Agroind. sci. 11(2): 201-210 (2021)

Agroindustrial Science

Website: http://revistas.unitru.edu.pe/index.php/agroindscience

Escuela de Ingeniería Agroindustrial

Universidad Nacional de Trujillo

Plant extracts of *Cleome viscosa* L. as biostimulants of the *in vitro* germination of two varieties of pepper (*Capsicum annuum* L.)

Extractos vegetales de *Cleome viscosa* L. como bioestimulantes de la germinación *in vitro* de dos variedades de pimiento (*Capsicum annuum* L.)

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ABSTRACT

The aim of the work was to evaluate the biostimulant effect of plant extracts of *Cleome viscosa* L. on the *in vitro* germination of two varieties of pepper (*Capsicum annuum* L.). A bioassay was set up on a completely randomized design with a control and 18 treatments with three samples each, evaluating the effect on the dynamics and percentage of germination, radicle length and hypocotyl length. The chemical families present in the extracts were determined by phytochemical characterization. For the length of the radicle and the hypocotyl, the extracts with the best potentialities in the Verano 1 variety are those obtained from the root and stem, although the concentrations with the best influence on both indicators are 1 and 3 of the root extracts. Differences in the richness of chemical families were verified in the extracts with the best results, although the best composition was obtained in those of the root and stem, showing the presence of free amino acids in all cases. *Cleome viscosa* is promising for obtaining products with a biostimulant effect on the varieties of pepper studied, obtaining a better effect when root, stem and whole plant extracts are applied.

Keywords: amino acids; dynamics; phytochemical; hypocotyl; radicle.

RESUMEN

El objetivo del trabajo fue evaluar el efecto bioestimulante de extractos vegetales de *Cleome viscosa* L. sobre la germinación *in vitro* de dos variedades de pimiento (*Capsicum annuum* L.). Se realizó un bioensayo en un diseño completamente al azar con un control y 18 tratamientos con tres muestras cada uno, evaluando el efecto sobre la dinámica y porcentaje de germinación, longitud de radícula y longitud de hipocótilo. Las familias químicas presentes en los extractos se determinaron mediante caracterización fitoquímica. Para la longitud de la radícula y el hipocótilo, los extractos con mejores potencialidades en la variedad Verano 1 son los obtenidos de raíz y tallo, aunque las concentraciones con mayor influencia en ambos indicadores son 1 y 3 de los extractos radiculares. Se verificaron diferencias en la riqueza de familias químicas en los extractos con mejores resultados, aunque la mejor composición se obtuvo en las de raíz y tallo, mostrando la presencia de aminoácidos libres en todos los casos. *Cleome viscosa* es prometedora para la obtención de productos con efecto bioestimulante sobre las variedades de pimiento estudiadas, obteniendo un mejor efecto al aplicar extractos de raíz, tallo y plantas enteras.

Palabras clave: aminoácidos; dinámica; fitoquímico; hipocótilo; radícula.

Recibido 12 marzo 2021
Aceptado 27 mayo 2021

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DOI: http://dx.doi.org/10.17268/agroind.sci.2021.02.10

1. Introduction

Cleome viscosa, (Synonyms: *C. icosandra* L., *Arivela viscosa* (L.) Raf., *Polanisia viscosa* (L.) DC., *P. icosandra* L.). Common names: Asian spider flower, Quitaruina, Pegajosa, Volantín, Frijolillo de playa, is an annual plant distributed throughout the world. It grows about 1 m tall, has yellow flowers, the fruits are long and cylindrical with light brown seeds. It develops in dry and humid conditions, appears on rocks, in calcareous soils and exposed to the sun (Mishra et al., 2011; Joshi et al., 2015; Osorio & Díaz, 2018).

As a medicinal plant, it has laxative, diuretic and anticancer properties (Chatterjee, 2014). It is anthelmintic and is used to treat flatulence, kidneys, colic, dyspepsia, bronchitis, heart disorders, infections, rheumatism, fever, headache, malaria fever and mental disorders. It is rubefacient, anti-inflammatory, expectorant and digestive stimulant (Joshi et al., 2015). Has analgesic, antidiarrheal, antipyretic, antiemetic, antimicrobial, hepatoprotective and diabetic neuroprotective effect (Faheemuddin et al., 2017), is antiscorbutic, antiseptic, cardiac stimulant, carminative and anticonvulsant (Singh, 2017). It has vesicant and sudorific properties (Lakshmanan, 2018).

