


In vitro allelopathy of the aqueous extract of *Calathea lutea* on the initial growth of *Lactuca sativa* L


Alelopatía in vitro del extracto acuoso de *Calathea lutea* sobre el crecimiento inicial de *Lactuca sativa* L.

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ABSTRACT

To determine the *in vitro* allelopathic effect of the aqueous extract of *Calathea lutea* (Aubl.) E.Mey. ex Schult. (*bijao*) on the initial growth of *Lactuca sativa* L. (lettuce) variety White Boston. An extract at 15% w/v was prepared with each part of the *bijao* plant (stem, leaf, and root), it was left to stand for 48 hours, then extracts at 2, 4, 6% concentrations were obtained by dilution. 10 lettuce seeds/petri dishes were sown, irrigating daily with 5 ml of each concentration of the extract. The control treatment received distilled water. The research was adjusted to a Complete Random Design (DCA), with a 3A x 4B factorial arrangement (A = parts of the plant and B = concentration of the extract), with four repetitions. At 10 days after germination, it was statistically evaluated and demonstrated ($p \leq 0.05$) that the germination percentage (7.5%), root length (1.23 cm), stem fresh weight (0.0085 g), dry weight of the stem (0.0012 g), fresh weight of the radicle (0.0010 g), and dry weight of the radicle (0.0005 g), were significant in the interaction of leaf extract at 6% concentration. It is concluded that the aqueous extract of leaf of *Calathea lutea* showed an allelopathic effect as the concentration increased, on the initial growth parameters in lettuce seedlings.

Mimosa pudica

Key words: Allelopathy, aqueous extract, concentration, *bijao*, *Calathea lutea*.

RESUMEN

Con el objetivo de determinar el efecto alelopático *in vitro* del extracto acuoso de *Calathea lutea* (*bijao*) sobre el crecimiento inicial de *Lactuca sativa* (lechuga) variedad White Boston. Se preparó un extracto al 15 % p/v con cada parte de la planta (tallo, hoja y raíz) del *bijao*, se dejó reposar por 48 horas luego se obtuvo por dilución extractos al 2, 4, 6 % de concentración. Se siembra 10 semillas de lechuga/placa petri, regando diariamente con 5 ml de cada concentración del extracto. El tratamiento control recibió agua destilada. La investigación se ajustó a un Diseño Completo al Azar (DCA), con arreglo factorial 3A x 4B (A = partes de la planta y B = concentración del extracto), con cuatro repeticiones. A los 10 días después de la germinación, se evaluó y se demostró estadísticamente ($p \leq 0,05$) que el porcentaje de germinación (7.5%), longitud radicular (1.23 cm), peso fresco del vástago (0.0085 g), peso seco del vástago (0.0012 g), peso fresco de la radícula (0.0010 g), y peso seco de la radícula (0.0005 g), fueron significativos en la interacción extracto de hoja al 6 % de concentración, Se concluye que el extracto acuoso de hoja de *Calathea lutea* mostró un efecto alelopático a medida que se incrementó la concentración, sobre los parámetros de crecimiento inicial en las plántulas de lechuga.

Palabras clave: Alelopatía, extracto acuoso, concentración, *bijao*, *Calathea lutea*.

INTRODUCCIÓN

Currently, the agents causing the greatest yield losses in crops are weed plants. Although its influence is highly variable in the different plant production systems, losses of around 15 percent of agricultural production are recognized in developed countries and higher than 25-30 percent in developing countries (Urbano 2002). The cost for weed control in agriculture normally represents up to 10 percent of the cost of production (Martinez 2009). In Peru, 37.7 percent of agricultural producers use chemical products (INEI 2013). Considering the use of chemical products to be a problem, this implies that it must be replaced by other control methods that do not involve the use of herbicides (FAO 2007).

