

Bioherbicidal Potential of Some Plant Extracts in Weed Control in West-Cameroon

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Abstract— The use of herbicides poses problems of multifaceted environmental pollution, toxicity and depletion of microflora and microfauna in the soil. Farmers and environmentalists are powerless in the face of this problem. However, certain plant extracts are known for their allelopathic properties through their allelochemicals, which is primarily demonstrated by their effectiveness on weeds. It is in this context that the present study was conducted, with the aim of evaluating the bioherbicidal efficacy of extracts of *Cupressus lusitanica*, *Bambusa vulgaris* and *Ricinus communis* on *Bidens pilosa*, a weed frequently found in cultivated plants and which causes enormous crop losses. To achieve this objective, 75 seeds of these weeds were sterilised using 0.5% sodium hypochlorite for 2 minutes. Subsequently, 15 Petri dishes were prepared with plant extract, then filter paper was moistened with 2 ml of distilled water and placed at the bottom of each Petri dish. Seventy-five (75) previously sterilised weed seeds were placed in each of these Petri dishes at a rate of 5 per dish. Each seed was sprayed with 1 ml of each extract at concentrations of 7.5, 15 and 30 mg/ml. The Petri dishes containing distilled water and those sprayed with the herbicide at the manufacturer's recommended dose were used as negative and positive controls, respectively. All of the dishes were placed in a growth chamber at $23 \pm 1^\circ\text{C}$ in the dark. From the second day of incubation, the seeds were carefully observed for any signs of germination, and when this was visible, it was counted and noted, and the lengths of the stems, the number of leaves and their surface areas were measured. The highest concentrations of the extracts that had shown some efficacy in vitro were brought back to the field for bioherbicide testing under pre-emergence conditions.

The results showed that all three plant extracts significantly inhibited the germination of *Bidens pilosa* seeds compared to the negative control. However, the percentage of inhibition of weed seed germination was significant at the highest concentrations. Thus, concentrations of 30 mg/ml of the three plant extracts were the most effective, with no germination of *Bidens pilosa* seeds, i.e. a rate of 0%. On the other hand, the highest germination rates were observed with concentrations of 7.5 and 15 mg/ml. These rates varied between 26.67 and 80% and were similar to those of the negative controls according to Duncan's 5% test. Of all three concentrations of the different plant extracts tested in the field, only those of *Cupressus lusitanica* were the most effective in significantly destroying the leaves of *Bidens pilosa*.

These results suggest the possibility of using extracts from this plant at a concentration of 30 mg/ml to control this weed, however further studies are needed to complete the information associated with this.

Keywords— Bioherbicide, plant extracts, *Cupressus lusitanica*, *Bambusa vulgaris*, *Ricinus communis*, weed.

I. INTRODUCTION

Weeds are one of the main causes of crop losses. These adventitious plants can compete with cultivated plants for nutrients or serve as shelters for pathogens that can attack these plants and cause considerable economic damage. In response to this damage, several weed control methods are available, including mechanical and thermal methods. However, these methods have proven ineffective, and farmers are still forced to resort to chemical herbicides. Furthermore, the application of these chemical herbicides on weeds poses risks to consumers and the environment, in addition to the development of resistant weeds (Uddin *et al.*, 2014; Sofiene, 2020). Furthermore, some herbicides are limited on the market and others, such as glyphosate, are gradually being withdrawn from the market. However, public demand for alternative herbicides that are biological in origin (bioherbicides) and more environmentally friendly is growing. Medicinal plants are known for their bioherbicidal properties and low or no toxicity. Researchers have been exploring this avenue for some time with a view to formulating bioherbicides that are safe for consumers and environmentally friendly. In fact, the work of Sofiene Ben Kaab (2020) has shown that *Rosmarinus officinalis* essential oil and *Cynara cardunculus* extract have high bioherbicidal activity compared to extracts of *Trifolium incarnatum* (*T. incarnatum*), *Sylibum marianum* (*S. marianum*) and *Phalaris minor*, which showed low activity on weeds.

Similarly, Claudia *et al.* (2022) on the bioherbicidal activity of *Campomanesia lineatifolia* seed extract on the weed *Sonchus oleraceus* L. showed a 100% incidence of chlorosis and necrosis symptoms with foliar application of this plant extract, but these applications did not result in complete plant death. However, all of these previous studies are limited in terms of both the plant extracts tested and the number and diversity of weeds on which they were tested. It is in this context that the present work was carried out with the aim of evaluating the bioherbicidal of plant extracts on *Bidens pilosa* under pre- and post-germination conditions.

