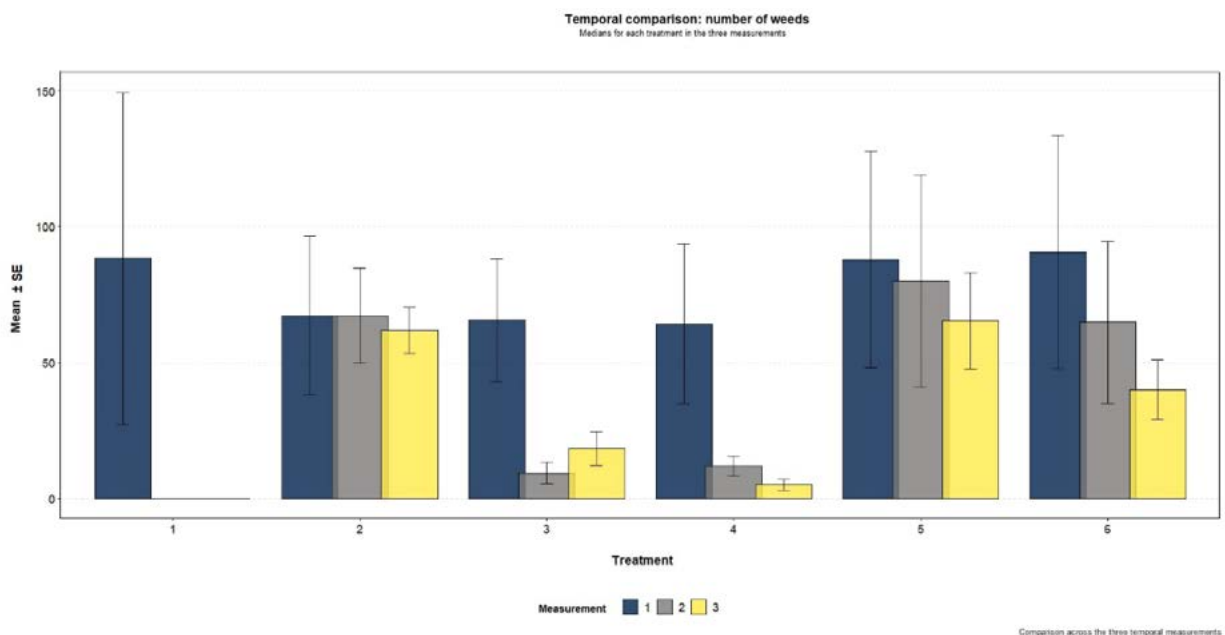


Figure 2. Number of weeds accumulated and encompassed without considering the type of leaf, during the three months recorded. 1 Clean control, 2 Weedy control, 3 Glufosinate ammonium, 4 Glyphosate, 5 Aqueous pine extract, 6 Alcoholic pine extract.

reduction of emergence are suggested, in this study, by reducing the number of plants with both solvents in different degrees (Treatments 5 and 6, Figure 2). Something else that can be seen is the effect on the reduction of weeds with Treatments 3 and 4, which are glufosinate ammonium and glyphosate, respectively. Although they do not completely eliminate the weeds, this is because of the presence of seasonal rains, since weeds continue to emerge when it is still raining, because glyphosate is a semi-systemic herbicide just like glufosinate ammonium, and its effect was not enough to eliminate the weeds.

The visual coverage expressed in percentage of soil covered (area) represents the vertical projection of the aerial part of the weeds and reflects the capacity for competition better than the number of weeds present, at the same time that it represents the control exerted by herbicides; it is a fast visual evaluation: with more coverage, less control, and vice versa. Regarding this variable (Figure 3), the Kruskal Wallis statistical analysis shows $p\text{-value}=1.08346^{e-11}$, indicating significant differences between treatments, although a statistically equal effect with both pine extracts. Herrera-Murillo and Picado-Arrollo (2024) mention that some herbicides do not cause chlorosis, but a reduction in coverage is observed which agrees with what was observed in the third measurement for the aqueous extract in narrow-leaf plants compared to the second and third measurements.