Allelopathy, is a term defined for the first time by Molish (1937) and is derived from the Greek words allelon (mutual) and pathos (prejudice), it is defined as the interaction between plants, or between other forms of life, through natural chemicals (allelochemicals); involving the direct inhibition or stimulation of one species by another. Based on scientific research, it is possible to take advantage of these natural substances to protect crops from certain pests, prevent their growth from being obstructed by weeds, and even accelerate their development (Sampietro 2003). These compounds are known as allelochemicals and can have beneficial effects (positive allelopathy) or detrimental effects (negative allelopathy) on recipient organisms (Suquilanda 2018).

Calathea lutea (*bijao*), which belongs to the Maranthaceae family, is a plant that grows and is generally found in humid forests, in open areas, and along rivers; Native to the Venezuelan Antilles,

Mexico, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, Honduras, Peru and throughout the American tropics (Quast 2008). In Peru it is distributed in areas of flooded forests from 0 - 1500 meters above sea level, they have been found in the regions of Amazonas, Cuzco, Huánuco, Junín, Loreto, Madre de Dios, San Martín and Ucayali. Known in the Ucayali region as white-breasted *bijao*, in Loreto as *huira bijao* (Vela et al. 2015). Aguirre et al. (2014) mentions the presence of natural substances such as coumarins, saponins, flavonoids, and a high content of phenolic compounds, including tannins. Studies carried out by Rodríguez et al. (2016), indicated that using the aqueous extract of leaves and stems of *Copaiba* (*Copaifera pubiflora*) at a concentration of 7 percent, caused 87 percent inhibition of the germination of the sensitive plant (*Mimosa pudica*) causing detrimental effects on the seedling height. In addition, with the preliminary tests, it was possible to determine the possible presence of secondary metabolites (coumarins, tannins, flavonoids, terpenoids) that are recognized for generating allelopathy.

In the same way, Ramirez (2014) mentions that using the aqueous extract of oat leaves (*Avena sativa*), at a concentration of 50 percent, he observed the effect of the plant, inhibiting the percentage of germination of the grass seeds of the buzzard (*Chenopodium murale*) and yuyo (*Amaranthus spinosus*) by 48 percent. While Laynez and Méndez (2013), using the aqueous extract of buttercup leaves (*Tithonia diversifolia*), caused 83 percent inhibition in the germination of *L. sativa* seeds, using concentrations of 1, 1.5, and 2 percent.

The increase in the use of *C. lutea* for various purposes such as food wrapping, crafts, medicine, and

the great importance in the industry due to the physical-chemical characteristics that the wax on the underside of the leaf presents, which is very similar to the wax of the palm (*Copernicia cerifera*), used in the production of bitumens, for which *Calathea lutea* can be an alternative resource for the manufacture of different products, floor waxes, furniture polish, bitumens, etc.

The wax of this species has also given excellent results for making patterns in dentistry (Suárez 2007). Among others, but mainly referring to the improvement in crop production, in Aguaytía it is associated with permanent crops (citrus, coffee, and cocoa) and in some areas of the Federico Basadre highway with temporary crops (banana and cassava), it is like this which requires a better understanding of its allelopathic potential to rule out its interference in agricultural production processes. Thus, the following

