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Research article

Allelopathic potential of *Petiveria alliacea* L.

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Abstract – *Petiveria alliacea* (Phytolaccacea) is a herbaceous plant of great importance in traditional medicine. It has been reported to be effective as an insecticide and acaricide; however, its allelopathic activity is unknown. The objective of this study was to evaluate its allelopathic activity in seeds of *Triticum aestivum*, *Oriza sativa*, *Lactuca sativa* and *Amaranthus hypochondriacus*. Tests were performed with different concentrations of the aqueous, methanolic and dichloromethanic leaf extracts. Total germination (G_t), accumulated germination velocity (AGV), length of the aerial portion (LAP) and radicular length (RL) were evaluated. It is demonstrated that the the organic extracts were more phytotoxic than the aqueous extract, producing a significant effect on lettuce and amaranth. However, in the evaluation carried out in the soil, no phytotoxic effect was observed in the germination of lettuce seeds. Therefore, the tested extracts of *Petiveria alliacea* were moderately phytotoxic.

allelopathy / phytotoxicity / *Petiveria alliacea* / *Phytolacaccea*e germination / extracts

1. INTRODUCTION

The potential damage of herbicides to human health has induced a great interest in carrying out intense research in this area. Alternatives such as the search for biodegradable compounds with herbicidal activity (Dudai et al., 1999) have been proposed. Therefore, the study of allelopathic activity offers useful clues in the investigation of new models of natural herbicides which are more specific and less harmful than the synthetic substances used in agriculture (Macías, 1992).

The literature describes the allelopathic activity of numerous plants (Chou et al., 1998; Dudai et al., 1999). However, the great diversity of plants on our planet justifies the need to investigate other species which have never been studied, especially those, such as *Petiveria alliacea*, which have been studied in their growing habitat, reporting diverse biological activities, being the point of departure of the present research.

P. alliacea is also known as skunk weed due to its characteristic odor resulting from the presence of sulfurate compounds (De Sousa et al., 1990). It belongs to the Phytolaccaceae family, which is widely distributed in Mexico, mainly in the states with warm climate, such as Veracruz, Yucatan and Guerrero, among others. Various uses are attributed to this plant, such as: antirheumatic, anticarcinogenic, antiflu, antitussive, analgesic and antiinflammatory (Villar et al., 1997). Insecticidal and acaricidal activities have also been reported, as well as bacterial (*Pasteurella multocida*), plaquetary antiaggregant, and inhibitory activity against the fungi *Cladosporium cladosporioides, Cladosporium sphaerospermum* and *Saccharomyces cerevisiae* (Benevides et al., 2001).

Based on the above, the purpose of the present study was to evaluate the allelopathic activity of aqueous and organic leaf extracts in four species of seeds.

2. MATERIALS AND METHODS

2.1. Plant samples

The plant material (leaves) was gathered from various adult specimens randomly distributed in the town of Rodríguez Clara, located in the southern part of the state of Veracruz. This area has a warm climate and is located at 18°00" latitude North and 95°24" longitude West, at an altitude of 95 m, with an average temperature of 25 °C and 1 266 mm average annual rainfall. The material was certified in the national herbarium (MEXU) of the Universidad Nacional Autónoma de México, and assigned the voucher number 1042314.

2.2. Preparation of extracts

The plant material was dried at room temperature and mechanically ground to obtain the aqueous and organic extracts. To prepare the different concentrations of aqueous

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extracts, the leaf was macerated for 24 h at room temperature. The organic extracts were obtained by macerating the plant material with methanol for three days at room temperature, and the dichloromethane extract was prepared in a similar way. The resulting raw extracts were obtained by evaporating the solvent under reduced pressure.

2.3. Treatments

From the aqueous extract, six treatments of different concentrations (0.0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0% w/w) were prepared: distilled water was used as control. The different treatments for each one of the organic extracts were obtained from a stock solution which had been previously prepared from each of the raw extracts. The concentrations were 0.0, 0.5, 1.5, 2.5, 3.5 and 5.0% v/v for the methanolic extract and 0.0, 0.5, 1.5, 2.5 and 3.5% v/v for the dichloromethane extract.