To visualize this variable better, a correlation was conducted between the coverage and the number of weeds or species present (Figure 4). It shows that, in general, there is a moderate positive correlation, since each treatment must be taken into account to analyze punctually; for example, in the regional control (glufosinate ammonium), the coverage is

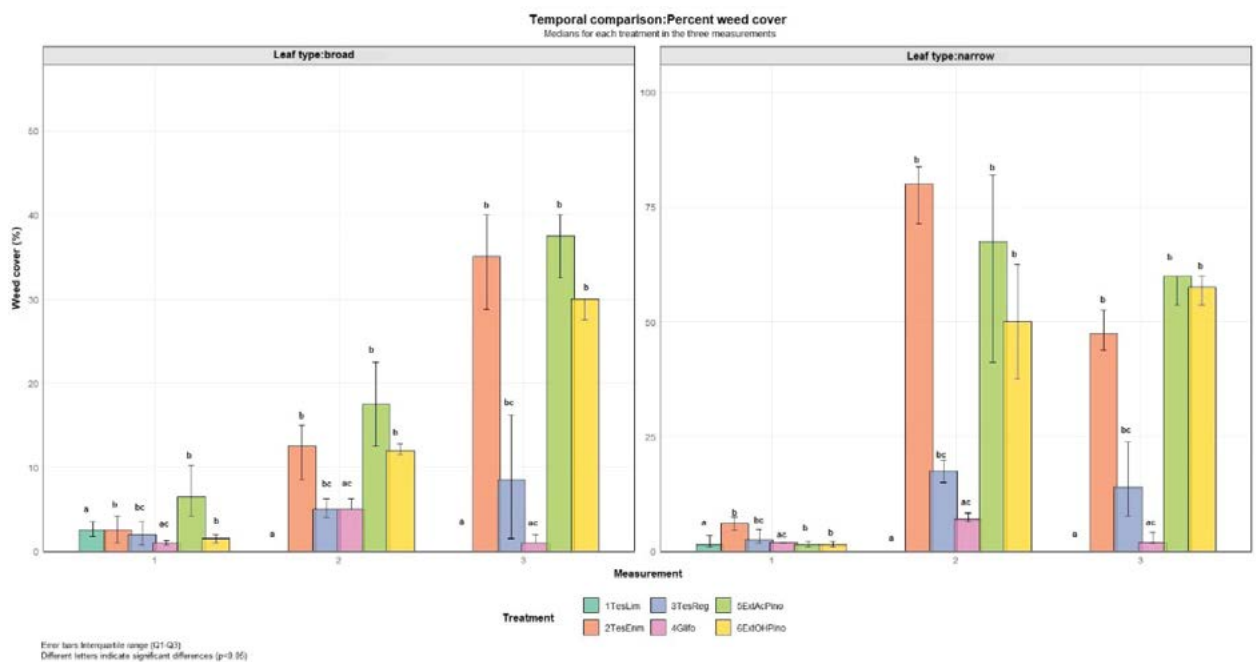


Figure 3. Means comparison of visual coverage of weeds (%) (control) present in the agave crop per treatment and type of leaf, in the three measurements 1, measurement 1, 2, measurement 2 and 3, measurement 3. 1TesLim: Clean control, 2TesEnm: Weedy control, 3TesReg: Glufosinate ammonium, 4TGlf: Glyphosate, 5ExAcPino: Aqueous pine extract, 6ExOHPino: Alcoholic pine extract.