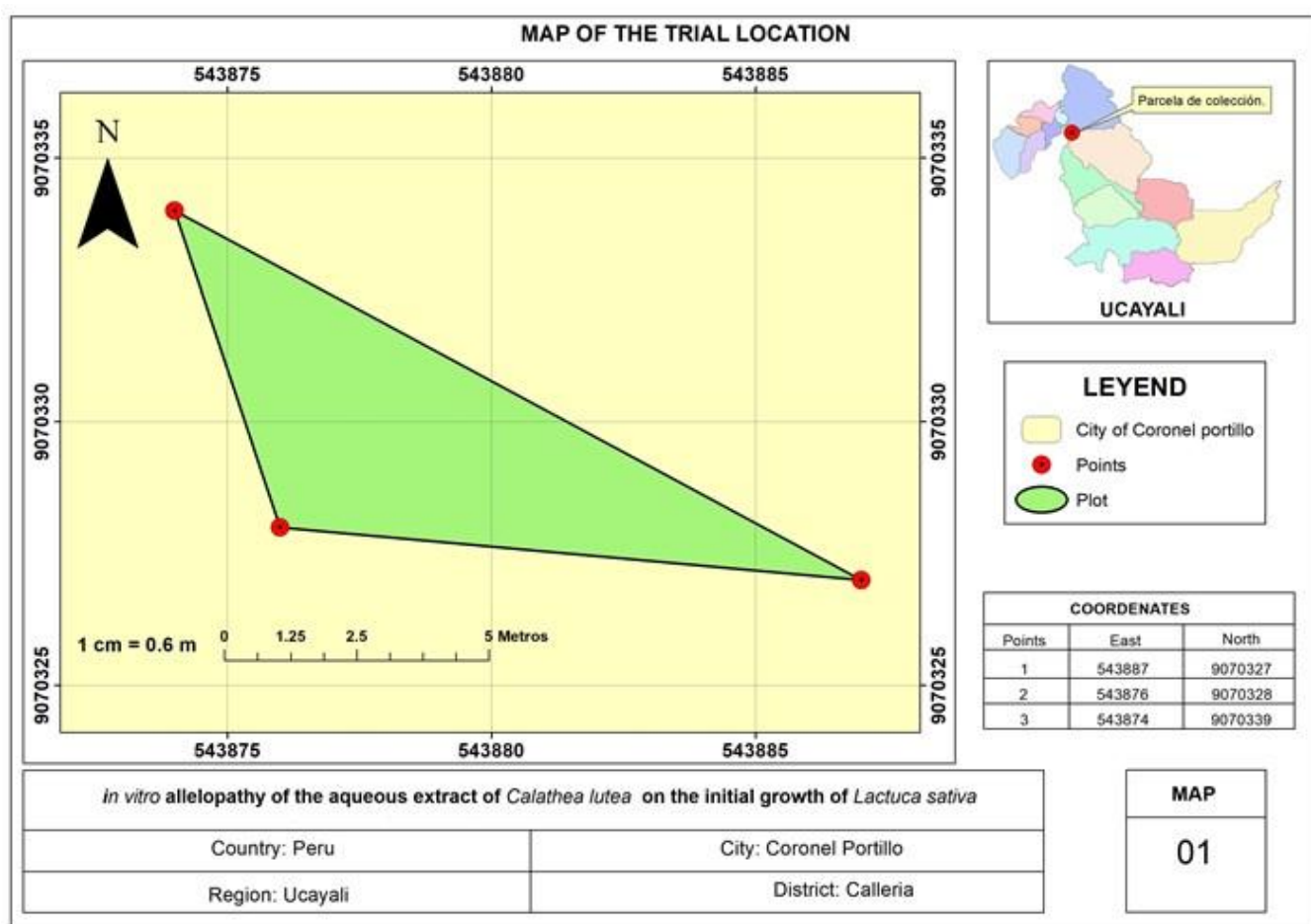
hypothesis was raised, there is a significant allelopathic effect when applying concentrations of the aqueous extract of *Calathea lutea* (*bijao*), on seed germination and growth of *Lactuca sativa* (lettuce) seedlings, considering the objective of determining the allelopathic effect *in vitro* of the aqueous extract of *Calathea lutea* (*bijao*) on the initial growth of *Lactuca sativa* (lettuce).

MATERIALS AND METHODS

The research was carried out in the Biochemistry Laboratory of the National Intercultural University of the Amazon, Yarinacocha, Ucayali, Peru. We worked with the leaf, stem, and root of *bijao* as donor species (Fig. 1), the action of the aqueous extract of this plant on the germination of seeds, and the growth of seedlings of lettuce variety White boston as recipient species was studied.