II. MATERIALS AND METHODS

2.1 Preparation of plant extracts:

The young leaves of the harvested plants were dried in the shade for two weeks, then finely ground in a mill, and the resulting powder was stored in jars away from light until use. Extraction was carried out at the University of Dschang's Research Unit for Microbiology and Antimicrobial Substances (URMSA), using ethanol as the extraction solvent for each plant. The extracts were obtained by macerating 250 g of powder from each plant for 48 hours in 500 ml of solvent. The mixture was first filtered using coffee filter paper and then Whatman No. 1 filter paper. The residues from the various ethanol extracts were placed in an evaporative rotator flask for partial evaporation of the solvent (ethanol) before being completely dried in an oven at a temperature of 40°C. The yield of the extract (Rdt%) was calculated using the following formula (Njimah, 2022)

$$\text{Rdt (\%)} = M_1/M_0 \times 100 \quad (1)$$

Where M_0 is the mass of the processed plant material, M_1 is the mass of the extract, and Rdt is the extraction yield.

2.2 Evaluation of the bioherbicidal activity of extracts from *Cupressus lusitanica*, *Bambusa vulgaris* and *Ricinus communis* on pre-emergence weeds

The *Bidens pilosa* seeds were sterilised using 0.5% sodium hypochlorite for 2 minutes. Subsequently, 15 Petri dishes were prepared with plant extract, then filter paper was moistened with 2 ml of distilled water and placed at the bottom of each Petri dish. Seventy-five (75) previously sterilised weed seeds were placed in each Petri dish at a rate of 5 per dish. Each seed was sprayed with 1 ml of each extract at concentrations of 7.5, 15 and 30 mg/ml. The Petri dishes containing distilled water and those sprayed with the herbicide at the manufacturer's recommended dose were used as negative and positive controls, respectively. All of the dishes were placed in a growth chamber at $23 \pm 1^\circ\text{C}$ in the dark. From the second day of incubation, the seeds were carefully observed for any signs of germination, which were counted and noted when visible. The length of the stem and the number of leaves were then measured until the seventh day using a graduated ruler (mm). The percentage of inhibition was determined using the following formula:

$$\text{Percentage inhibition (\%)} = n/N \times 100 \quad (2)$$

Where n represents the number of seeds that germinated, and N represents the total number of seeds sown.

2.3 Evaluation of the bioherbicidal activity of plant extracts on weeds under post-emergence conditions:

Twenty-four (24) *Bidens pilosa* seeds were sown separately in bags containing soil that had been disinfected in a Chamberlain autoclave at 121°C for 15 minutes. The seeds were watered daily from the second day onwards and after germination and plant development until 2 to 3 leaves appeared. Nine of these adventitious plants were sprayed with each plant extract at concentrations of 7.5, 15 and 30 mg/ml. Three others were also watered with 1 ml of water, and those treated with herbicides served as negative and positive controls, respectively. The experiment was carried out in triplicate. Data on the development of physiological parameters such as necrosis were recorded from the second day of treatment until the seventh day. The data collected were used to calculate the percentage of efficacy according to the following formula:

$$(\%) \text{ Efficacy} = N / T \times 100 \quad (3)$$

Where N represents the number of necrotic or wilted leaves, and T represents the total number of leaves.

III. RESULTS AND DISCUSSION

3.1 Characteristics and yield of plant extracts:

The table below shows the characteristics and yield of the plant extracts used for the bioherbicide test. This yield varied depending on the extract, the harvest period and the harvest location. The table shows that *Bambusa vulgaris* extracts had the highest extraction yield (11.11). On the other hand, the lowest yields were obtained with *Cupressus lusitanica*. In terms of appearance and colour, *Cupressus lusitanica* was creamy with a brown colour, while *Bambusa vulgaris* was thick and black. *Ricinus communis* had a black-brown colour and a thick appearance. The variation observed in yields could be attributed, on the one hand, to intrinsic factors of the plant and, on the other hand, to the plant species, the period of the plant's vegetative cycle and/or the plant family. Indeed, Smallfield (2001) reported that environmental conditions, harvest period and age of plant material can influence extraction yields. Djeugap *et al.* (2011) showed that certain plant families offer high yields compared to others that give low yields: *Annonaceae*, *Myrtaceae*, *Asteraceae* and *Zingiberaceae* generally offer very high yields, unlike *Poaceae* (*Cymbopogon citratus*), which has very low yields.