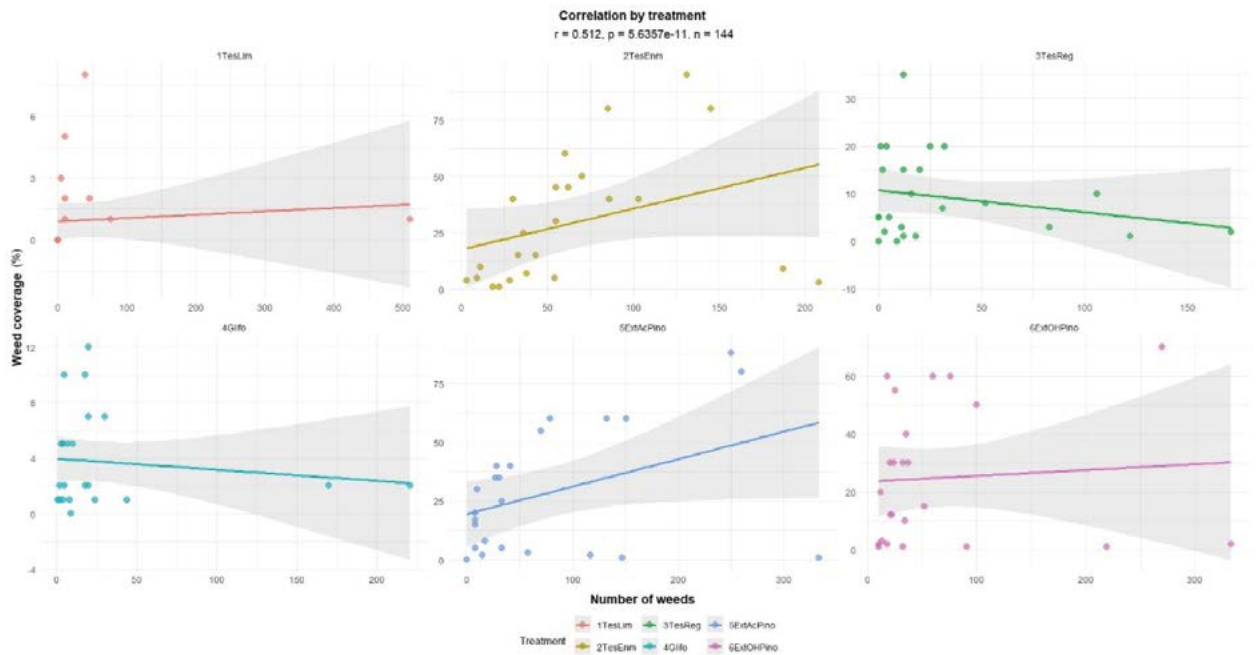


Figure 4. Correlation between number of weeds and coverage. 1TesLim: Clean control, 2TesEnm: Weedy control, 3TesReg: Glufosinate ammonium, 4TGlif: Glyphosate, 5ExAcPino: Aqueous pine extract, 6ExOHpino: Alcoholic pine extract.

generally low and when it comes to the number of weeds present in the case of broad leaf, it ranges between 3 and 10 individuals, while for the narrow leaf the number fluctuates between 110 individuals in the first measurement and 20 in the last. Regarding the behavior of ethanolic and aqueous extracts, the trend was positively moderate but with variable points, so the effectiveness is qualified as weak for both, with their particularities, which will be discussed when addressing the species present in each treatment. This is probably because of the type of extraction performed; by using only water, certain metabolites can be extracted (acids, amines, ketones, aldehydes, tannins, lignans, stilbenes, and sugars), which will probably not be extracted with the alcohol solvent used (terpenes, tannins, phenols, siloxanes, alkanes) (Amri *et al.*, 2011).

However, Alam *et al.* (2022) mention that in extracts of needles from *Pinus roxburghii* Sargent, elaborated with alcohol and aqueous solvent, the same metabolites were obtained, except more concentrated with the organic solvent (ethanol). When it comes to plant extracts, their action can be due to the compounds that the bibliography refers are found in *Pinus patula*, such as alpha-pinene, limonene, among others (Amri *et al.*, 2011; Iñíguez-Guillén *et al.*, 2007; Portuguese-García *et al.*, 2020). However, it is necessary to work on elaborating more concentrated extracts. For the chemical-synthetic treatments, the values are shown for glyphosate in the base of the figure compared to the regional control (glufosinate ammonium), so glyphosate is qualified as efficient when presenting a lower number of weeds and less coverage. Regarding both extracts, the percentage of coverage was similar, that is, without significant difference between them (Figure 3).