Figure 1

Trial location



The samples were collected from three strains (set of five to 10 plants), in this process four adult plants of 3 m each strain were selected; Then, with the help of a

hoe, the soil was excavated until the entire root of the plant was extracted. The collection was carried out in October 2016, in the District of Manantay, km. 9 left

margins entering 2 km from the Federico Basadre highway. The samples were washed separately and disinfected with sodium hypochlorite, then dehydrated at room temperature for 24 h, and then in an oven for 72 h at 50 °C, with scissors the samples were cut into pieces no larger than 3 cm and were dry crushed separately without pulverizing, then left to macerate for 48 h in 3 covered dark glass containers. Subsequently, the liquid from the solid part was filtered with aluminum sieves of 2 mm, 1 mm, 425 µm mesh, obtaining the aqueous extract that was diluted with distilled water until concentrations of 0, 2, 4, and 6% were obtained. For the control treatment, distilled water was used. Sowing was carried out in a dark space on a table disinfected with sodium hypochlorite, using 48 petri dishes labeled indicating the type of aqueous extract and concentration. Before sowing, two layers of filter paper were placed at the base of each plate, using gloves, in such a way to reduce the transmission of microorganisms and with a sterilized tweezer, 10 lettuce seeds/petri dish were planted and with a millimeter pipette they were soaked with 2.5 ml of each concentration of aqueous extract at each treatment to finally cover each plate with the lid, the irrigations were carried out twice a day (8:00 am and 5:00 pm), applying a total of 5 ml of aqueous extract per day, for the control treatment the same procedure was carried out but irrigating with distilled water.

Table 1

Tukey's multiple range test for the percentage of germination in lettuce due to the effect of the part or organ of the plant and concentrations of the aqueous extract of Calathea lutea

Plant organ	Concentrations (%)	Germination (%)	
Leaf	6	7.5	a
Leaf	4	32.5	b
Leaf	2	70.0	c
Root	4	92.5	d
Root	6	92.5	d
Stem	6	95.0	d
Stem	4	95.0	d
Stem	2	95.0	d
Leaf	0	100.0	d
Stem	0	100.0	d
Root	2	100.0	d
Root	0	100.0	d

Means with a common letter are not significantly different ($p > 0.05$)

Allelopathic effect on the growth parameters of *L. sativa*

The variables root length (cm), stem fresh weight (g), stem dry weight (g), root fresh weight (g), and root dry

After 10 days of sowing, the variables were evaluated: Germination percentage (%), growth parameters: seedling height (cm), radicle length (cm), stem diameter (mm), radicle diameter (mm), fresh weight of stem and radicle (g), dry weight of stem and radicle (g). The Complete Random Design (DCA) was used, with a 3x4 factorial arrangement, being factor A, composed of 3 parts or organs (leaf, stem, root) and factor B composed of 4 concentrations (0, 2, 4, 6 percent), making 12 treatments with 4 repetitions and 10 seeds per experimental unit. Finally, the percentage of *in vitro* inhibition of *Calathea lutea* (*bijao*) extracts on the radicle and hypocotyl of *Lactuca sativa* was calculated.

RESULTS

Allelopathic effect on the germination percentage of lettuce seeds.

The interaction between part or organ of the plant (leaf, stem and root) and the concentrations of the aqueous extract of *Calathea lutea* on the variable percentage of germination of the lettuce seeds presented significant differences ($p \leq 0.05$), see Table 1. Therefore, Tukey's test shows that the treatment with the aqueous extract *bijao* leaf interaction at six percent concentration inhibited the germination of lettuce reaching 7.5 percent.

weight (g), showed significant differences ($p \leq 0.05$) in the interaction (Table 2). Tukey's average test showed that the *Calathea lutea* leaf level with six percent concentration prevented the manifestation of the initial growth parameters of lettuce (Table 3).

Table 2

Analysis of variance for seedling height (AP), root length (LR), stem diameter (DV), root diameter (DR), stem fresh weight (PFV), stem dry weight (PSV), weight root fresh weight (PFR) and root dry weight (PSR) in lettuce by the effect of the part or organ of the plant and concentrations of the aqueous extract of *Calathea lutea*

Variation Source	Degree of freedom	Mean squares							
		AP (cm)	LR (cm)	DV (mm)	DR (mm)	PFV(g)	PSV(g)	PFR (g)	PSR (g)
Plant organ (O)	2	2.62*	7.72**	0.004 ^{NS}	0.03**	0.01**	0.000006**	0.006**	0.000013**
Concentration (C)	3	2.00*	65.86**	0.101**	0.10**	0.06**	0.000017**	0.003**	0.00001**
OxC	6	0.71 ^{NS}	1.07**	0.001 ^{NS}	0.004 ^{NS}	0.004**	0.000004**	0.002**	0.000004**
Residue	36	0.36	0.13	0.006	0.003	0	1.14E-07	0	5.87E-08
CV (%)		15.87	9.31	17.35	24.41	8.98	10.84	9.53	12.05