TABLE 1
CHARACTERISTICS AND YIELD OF PLANT EXTRACTS USED

Plants used	Extraction yield	Physical appearance	Color
<i>Bambusa vulgaris</i>	11,11	Thick	black
<i>Ricinus communis</i>	7,2	thick	black brown
<i>Cupresus lustanica</i>	7,45	creamy	brown

3.2 Effect of extract concentrations on germination percentage:

Table 2 presents the results of the effect of ethanol extracts of *Cupressus lusitanica*, *Rinusus communis* and *Bambusa vulgaris* on the germination of *Bidens pilosa* seeds on the 7th day. In general, all three plant extracts significantly inhibited the germination of *Bidens pilosa* seeds compared to the negative control. The work of Sofiene Bens Kaab (2020) on the study of the herbicidal potential of plant extracts from Tunisian xero-halophytic species and the determination of their modes of action revealed that phenolic extracts from *Cynara cardunculus* and essential oil from *Rosmarinus officinalis* have bioherbicidal properties. The percentages of inhibition of weed seed germination were significant at the highest concentrations. Thus, concentrations of 3 mg/ml of extracts from three plants were the most effective, showing the lowest germination rate of *Bidens pilosa* seeds, i.e. 0%. These results are similar to those of Melakhessou *et al.* (2023), who also noted in their experiment that the inhibition of *Bromus rubens* and durum wheat growth increases as the concentration of the extract applied increases. On the other hand, the highest germination rates were observed with concentrations of 7.5 and 15 mg/ml. These rates varied between 26.67 and 80% and were similar to those of the negative controls according to Duncan's test at 5%.

However, weed seeds treated with Supermachette herbicide (positive controls) at the concentration recommended by the manufacturer showed high germination rates (73.33%) on the seventh day of the experiment (Figure 1). Nevertheless, although these seeds treated with herbicides germinated, they did not grow but instead withered and dried out (Table 3). This trend suggests that the herbicide used does not act on germination but rather on the plant itself. This could also be explained by the fact that the various seeds germinated before the various active ingredients in the herbicide penetrated the weed seeds, especially since their walls did not facilitate the rapid entry of these active ingredients. These observations are consistent with those of Macharia & Peffley (1995), who found in their work that winter onion extract (*Allium fistulosum*) does not affect the germination of *Kochia scoparia* seeds, although it significantly reduces the biomass of these seedlings.

TABLE 2
EFFECTS OF DIFFERENT PLANT EXTRACTS ON THE GERMINATION RATE OF WEED SEEDS

Concentrations (mg/ml)	Plant Extracts		
	<i>Cupressus lusitanica</i>	<i>Ricinus communis</i>	<i>Bambusa vulgaris</i>
7,5	26,67±11,55 ^{bA}	53,33±11,55 ^{bAB}	60,00±20,00 ^{bB}
15	53,33±11,55 ^{cAB}	80,00±20,00 ^{cBC}	13,33±11,54 ^{aA}
30	00,00±00,00 ^{aA}	00,00±00,00 ^{aA}	00,00±00,00 ^{aA}
T+	73,33±11,55 ^{dBC}	73,33±11,55 ^{bcBC}	73,33±11,55 ^{bcBC}
T-	93,33±11,55 ^{eC}	93,33±11,55 ^{eC}	93,33±11,55 ^{eC}

a, b, c and d: comparison of the percentages of inhibition of weed seed germination by plant extracts. Averages marked with the same letter in the same row are not significantly different according to Duncan's test at 5%. A, B, C and D: comparison of the percentages of inhibition of weed seed germination by plant extracts. Averages marked with the same letter in the same column are not significantly different according to Duncan's test at 5%. T+: Positive controls (Supermachette herbicide at a concentration of 5.33 mg/ml); T-: Negative controls (1 ml of distilled water)