According to the European scale (EWRS), the levels of activity decrease as the effectiveness of the herbicide increases, so that, with less visual coverage, the effect is

greater. And in this sense, glufosinate ammonium has a classification of 3 and glyphosate of 2, the aqueous pine extract 8, and the ethanolic extract 7.

No impacts were seen on the agave crop and symptoms were observed in the weeds such as chlorosis, stunting, and in some cases necrosis; this is the first report of phytotoxic or allelopathic action of *Pinus patula* elaborated in a proportion of 1:100 (p-v) applied at 25%. Regarding the species that were present in the different treatments, the Shannon index was calculated, where the low diversity there is in broad-leaf weeds (Figure 5) and narrow-leaf weeds (Figure 6) is shown; clear differences are seen between the diversity present per treatment, and the value of 1 is not even reached, which is the lowest value mentioned for this index; the herbicide that decreases diversity the most is glyphosate.

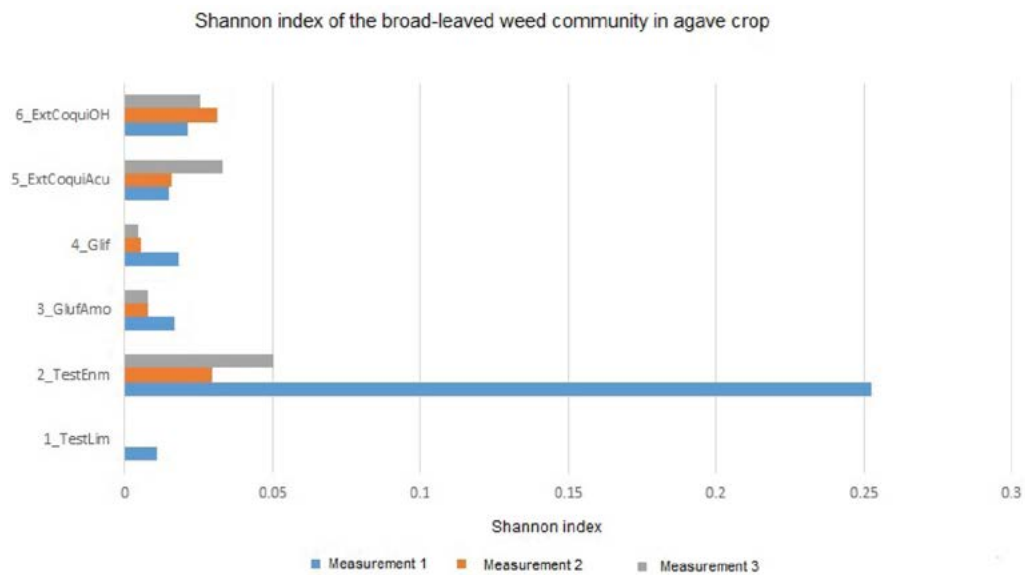


Figure 5. Shannon index in the treatments applied with broad-leaf weeds.