Misra & Misra (2014) indicated that the leaves have a good content of proteins, vitamins and lipids. According to Joshi et al. (2015), cooked are consumed as vegetables. Of the seeds, Saroop (2016) referred their nutritional value, they are used as a condiment and, when they are commercialized, they report economic benefits. In the agricultural field Pupo et al. (2011) and Alamo (2014) reported that their extracts were promising against *Alternaria solani* (E. & M.) J. & G. in tomato. Deventhiran (2017) reported a similar effect against viral diseases that affect melon (*Cucumis melo* L.).

Other benefits that this plant has, according to Vargas et al. (2014) are related to its bactericidal, nematicidal and insecticidal properties, it has good biomass production and does not have a phytotoxic effect on some crops. All the aspects mentioned above suggest that *C. viscosa*, given its bioactivity, is a good candidate to expand studies in the search for natural products for agricultural use, as this has been one of the least studied fields related to this species. However, Singh et al. (2017), Deventhiran et al. (2017), Uraku & Uraku (2018), Elahifard & Derakhshan (2018), reported that it is commonly found as a weed and that it shows a pantropical distribution.

In Cuba, *C. viscosa* has been reported as abundant in different agricultural systems but, despite its multiple benefits, it is eliminated from agroecosystems and its benefits are not used. Hence the importance of carrying out studies that make it possible to value this plant in such a way that its benefits are enhanced, and its risks are minimized. For all the above, the present work aims to evaluate the biostimulant potentialities of plant extracts of *Cleome viscosa* L. on the *in vitro* germination of two varieties of pepper (*Capsicum annuum* L.).

2. Materials and methods

The work was developed in the Department of Agronomy and Biology Laboratory (Universidad de Oriente) and in the Natural Products Laboratory of the Center for Applied Chemistry Studies (CEQA) (Universidad de Granma). First, a deep bibliographic analysis of the specialized literature on the subject was carried out, which allowed the selection of the plant species, as well as the crop and varieties to work with. Then, tours were carried out to verify the existence of the selected plant species as source for obtaining the extracts.

Collection

The collection of *C. viscosa* took place in an area near the National Center for Applied Electromagnetism (CNEA) located on the Julio Antonio Mella Campus of the Universidad de Oriente, Santiago de Cuba, Cuba. Healthy, vigorous plant material was collected, in the phenological flowering-fruiting stage and in the morning hours (preferably before 10:00 AM). The different organs (root, stem, leaf, flower, fruits) were separated and wrapped in paper, they were dried in an INMERTF 760416 oven at 35 °C, until by inspection to the touch, and the dehydrated material could easily disintegrate into particles with maximum diameter 2.5 mm. A temperature rises above 50 °C was always avoided. Subsequently, the extracts were obtained according to the organs of the plant.

Extraction

The extraction was carried out with distilled water by maceration for 24 h, stirring manually every 10 min by 12 h, then it was left at rest in dark conditions for 12 h and after that time the same process was repeated. Tinctures were prepared at 30% by mass (30 g of dehydrated plant material in 100 mL of solvent). In the case of the whole plant extract, the mass consisted of 6 g of each of the organs. After this time, the liquid was decanted, which was then filtered through Double Rings filter paper with 102 pores, 11 cm in diameter and medium quality. The extract obtained was kept refrigerated until use.

Dilution preparation

For the evaluation of the biostimulant potentiallities of the obtained extracts, dilutions were prepared at different concentrations. Knowing the total solids extracted from 5 mL of the extract, the concentration of each of the extracts (root, stem, leaf, flower, fruit, and whole plant) was determined. With these data and applying the fundamental equation of volumetry, the necessary volume to be used of the extracted fluid was calculated to obtain dilutions of 1, 2 and 3 g.L^{-1}, which were the concentrations used in the assembly of the bioassays. The volume used of the aqueous extract of each one of the organs was variable and RONGTAI micropipettes of 20 to 200 µl and 100 to 1000 µl were used.