2.4. In vitro biotest

The treatments were tested on seeds of two species of monocotyledoneae; wheat (*Triticum aestivum* L.) and rice (*Oriza sativa* L.), and two species of dicotyledoneae; lettuce (*Lactuca sativa* L.) and amaranth (*Amaranthus hypocondriacus* L.). Viability tests were performed prior to the biotest, selecting those with a 95% viability. The experiment of phytotoxicity was carried out according to the method described by Anaya et al. (1999); 2.5 mL of each solution of aqueous extract were added separately over No. 1 Whatman filter paper to Petri dishes 10 cm in diameter, and 50 seeds per treatment were used. For the organic extracts, in contrast to the above, 1.5 mL of each concentration were added, and allowed to evaporate before depositing the seeds, and finally, 2.5 mL of distilled water were added to each dish. The control was prepared in a similar way with pure solvent, allowing it to evaporate. The treatments were placed in a germinator under continuous light conditions, 15.39 μmoL s^{-1} m^{-2}, 80% relative humidity and a temperature of 25 °C.

The variables used to evaluate the phytotoxicity according to Chiapusio et al. (1997) were total germination (Gt; percentage of germinated seeds) and accumulated germination velocity (AGV; number of seeds germinated per time). The experiment was carried out with four repetitions per treatment. The readings were made every 24 hours. The final reading was made according to the germination cycle established for each seed according to the International Rules for Seed Testing (Anónimo, 1976).

2.5. Biotest in the soil

Two tests were done; one with leaves which had been dried and ground, and the other with aqueous leaf extracts. According to the method proposed by Chou et al. (1998), the plant material in different proportions (0.0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g) was mixed separately with 10 g of argillaceous soil ("Vertisol pélico", pH = 7.87, OC = 1.19% and C/N = 10.9) for each treatment, collected at a depth of 15 cm in the Chapingo experimental field (located at 19°29' North latitude and 98°53' West longitude, and 2250 m above sea level with a mean rainfall of 691 mm) and previously dried and sieved to obtain particles of 2 mm in diameter, and to remove plant residues. Polypropylene boxes of 12 × 12 × 6 cm were used for the mixture. Soil moisture was maintained by adding distilled water at field capacity. After the decomposition period had passed (2, 4, 12, 24, 32 and 64 days for each treatment, corresponding to the lowest concentration, the shortest time of decomposition, and so on), 50 lettuce seeds were planted.

The second test was carried out by preparing different aqueous extracts (0.0, 0.5, 1.0, 2.0, 4.0 and 5.0% w/w) from the dried and ground plant material. In containers similar to those used in the previous test, 10 mL of each extract were mixed separately with 100 g of argillaceous soil; 50 lettuce seeds were planted afterwards.

Seven days after planting, measurements of the percentage of germination (Gt), length of the aerial portion (LAP) (primary leaves and stem), accumulated germination velocity (AGV) and radicular length (RL) were taken in both tests. The experiment was carried out with four repetitions per treatment at a temperature of 25 °C and 80% relative humidity.

2.6. Statistical analysis

The data obtained were analyzed, employing the ANOVA and a Tukey means comparison using SAS software, version 8.0, 2002.

3. RESULTS AND DISCUSSION

3.1. Percentage of in vitro germination

When the phytotoxicity of the aqueous extract was evaluated considering the percentage of inhibition of germination in the two species of monocotyledoneae seeds, it was found that in wheat and rice, there are statistical differences between treatments with respect to the control. In the lowest concentration of 0.5%, a percentage of inhibition of germination in rice of 4.6%, and 11.0% in wheat, was observed. When the same extract was tested in the dicotyledoneae seeds of lettuce and amaranth, from the concentration of 2.0% on, statistical differences were found between the treatments with respect to the control with percentages of inhibition of 31.0 and 10.6%, respectively (Fig. 1).

When this variable was compared with the highest concentration of 5.0% in the aqueous extract in the four species, it was found that the seeds of lettuce and amaranth presented 98.5 and 90.6% inhibition, and in rice and wheat 17.0 and 33.5%, respectively. However, the percentage of germination inhibition increased suddenly in lettuce and amaranth only with higher concentrations (4.0 and 5.0%, respectively) (Fig. 1). In the monocotyledonae, compared with the dicotyledonae there was not a tendency in the percentage of germination inhibition to increase as the concentration was increased.

There are numerous investigations with extracts of diverse species which consistently point to the phytotoxic and allelopathic effect of extracts and their components; notably, *Artemisia princes* (Bong-Seop and Kyeong, 1992), *Delonix regia* (Chou and Lih-Ling, 1992), *Melilotus messanensis* (Macías et al., 1997), sorghum (Einhelling and Souza, 1992), cruciferae (Steven and Boydston, 1997); specifically in Mexico *Piper auritum*