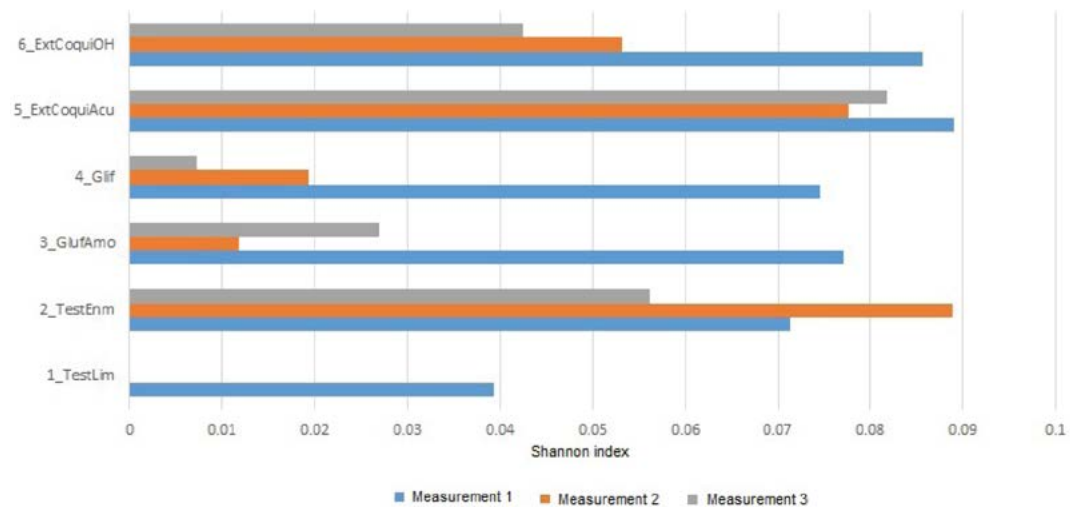


Figure 6. Shannon index in the treatments applied with narrow-leaf weeds.

All the treatments reduce the diversity in both types of leaf, including the weedy control; this is because of the environmental conditions and the life cycle of some weeds that appear and disappear according to climate conditions, and this treatment has the highest values in diversity.

Although the areas sampled are relatively small to estimate this index, this gives us an idea of the decrease in diversity that is caused by using herbicides; in many cases they are species with medicinal or allelopathic properties that could well be used. For example, in the species of broad-leaf weeds that were identified because they were not controlled (Figure 7), without this indicating resistance, *Salvia tilifolia* Vahlque was found, which is reported as forage, edible and medicinal (Vibrans, 2009c). *Mentha rotundifolia* L. was also identified, which has medicinal properties and is considered an aromatic plant similar to another *Salvia* species (Vibrans, 2009b). One species that is tolerant or resistant to herbicides applied is *Anoda cristata* L., which has been seen in the region that tolerates their application and was not the exception in this study; in addition, it is medicinal, forage, edible, and melliferous (Vibrans, 2009a).

When it comes to the presence of weeds per species, treatment and measurement (Figure 7), it was seen that there was a decrease in the number of species in the third measurement, just as with glyphosate, but more gradual or slower with the extracts. The grasses that were identified because they could not be controlled completely (Figure 7), agree with what was reported by Pimienta *et al.* (2005); some species present resistance to both glyphosate and glufosinate ammonium, and they were *Digitaria ciliaris* (Ketz.) Koeler, *Paspalum lividum* Trin. Ex Schtdl. and *Chloris virgate* Sw, although further studies must be done due to the