Bioassay setup

To evaluate the effect of the extracts, certified seeds of *C. annuum* varieties Verano 1 (84% germination percentage) and Española-16 (75% germination percentage) were used. Once the seeds were obtained, 115 mm diameter Petri dishes were taken which were covered with filter paper (with the same characteristics as those used in the filtering of the extracts) and duly labeled to identify the treatment to which they belonged. Two experiments were set up in correspondence with the varieties used. Considering all the treatments and the control, a total of 57 plates were used in each experiment.

Each bioassay was mounted on a completely randomized design with a control and 18 treatments (Table 1) and each treatment with three samples each. Only 2 mL of distilled water were added to the control and in the case of the treatments 2 mL according to extract and concentration, in all cases it was always verified that the paper was well moistened, and that the liquid was distributed homogeneously. RONGTAI micropipettes from 100 to 1000 µl were used for the application. After mounting the experiment every day, 1 mL of distilled water was added to each sample.

Subsequently, the seeds were placed in each of the plates on the already moistened paper at the rate of 23 seeds of the Verano 1 variety and 15 seeds of the Española-16 variety. After all the procedure described above, the plates were placed in drawers to fulfill the dark phase within the germination process between 24 and 48 hours. From this period on, they were placed on a shelf under similar lighting and temperature conditions according to the experimental design used.

Table 1

Description and coding of each of the treatments used in the experiments developed during the investigation

Treat-ments	Description	Code
Control	Only distilled water was added	CTRL
T1	Root extract concentration 1 (1 g.L^{-1})	RD1
T2	Root extract concentration 2 (2 g.L^{-1})	RD2
T3	Root extract concentration 3 (3 g.L^{-1})	RD3
T4	Stem extract concentration 1 (1 g.L^{-1})	TD1
T5	Stem extract concentration 2 (2 g.L^{-1})	TD2
T6	Stem extract concentration 3 (3 g.L^{-1})	TD3
T7	Leaf extract concentration 1 (1 g.L^{-1})	HD1
T8	Leaf extract concentration 2 (2 g.L^{-1})	HD2
T9	Leaf extract concentration 3 (3 g.L^{-1})	HD3
T10	Flower extract concentration 1 (1 g.L^{-1})	FD1
T11	Flower extract concentration 2 (2 g.L^{-1})	FD2
T12	Flower extract concentration 3 (3 g.L^{-1})	FD3
T13	Fruit extract concentration 1 (1 g.L^{-1})	FrD1
T14	Fruit extract concentration 2 (2 g.L^{-1})	FrD2
T15	Fruit extract concentration 3 (3 g.L^{-1})	FrD3
T16	Whole plant extract concentration 1 (1 g.L^{-1})	PED1
T17	Whole plant extract concentration 2 (2 g.L^{-1})	PED2
T18	Whole plant extract concentration 3 (3 g.L^{-1})	PED3

Variables evaluated and statistical processing

Related to germination, the dynamics and germination percentage were considered by counting the number of germinated seeds. They were evaluated from 24 hours after setting up the experiment and up to 13 days. Once the time limit was fulfilled, 15 seedlings were taken per treatment randomly selected to measure the length of the radicle and hypocotyl with a millimeter rule of 60 cm. A Multiple Range test was performed, using Fisher's LSD test for a 95% confidence level ($p \leq 0.05$). For data processing, the statistical package was used StatSoft. STATISTICA v10.0.228.8.

Phytochemical evaluation

From the procedure carried out previously, the organs from which the best performing extracts and concentrations were obtained were selected to carry out a phytochemical characterization and elucidate the families of compounds present that could influence the effect obtained. The phytochemical screening was carried out according to the methodology of Miranda & Cuéllar (2000).

3. Results and Discussion

Related to the effect of *C. viscosa* extracts on germination dynamics, it is found that for the Verano 1 variety (Figure 1) the trend over time of the treatments with the extracts is similar to that of the control. From the first day of evaluation, germinated seeds were reported and, although in the five successive days it showed an increase, a flare was never experienced. From the fifth to the sixth day there is an increase and until the 9th day, in all cases, a constant value of germinated seeds appears. In this period, all the concentrations of the fruit extract and the concentration 3 of the flower extract exceed the control, while the concentration 2 of the whole plant shows a behavior similar to this.