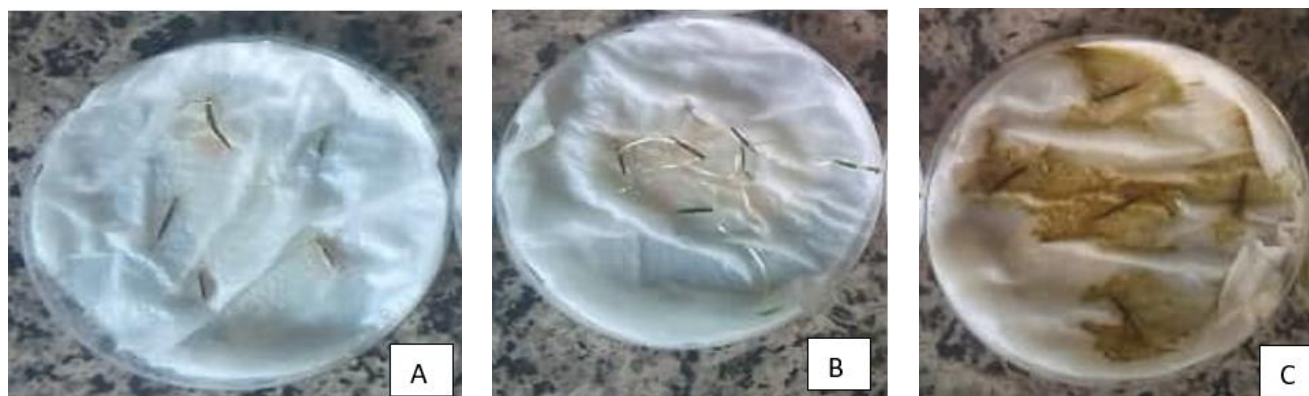


FIGURE 1: Seeds of the weed that underwent different treatments; (A): Seeds treated with Supermachette herbicide (positive control); (B): Seeds that underwent no treatment (negative control) and (C): Seeds treated with *Cupressus lusitanica* extracts at a concentration of 30mg/ml

3.3 Effect of different concentrations of extracts on the length of *Bidens pilosa* stems:

On the seventh day of the experiment, we also evaluated the effect of different concentrations of plant extracts on the length of the weed stems, and the results are presented in the table below. We observed a significant difference in the length of the stems of *Bidens pilosa* seedlings treated with different concentrations of ethanol extracts compared to those treated with herbicide and water. The seeds treated with the highest concentration of the three plant extracts had the shortest stems, similar to those of the positive controls. This part of the weed appears to be one of the most sensitive to the various plant extracts. These results corroborate those of previous studies, which also showed that bioherbicidal activity increases with the concentration of the plant extracts used (Lamia *et al.*, 2017 & Melakhessou *et al.*, 2023).

TABLE 3
EFFECT OF DIFFERENT CONCENTRATIONS OF EXTRACTS ON THE LENGTH OF *BIDENS PILOSA* STEMS

Concentrations (mg/ml)	Plant extracts		
	<i>Cupressus lusitanica</i>	<i>Ricinus communis</i>	<i>Bambusa vulgaris</i>
7,5	02,77±00,49 ^b	03,97±00,45 ^c	02,63±01,29 ^b
15	02,58±00,73 ^b	02,80±01,00 ^{bc}	00,17±00,29 ^a
30	00,00±00,00 ^a	00,62±00,60 ^a	00,00±00,00 ^a
T+	00,62±00,60 ^a	00,62±00,60 ^a	00,62±00,60 ^a
T-	03,17±00,40 ^b	03,17±00,40 ^b	03,17±00,40 ^b

a, b, c and d: comparison of the effect of plant extracts on the length of Bidens pilosa stems. Means marked with the same letter in the same row are not significantly different according to Duncan's test at 5%. T+: Positive controls (Supermachette herbicide at a concentration of 5.33 mg/ml); T-: Negative controls (1 ml of distilled water)

3.4 Effect of different concentrations of extracts on the number of leaves of *Bidens pilosa*:

Table 4 shows the effect of different concentrations of extracts on the number of *Bidens pilosa* leaves. This table shows that all extracts significantly reduced leaf growth. However, all three extracts at the highest concentration (30 mg/ml) completely prevented the development of weed leaves according to Duncan's 5% test compared to the positive controls. These results are similar to those of Fatima *et al.* (2011), who demonstrated that at high concentrations, *Peganum harmala* extracts had a suppressive effect on weeds.

TABLE 4
EFFECT OF DIFFERENT CONCENTRATIONS OF EXTRACTS ON THE NUMBER OF LEAVES OF *BIDENS PILOSA*

Concentrations (mg/ml)	Plant Extracts		
	<i>Cupressus lusitanica</i>	<i>Ricinus communis</i>	<i>Bambusa vulgaris</i>
7,5	01,17±00,29 ^b	01,89±00,20 ^b	01,83±00,29 ^b
15	01,61±00,35 ^{bc}	01,83±00,29 ^b	01,33±01,15 ^b
30	00,44±00,76 ^a	00,00±00,00 ^a	00,00±00,00 ^a
T+	02,00±00,00 ^c	02,00±00,00 ^c	02,00±00,00 ^c
T-	02,00±00,00 ^c	02,00±00,00 ^c	02,00±00,00 ^c

a, b and c: comparison of leaf growth of weed seeds treated with plant extracts. Averages marked with the same letter in the same row are not significantly different according to Duncan's test at 5%. T+: Positive controls (Supermachette herbicide at a concentration of 5.33 mg/ml); T-: Negative controls (1 ml of distilled water).