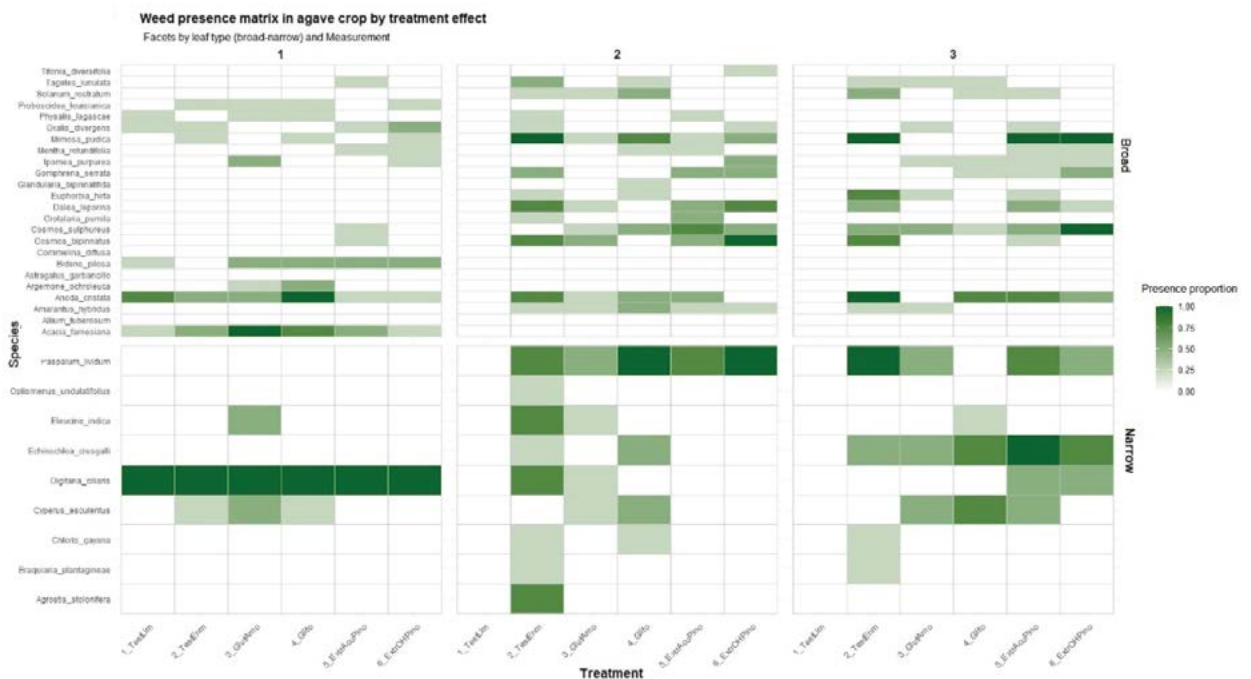


Figure 7. Presence of species per Measurement (1, 2, 3) and Treatment. Trat 1: Clean control, Trat 2: Weedy control, Trat 3: Glufosinate ammonium, Trat 4: Glyphosate, Trat 5: Aqueous pine extract, Trat 6: Alcoholic pine extract.

existence of ecotypes. Glufosinate ammonium is used to control weeds that are resistant to glyphosate (Takano and Dayan, 2020), and in this study species with resistance to both herbicides applied were found, such as those mentioned before. The total species in the aqueous pine extract were 31, and from these only eight could be controlled (*Proboscidea lousitanica* (Mill.) Thell., *Argemone ochroleuca* Swet, *Eleusine indica* L., *Chloris gayana* Kunt and *Agrostis stolonifera* L., among others) and there were 25 species reported for the ethanolic pine extract, of which 15 could be controlled (*Physalis lagascae* Roem. & Schult, *Argemone ochroleuca*, *Cyperus esculentum* L. and *Eleusine indica*, among others); regarding the 33 species reported in the treatment with glyphosate, nine could be controlled (*Oxalis divergens* Benth. & Lindl., *Cosmos bipinnatus* Cav., *Dalea leporine* (Ait.) Bullock and *Crotalaria pumila* Ort.).

Regarding plant herbicides, it is reported that when *Pinus caribean* Morelet var. *caribaea*. needles decompose, they form a compound called pycnogenol that can inhibit the germination of some plants considered weeds, since this compound inhibits the activity of the indole-acetic-oxidase acid, preventing seeds from germinating (Jiménez Ferrer *et al.*, 2006). For *Pinus patula*, ethanolic extract components reported include 62.4% of monoterpene hydrocarbons, α -pinene (35.2%), and β -phellandrene (19.5%) (Amri *et al.*, 2011). However, as a second study, it is necessary to evaluate the compounds of the extract. In a study, CO₂ was used to apply herbicides with pine oil and ensure the entry of the organic herbicide to the vascular system of the weed; it was reported that the pine oil had a good performance for weed control (James *et al.*, 2002).

Analysis of the agave plant

Concerning the statistical analysis of the parameters measured in the agave plant, the results showed significant differences between treatments (Table 2, leaf width, 4a, detached leaves, 4b, leaf length, 4c, plant height), which indicate that the treatments applied exert a different effect on the growth variables of the agave plant found.

Table 2. Analysis of variance for measurements repeated with non-parametric analyses of the variable leaf width.