*** NS Significant at 1%, 5% and not significant according to the F

Table 3

Tukey's multiple range test for root length (LR), stem fresh weight (PFV), stem dry weight (PSV), root fresh weight (PFR) and root dry weight (PSR) in lettuce due to the effect of part or organ of the plant and concentrations of the aqueous extract of *Calathea lutea*

Plant organ	Concentration (%)	Root length - LR (cm)	Stem fresh weight - PFV (g)	Stem dry weight - PSV (g)	Root fresh weight - PFR (g)	Root dry weight - PSR (g)
		\bar{x}	\bar{x}	\bar{x}	\bar{x}	\bar{x}
Leaf	6	1.2 a	0.01 a	1.2 E-03 b	0.00 a	5.3 E-04 a
Leaf	4	1.9 a b	0.04 b c d	2.8 E-04 a	0.01 a b	1.3 E-03 b c
Leaf	2	2.4 b c	0.07 e	4.0 E-03 d e	0.00 a	9.8 E-04 a b c
Stem	4	2.5 b c	0.04 c d e	3.2 E-03 d	0.00 a	7.5 E-04 a b
Stem	6	2.6 b c	0.02 a b c	2.3 E-03 c	0.00 a	5.0 E-04 a
Stem	2	2.8 b c d	0.06 d e	3.4 E-03 d	0.01 b	1.3 E-03 b c
Root	6	3.2 c d	0.01 a b	1.9 E-03 b c	0.09 d	4.2 E-03 e
Root	4	3.5 d e	0.09 f	3.9 E-03 d e	0.01 b	1.5 E-03 c
Root	2	4.3 e	0.10 f	4.3 E-03 e	0.04 c	3.3 E-03 d
Stem	0	7.4 f	0.18 g	4.3 E-03 e	0.04 c	3.3 E-03 d
Leaf	0	7.4 f	0.18 g	4.3 E-03 e	0.04 c	3.3 E-03 d
Root	0	7.4 f	0.18 q	4.3 E-03 e	0.04 c	3.3 E-03 d

Means with a common letter are not significantly different ($p > 0.05$)

Means with a common letter are not significantly different ($p > 0.05$)

In vitro inhibition of *Calathea lutea* (bijao) extracts on the radicle and hypocotyl of *Lactuca sativa*.

In Table 4, it is observed that the aqueous extract of the *Calathea lutea* leaf at six percent concentration inhibits the growth of the hypocotyl at 39.8%, while at 83.3% at the level of the radicle.

Table 4

Percentage of inhibition of the hypocotyl and radicle of *Lactuca sativa* by the effect of the plant organs and concentration of the aqueous extract of *Calathea lutea*

Plant organ	Concentration (%)	Hypocotyl (\bar{X})	Inhibition of the hypocotyl (%)	Radicle (\bar{X})	Inhibition of the radicle (%)
Leaf	0	4.1	0	7.4	0
Leaf	2	3.5	13.9	2.4	67.8
Leaf	4	3.6	11.5	1.9	74.1
Leaf	6	2.5	39.8	1.2	83.3
Steam	0	4.1	0	7.4	0
Steam	2	3.7	9.2	2.8	62.2
Steam	4	3.4	16.5	2.5	66.1
Steam	6	3.5	15.3	2.6	64.7
Root	0	4.1	0	7.4	0
Root	2	5	-21.6	4.3	42
Root	4	4	1.5	3.5	51.9
Root	6	3.8	8.4	3.2	56

DISCUSSION

Allelopathic effect on the germination percentage of lettuce seeds.