3.5 Effectiveness of the most effective plant extract concentrations in pre-germination conditions on weeds in post-germination conditions:

The table below (Table 5) shows the effect of the highest concentration of the three plant extracts on the leaves and the areas of damage caused by each extract. This table shows that all three extracts damaged the weed leaves to the same extent as the positive control. With regard to the lesion area, no lesions were observed on the leaves treated with *Bambusa vulgaris* extracts. However, the other two extracts significantly reduced the leaf surface area, with lesion areas between 0.23 and 0.24 cm² greater than those of the negative controls but less than those of the positive controls according to Duncan's 5% test (Figure 2). Bens *et al.* (2020) also showed that *Cynara cardunculus* extracts and *Rosmarinus officinalis* essential oil had bioherbicidal properties under post-germination conditions. Similarly, the work of Claudia *et al.* (2022) on the bioherbicidal activity of *Campomanesia lineatifolia* seed extract on the weed *Sonchus oleraceus* L. showed a 100% incidence of chlorosis and necrosis symptoms with foliar application of this plant extract, but these applications did not result in plant death. The bioherbicidal activity observed in *Cupressus lusitanica* could be attributed to its high content of secondary metabolites. (Njimah *et al.*, 2024) had already reported in their work that extracts from this plant are rich in secondary metabolites such as tannins, phenols, flavonoids, saponins, triterpenoids, anthocyanins and anthraquinones.

TABLE 5

EFFECTIVENESS OF THE 30 MG/ML CONCENTRATION OF THE THREE PLANT EXTRACTS ON THE LEAVES AND LESION AREAS (cm²) INDUCED BY EACH EXTRACT ON THE FIFTH DAY OF TREATMENT

Treatments	Efficacy of extracts (%)	Lesion area (cm ²)
<i>Cupressus lusitanica</i>	02,78±01,07 ^{bc}	00,24±00,05 ^b
<i>Ricinus communis</i>	02,67±01,15 ^{bc}	00,23±00,03 ^b
<i>Bambusa vulgaris</i>	01,17±00,29 ^{ab}	00,00±00,00 ^a
T+	03,33±01,33 ^c	100,00±00,00 ^c
T-	00,00±00,00 ^a	00,00±00,00 ^a

a, b and c: comparison of the effectiveness of the extracts on the number of leaves and on the surface area of lesions induced on the leaves of weeds after spraying with plant extracts. Means marked with the same letter in the same row are not significantly different according to Duncan's test at 5%. T+: Positive controls (Supermachette herbicide at a concentration of 5.33 mg/ml); T-: Negative controls (1 ml of distilled water)



FIGURE 2: Lesion areas caused by *Cupressus lusitanica* extracts on *Bidens pilosa* leaves at a concentration of 30 mg/ml, (A): Positive and negative controls, (B): Plants treated with *Cupressus lusitanica* extracts and (C): Lesion area induced by *Cupressus lusitanica* extracts on leaves on the fifth day of treatment

IV. CONCLUSION

Having completed this work, the objective of which was to evaluate the bioherbicidal efficacy of extracts of *Cupressus lusitanica*, *Bambusa vulgaris* and *Ricinus communis* on *Bidens pilosa*, a weed frequently found in cultivated plants and causing enormous crop losses, it appears that all three plant extracts significantly inhibited the germination of *Bidens pilosa* seeds compared to the negative control. However, the percentages of inhibition of weed seed germination were significant at the highest concentrations. Thus, concentrations of 30 mg/ml of the three plant extracts were the most effective, with no germination of *Bidens pilosa* seeds, i.e. a rate of 0%. On the other hand, the highest germination rates were observed with concentrations of 7.5 and 15 mg/ml. These rates varied between 26.67 and 80% and were similar to those of the negative controls according to Duncan's 5% test. Of all three concentrations of the different plant extracts tested in the field, only those from *Cupressus lusitanica* were the most effective in significantly destroying *Bidens pilosa* leaves compared to those from *Bambusa vulgaris*, *Ricinus communis* and the negative controls. These results suggest the possibility of using extracts from this plant at the recommended concentration to control this weed, but further studies are needed to supplement the data obtained in this research.

CONFLICT OF INTEREST STATEMENT

Authors declare that they have no conflict of interest.

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