	Error	DF	Df. res	F value	Pr(>F)
Measurement	Withn	1	2	5.7250	0.1391
Treatment	Withn	5	472	3.3291	0.00576**
4a. Repeated measures analysis of variance with non-parametric analysis for the detached agave leaves variable					
	Error	DF	Df. res	F value	Pr(>F)
Measurement	Withn	1	2	0.21875	0.68601
Treatment	Withn	5	472	2.64343	0.02267*
4b. Repeated measures analysis of variance with non-parametric analysis for the leaf's length variable					
	Error	DF	Df. res	F value	Pr(>F)
Measurement	Withn	1	2	4.2061	0.1767
Treatment	Withn	5	472	2.5963	0.024857*
4c. Repeated measures analysis of variance with non-parametric analysis for the plant height variable					
	Error	DF	Df. res	F value	Pr(>F)
Measurement	Withn	1	2	4.8333	0.1589
Treatment	Withn	5	472	2.4689	0.0318*

The four parameters measured indicate significant differences in all the parameters, although when the post hoc analysis is conducted (Figure 8), differences are only found in the variable detached leaves. It is seen that the treatment with glyphosate has greater leaf detachment, and this is explained because glyphosate is the only treatment that was not applied on the plant, because of its well-known effects, so there was no toxic effect on the crop; glyphosate has been used in other crops and it affects the seedlings due to the movement of this product in the soil and its contact with roots (Tofiño Rivera *et al.*, 2020). In addition, in this study, when it comes to plant growth, no impact was observed with the variables measured; this is because it is applied on the roads and with a hood, taking care that the glyphosate does not touch the plants. So its effect on its physiology is non-existent in the time of observation, although it was also observed that it does not affect the roots; this could be because of the low electrical conductivity recorded in dS/m (0.1) which was obtained with the application of this herbicide. The pH found was 6.03, or due to the low amount of organic matter present in the plot soils; something to remember is that the soils are clayey Luvisols, and another aspect to mention is that the pH interferes in the water quality and at the same time in the efficiency of the herbicide, where if the water is more alkaline, the efficiency decreases (García Ruiz and Sánchez Ortiz, 2005).

For the aqueous pine extract, these values were EC (0.7) and pH (6.43), and this treatment was the one that resulted in the lowest number of leaves detached, which could be due to a higher concentration of salts, given the EC recorded and this could cause a slight osmotic stress in the plant, in addition to the pH closer to 7 that can help to achieve better nutrient absorption; the compounds obtained are also water-soluble and possibly more persistent. When it comes to the ethanolic extract, with EC (0.8) and pH (6.7), in this