Similar results were found by Ramírez (2014), who using the aqueous extract of oat leaves (*Avena sativa*), at a concentration of 50 percent, inhibited the percentage of germination of the grass seeds of the buzzard (*Chenopodium murale*) and yuyo (*Amaranthus spinosus*) by 48 percent. Likewise, Laynez and Méndez (2013), showed that using the aqueous extract of buttercup leaves (*Tithonia diversifolia*), caused a greater inhibitory effect on the germination of *L. sativa* seeds, using concentrations of 1, 1.5, and 2 percent. In addition, the authors mention that the effect probably occurred, because the buttercup leaf contains a high allelopathic potential. For its part, the study conducted by Rimando et al. (2001) found that p-coumaric acid, a known allelochemical compound, inhibited the germination of lettuce seeds at a concentration of 1 milliMol (mM). On the other hand, Souza et al. (2009), report that monoterpenes are major components of the essential oils of a large number of species and have been related as effective allelochemicals with a toxic effect on seed germination. Likewise, Rodríguez et al. (2014), point out that applying the aqueous extract of sunflower leaves (*Helianthus annuus*), at a concentration of 50 and 100 percent, produces inhibition causing negative and aggressive effects on the grass seeds of the buzzard (*Chenopodium murale*) and rabo de fox (*Setaria unguolata*). Similar results were obtained by Rodríguez (2016), who used the aqueous extract of leaves and stems of copaiba (*Copaifera pubiflora*), at a

concentration of 7 percent, caused inhibition of 87 percent on the germination of the sensitive plant (*Mimosa pudica*), causing detrimental effects on the height of seedlings. Some compounds have been reported in the *Calathea lutea* (*bijao*) leaf with allelopathic properties, as demonstrated by Aguirre et al. (2014), verifying that the allelopathic power is due to the presence of allelochemicals such as (saponins, flavonoids, coumarins, alkaloids, and phenolic compounds: catechic tannins), results that were determined through a phytochemical analysis of the leaves. According to the authors, these natural chemical compounds are capable of inhibiting seed germination, causing adverse effects on the development of a plant. In the present investigation, the metabolites (tannins, saponins, and flavonoids) existing in the aqueous extract of the *bijao* leaf, not only reduced the germination of the seeds but also caused changes in their morphological appearance, such as the fragility of the stems, short, atrophied roots and blackening of the tissues, hindering the normal growth of lettuce seedlings.

Allelopathic effect on the growth parameters of *L. sativa*

Regarding plant height, Martínez et al. (2016), indicate that using the aqueous extract of stem and leaf of crotalaria (*Crotalaria juncea*) and canavalia (*Canavalia ensiformes*), in concentrations of 3.3 percent, they inhibited 58 and 64 percent of the growth of the hypocotyl of lettuce seedlings. On the other hand, Cruz (2012) pointed out that applying the aqueous extract of the sweet potato leaf (*Ipomoea batatas*) inhibited the longitudinal growth of the bean