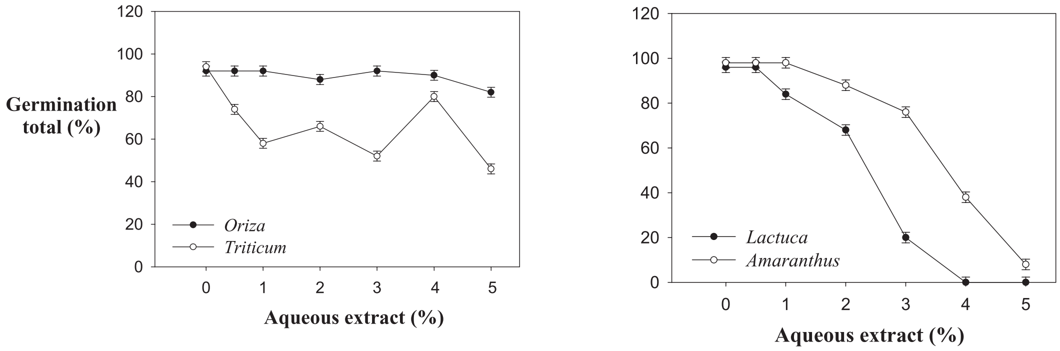

Figure 1. Effects of aqueous extract of *P. alliacea* on the germination total of the four species tested. ESTD according to Tukey's multiple range test ($P =$ 0.05).

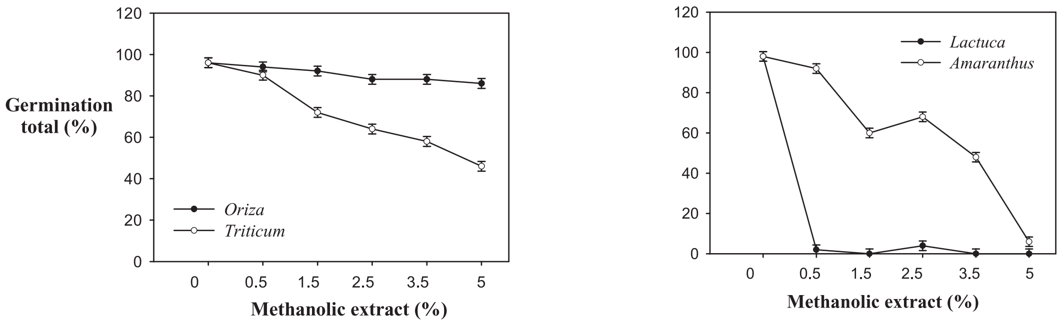

Figure 2. Effects of methanolic extract of *P. alliacea* on the germination total of the four species tested. ESTD according to Tukey's multiple range test ($P =$ 0.05).

(Anaya et al., 1974), *Ambrosia cumanensis* (Anaya and Del Amo, 1978), *Ipomea tricolor* (Anaya et al., 1990), *Malmea depressa* and *Stauranthus perforatus* (Anaya et al., 1994) and *Metopium brownie* (Anaya et al., 1999), among others. However, no documented studies have been found on the phytotoxic effects of *P. alliacea.*

A similar study carried out by Chou and Yang (1982) in which an evaluation was made of the toxicity of aqueous extracts of bamboo leaves (*Phyllostachys edulis*) and the conifer (*Cryptomeria japonica*) in seeds of lettuce, rice and rye grass, reports high toxicity of the bamboo extracts at a concentration of 5.0%, in contrast to that of the conifer. Therefore, it can be inferred that the aqueous extract of *P. alliacae* is also phytotoxic. The differences in germination observed in wheat, rice, lettuce and amaranth also coincide with what was described by Chou and Yang (1982), in which they point out that when they measured radicular growth and germination, differences were found not only among species, but also among varieties, although in this research only one variety per species was tested.

In the methanolic extract, significant differences were observed among treatments and the control of the four species. Contrary to the other species, lettuce seeds presented the greatest percentage of inhibition (97.6%) at the lowest concentration of 0.5%, which was 6.0, 10.0 and 6.6% for rice, wheat and amaranth, respectively (Fig. 2).

With respect to the extract of dichloromethane, no statistical differences were observed in rice and wheat, as opposed to the case of lettuce and amaranth, where differences were found starting with the lowest concentration, with a percentage of inhibition for the latter two species of 27.0 and 31.6%, respectively. As with the aqueous extract, in this case the percentage of inhibition of germination in lettuce and amaranth increased suddenly, but starting from the concentrations of 1.5 and 2.5%, respectively (Fig. 3).

An additional observation can be made with respect to the effect of the aqueous extract; in Table I, a comparison is made of the percentage of germination with the percentage of developing seedlings in rice, wheat, lettuce and amaranth. It is evident that not all of the germinated wheat and lettuce seeds presented optimum conditions for development; that is, there was lack of development of the apical meristem in roots and primary leaves. However, this was not true of the organic extracts. In the methanolic extract, there was germination and development of the seedlings of rice, lettuce and amaranth; only the length and aerial portion of wheat was affected. In the case of dichloromethane extract, none of these effects was observed.