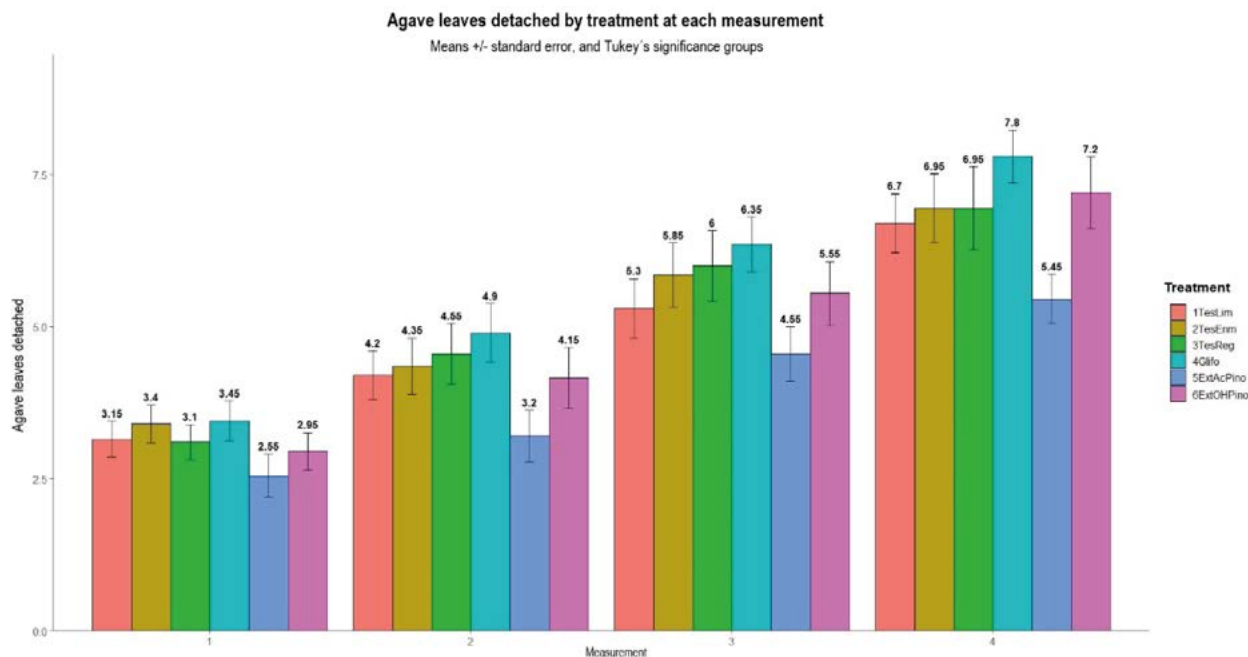


Figure 8. Means comparison of leaves detached in four months of records. 1TesLim: Clean control, 2TesEnm: Weedy control, 3TesReg: Glufosinate ammonium, 4TGliF: Glyphosate, 5ExAcPino: Aqueous pine extract, 6ExOHPino: Alcoholic pine extract.

case the compounds that were obtained are more volatile, so they exert their ephemeral allelopathic effect. The compounds that are obtained with the different solvents depend on their affinity with the polarity of the solvent; ethanol is catalogued by Adam *et al.* (2022) as one of the best solvents to extract compounds from the pine needles. Regarding the regional control, which was glufosinate ammonium, the EC was 0.16 and the pH 6.37; for the clean control where the weeds were only removed manually, the EC was 0.13 and the pH 6.3; the weedy control showed EC of 0.11 and pH of 6.67. The variations caused by the application of the treatments are slight and a study should be conducted considering the persistence and the identification of the metabolites extracted. However, this is a first approximation to the activity of *Pinus patula* needle extract in the field. This variation in pH could affect the soil microbiota, not only the variation but also the application of glyphosate, since the shikimate pathway that this herbicide impacts is in the metabolism of microorganisms, so they are also affected (González Ortega *et al.*, 2022), and this aspect should be studied further.

Regarding the costs that each treatment represents (Table 3), the extracts have a low cost, and what increases the cost is the workday that must be paid for any application. And given the total cost and the efficacy of glyphosate, it continues to be first place in use.

Table 3. Cost of treatments applied.

Treatments	Cost of supplies/ Daily labor cost	Total
1-Clean control	\$0.00/\$500	\$500.00
2- Weedy control	\$0.00/\$0.00	\$0.00
3- Glufosinate-ammonium	\$797.00/\$500	\$1,297
4- Glyphosate	\$350.00/\$500	\$850.00
5- Aqueous pine extract	\$5.00/\$500	\$505.00
6- Alcoholic pine extract	\$45.00/\$500	\$545.00

CONCLUSIONS

This is the first report of phytotoxic activity for *Pinus patula*. The pine extract with both solvents, in the dose used in this study, showed phytotoxic symptoms in weeds such as stunting, chlorosis, and to a lesser extent, necrosis, as well as a decrease in emergence. The aqueous and ethanolic pine extracts cause changes in number of species and coverage of weeds. The extracts from pine needles with both solvents and in the dose used do not match the effect of a synthetic herbicide. The variation in their performance in the last month of record, even in grasses, suggests that they are extracts that should still be studied. The species *Digitaria ciliaris*, *Paspalum lividum* and *Chloris virgata* are reported as not being able to be controlled with glyphosate or glufosinate ammonium. The type of leaf (broad or narrow) is determinant in the efficacy of herbicides.

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