seedling and radicle by 44.4 percent compared to the control plants, with greater inhibition being observed of height with the increase in concentration, corroborating with works by Laynez and Méndez (2013) and Blanco et al. (2007). Regarding root length, studies carried out by Cruz (2012), mention that applying the aqueous extract of sweet potato (*Ipomoea batatas*) leaf and stem, at a concentration of 50%, inhibited the longitudinal growth of the bean radicle up to 44.4 percent, likewise the author mentions that lower concentrations stimulated the growth of the radicle of bean seedlings in agreement with Gatti et al. (2004), Della et al. (2009) and Islam and Noguchi (2013). Regarding stem diameter of lettuce seedlings, Periotto et al. (2004), observed that the aqueous extract of leaf and stem of (*Andira humilis*), reduced the development of the diameter of lettuce seedlings, applying concentrations of 4, 8, 12, and 16 percent, coinciding with the investigations carried out by Ramírez (2014), Borghetti et al. (2005) and Ferguson and Rathinasabapath (2009). Evaluating the root diameter, Guzmán et al (2009), using the aqueous extract of the leaves of (*Baccharis dracunculifolia*) both *in vitro* and *ex vitro*, inhibited 50 percent of the initial growth and the diameter of the root system of mustard and cabbage, applying a concentration of 30 percent, similar results were obtained by Ramírez (2014), Gonzales et al. (2015), Tucat et al. (2013), Otusanya and Llori (2012), and Aguirre et al (2014). Regarding the fresh and dry weight of the stem of the lettuce seedlings, Laynez and Méndez (2013), mention that by using the aqueous extract of the buttercup leaf at concentrations of 1; 1.5; and 2 percent, the reduction of the fresh and dry weight of the stem of the lettuce and sesame seedlings is observed, following the same effect reported by Vásquez and Torres (2006) and Calabrese and Baldwin (2003). In the same way, the fresh and dry weight of the radicle of the lettuce seedlings was studied by Ramírez (2014), where applying the aqueous extract of the oat leaf, the fresh and dry weight of the gallinazo grass and weed, presented a decrease as the concentration increased, obtaining a very low dry weight in the two species studied, which would mean that the aqueous extract of oats affected the root causing necrosis at its apices, reducing the absorption of water and nutrients, causing the decrease of the mass in the seedlings, thus affecting their growth and development, authors such as Laynez and Méndez (2013), Vera (1995), Rodríguez (2008), Thompson (1985) and Sáenz et al. (2010) coincide in pointing out that foliar extracts cause an allelopathic effect *in vitro*, using sensitive plants. In this investigation, it was possible to show negative effects (weakness, deformity, and necrosis) in the initial growth of lettuce seedlings.

***In vitro* inhibition of *Calathea lutea* (bijao) extracts on the radicle and hypocotyl of *Lactuca sativa*.**

Regarding hypocotyl inhibition, similar results were found by Laynez and Méndez (2013), who determined that using the aqueous extract of buttercup leaves at a concentration of 2 percent, caused weakness in the stems caused by the loss of their size. erect, followed by a browning of the tissues and finally the death of the seedlings. Likewise, the authors point out the existence of existing metabolites in the buttercup extracts. Likewise, Blanco et al. (2007), determined that applying the aqueous extract of sunflower leaves (*Heliantus annus*) at a concentration of 15 percent, caused a negative effect causing susceptibility in the length of the bean stem. These results agree with what was obtained in the present investigation, where using 6 percent concentration of the extract water content of the *bijao* leaf, negative effects (weakness, deformity and necrosis) were observed in the growth of the height of the lettuce seedlings. Likewise, Martínez et al. (2016), indicate that using the aqueous extract of stem and leaf of crotalaria (*Crotalaria júncea*) and canavalia (*Canavalia ensiformes*), in concentrations of 3.3 percent, they inhibited 58 and 64 percent of the growth of the hypocotyl of lettuce seedlings.

Likewise, the inhibition of the radicle, research carried out by Gatti et al. (2004), mention that using the aqueous extract of stem and root of *Aristolochia esperanzae* (calico flower), in concentrations of 50 and 100 percent, caused damage to the root system of lettuce seedlings, showing atrophied primary roots, defective, at sometimes absent, roots short and disproportionate in relation to other plant structures. On the other hand, Della et al. (2009), determined that applying the aqueous extract of the adult leaves of cerraja (*Sonchus oleraceus*), in concentrations of 20 and 50 percent, they observed a decrease in root elongation of chicory (*Cichorium intybus*) and green onion (*Allium schoenoprasun*). On the other hand, research carried out by Islam and Noguchi (2013), indicate that the greater inhibition of the root compared to the aerial parts may be due to the fact that in the initial development, the coleoptile and the hypocotyl depend mainly on the expansion of cells that they are relatively insensitive to allelochemicals. On the contrary, the rhizogenesis process is associated with cell division that is sensitive to chemical alleles, exerting greater inhibition of radicle growth. Similarly, the roots have greater contact with allelochemicals because it is the first organ to absorb substances from the environment.

CONCLUSIONS

The aqueous extract of *Calathea lutea* (*bijao*) leaves has a negative allelopathic effect and inhibits the germination and growth parameters of *Lactuca sativa* (lettuce) seedlings under *in vitro* conditions.

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