When a comparison is made of the phytotoxic effect on germination of the three extracts on the four species, it is evident that the aqueous extract is the least toxic. Of the organic extracts, the methanolic extract was most toxic for the lettuce seeds and the dichloromethane was most toxic for amaranth.

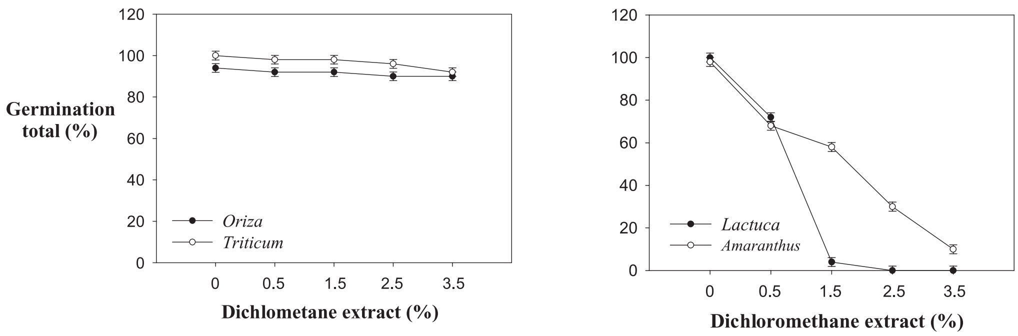

Figure 3. Effects of dichloromethane extract of *P. alliacea* on the germination total of the four species tested. ESTD according to Tukey's multiple range test ($P = 0.05$).

Table I. Germination percentage and developing seedlings of the four species tested at different concentrations of aqueous extract of *P. alliacea*.

	Aqueous extract							
	Oriza		Titricum		Lactuca		Amaranthus	
(%)	Gt (%)*	Developing seedlings (%)	Gt (%)*	Developing seedlings (%)	Gt (%)*	Developing seedlings (%)	Gt (%)*	Developing seedlings (%)
0.0	93.0a	100.0a	95.5a	100.0a	96.5a	100.0a	98.0a	100.0a
0.5	92.0ab	100.l0a	78.5ab	92.3ab	96.5a	100.0a	99.5a	100.0a
1.0	93.0a	100.0a	58.0ab	88.6b	85.0a	100.0a	99.5a	100.0a
2.0	88.0ab	100.0a	67.0ab	94.7ab	69.0b	44.2b	89.5ab	100.0a
3.0	92.0ab	100.0a	52.0b	18.3c	20.5c	0.0c	76.5b	100.0a
4.0	91.4ab	100.0a	81.0ab	2.5c	1.5d	0.0c	39.0c	100.0a
5.0	82.5b	94.5b	46.0b	0.0c	1.5d	0.0c	9.5d	100.0a

* Values in columns with the same letters are not significantly different at the $P = 0.05$ level by Tukey's test.

From the above results, it can be inferred that the germination of lettuce seeds was the most affected by the three extracts, possibly because they are the most sensitive. Furthermore, the dichloromethane extract selectively affected the dicotyledoneae, perhaps due to (1) low polarity nature; (2) the presence of metabolites of a different chemical nature; (3) its capacity to penetrate the seed and tissue; (4) dispersion and accumulation in the intracellular compartments, and (5) the different solubility of the fractions in water.

In the present study, the environmental conditions (temperature, humidity and light intensity) were maintained under strict control. The literature points out that physical factors could interact synergically with substances with allelopathic activity to produce more complex interactions; however, for this study these can be discarded.

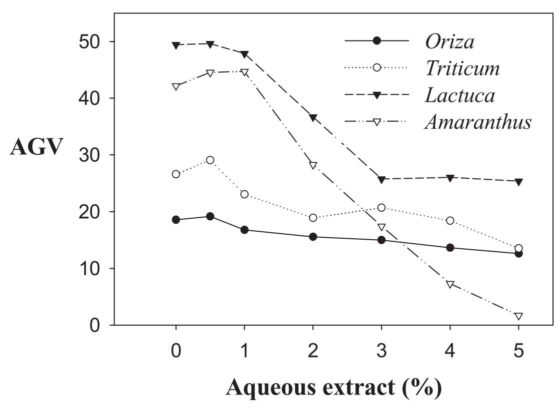

Figure 4. Effect of aqueous extract of *P. alliacea* on the accumulated germination velocity of the four species tested.

3.2. In vitro accumulated germination velocity

In addition, a calculation was made of the accumulated germination velocity (AGV) (Chiapusio et al., 1997), and it was found that for the aqueous extract, from a 2.0% concentration, a reduction of the AGV was observed only in amaranth. The organic extracts, methanol and dichloromethane had equal effect, with a drastic effect in lettuce and amaranth starting at the lowest concentration of 0.5 and 1.5%, respectively. Finally, wheat and rice showed a slight decrease when treated with both (Figs. 4–6); these results coincide with those observed for Gt, due to the fact that the Tukey means comparison presented a similar behavior for each index.

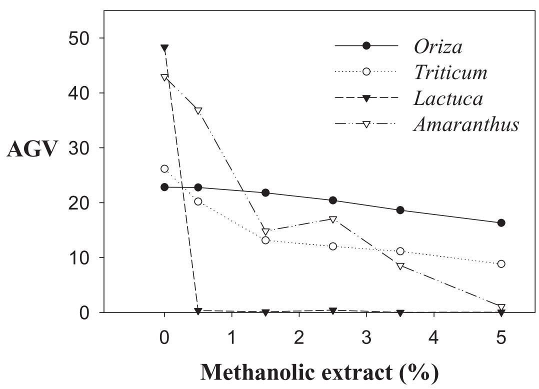

Figure 5. Effect of methanolic extract of *P. alliacea* on the accumulated germination velocity of the four species tested.

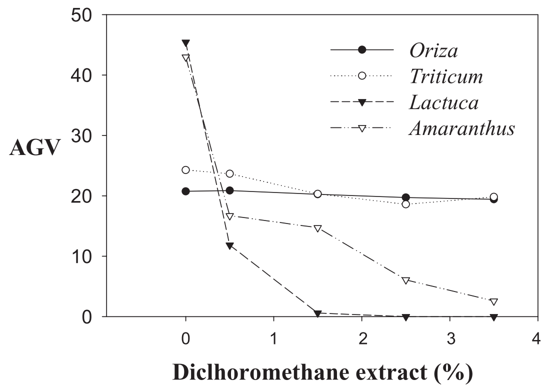

Figure 6. Effect of diclhoromethane extract of *P. alliacea* on the accumulated germination velocity of the four species tested.

On the other hand, although very low phytotoxicity was observed for the aqueous extract in comparison with the organic extracts, the prior has fewer ecological implications, for which there have been few studies done with extracts of an organic nature to establish comparisons, although the study of the organic extracts makes it possible to identify substances or metabolites, in some cases with a greater phytotoxic potential, that may serve as a model for the synthesis of new natural herbicides.

3.3. Biotest in the soil

The results of the phytotoxicity of the aqueous extracts and ground leaf mixed with soil in lettuce seeds are described. Although a slightly different tendency was observed, the statistical analysis demonstrated the absence of significant differences between treatments and the control, with respect to the percentage of germination for both. On the other hand, there were significant statistical differences between treatments and the control in the elongation of lettuce seedlings. Contrary to the effects observed in vitro, here the seedlings showed optimal elongation, making it possible to infer that the soil may be a determining variable in the phytotoxic effect of some extracts in the germination process, which coincides with what was pointed out by Chou and Yang (1982).

In addition, Chou and Kuo (1986) report that the mimosine isolated from leaves of *Leucaena leucocephala* inhibits cellular division and the synthesis of DNA, RNA and proteins of *Paramecium tetraurellia*, presenting an important phytotoxic effect in seeds of lettuce, rice, and some species of *Acacia, Casuarine, Liquidambar* and *Mimosa*. The literature describes different biological activity for *P. alliacea*; in this respect Malpezzi et al. (1994), after studying the development of the sea urchin embryo (*Lytechinus variegates*), also described a probable inhibition of DNA synthesis during cell division, which could justify the phytotoxic effect in lettuce, amaranth, rice and wheat seeds, described in the present investigation as indicated by Chou and Kuo (1986).

4. CONCLUSIONS

The calculation of the accumulated germination velocity made it possible to observe a severe phytotoxic effect of the organic extracts in lettuce and amaranth according to the concentration, finding the same effect in the evaluation of total germination, in contrast to the moderately toxic effect of the aqueous extract, possibly due to the chemical nature of the metabolites present in the extracts. Not all of the wheat and lettuce seeds that germinated in the aqueous extract developed into seedlings.

With respect to the studies of ground leaf and the aqueous extract in soil, no phytotoxic effect was observed in the total germination of lettuce seeds; however, in both studies the length of the aerial portion in lettuce seedlings was significantly affected. It can be concluded from the present investigation that the extracts tested of *Petiveria alliacea* were moderately phytotoxic.

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