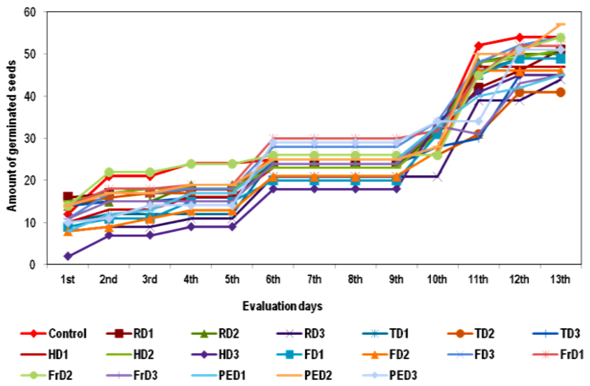

Figure 1. Effect of *C. viscosa* extracts on the germination dynamics of the pepper variety Verano 1.

From the ninth day onwards, there is an increase in the total number of germinated seeds in the form of a flare with a tendency to stability in the last two days where the control (not significantly) exceeds the extracts. In general, throughout the evaluation period, the control was superior to the extracts, although it must be borne in mind that the number of germinated seeds between the first (control) and the last (extracts) was quite similar. The number of germinated seeds in the extracts fluctuated between 41 and 54 while, in the control, a total of 54 germinated, only in the concentration 2 of whole plant a quantity of 57 germinated seeds was obtained.

In the Española-16 variety (Figure 2), the observed behavior is completely different. Between the first and third day of measurement, no germinated seeds were counted except in concentration 1 of the leaf extract. From the third day until the sixth there is a flare in the germination quite differentiated between the control and the extracts and then stability around a value of germinated seeds appears until the ninth day. Finally, there is an increase from the ninth day onwards with a germination flare, less marked, reaching stability again in the last two days.

This variety seems to have shown a better response for this indicator in the presence of plant extracts. From the fourth day, most of the treatments where the extract was applied exceeded the control, an effect that became more evident with the passage of time. Only concentration 1 of the flower extract showed lower results than the control. The best values regarding the quantity of germinated seeds for this variety were reached when concentrations 1 and 3 of the root, stem and leaf extracts were applied. The total number of seeds germinated on day 13 ranged between 28 and 38 for the extracts, while in the control only 25 germinated one more than in concentration 1 of the flower extract.

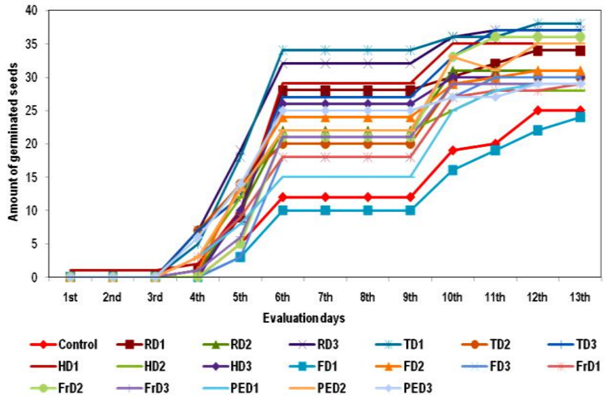

Figure 2. Effect of *C. viscosa* extracts on the germination dynamics of the pepper variety Española-16.

The germination percentage is related to the results obtained previously, which is assumed to be also favored with the application of the different extracts. On the fifth day, the highest germination percentage for the Verano 1 variety (Table 2) was observed in the control and in the concentration 2 of the fruit extract (34.78%), in the rest of the treatments the percentages of germinated seeds were below. At 10 days, the results obtained were different because, except with concentration 3 of the root, concentrations 2 and 3 of the stem, as well as concentration 2 of flowers, fruits and whole plant, in the rest it exceeded or equaled the control in the germination percentage. After the last evaluation was carried out, the control once again increased its germination percentage with respect to the extracts, exceeded only by concentration 2 of the whole plant, and equaled by concentration 3 and 2 of flowers and fruits, respectively.

The seeds used in the experiment had a certificate of 84% germination, so it can be argued that both the control and the applied extracts did not reach adequate values for this parameter. This could be related to internal propagation material problems such as viability.

Therefore, it is considered that the results achieved with concentrations 1 and 2 of root, 1 of stem, 2 of leaves, 1 of flowers and fruits, as well as 3 of whole plant, are encouraging as they exceed 70% of germinated seeds.

Table 2
Effect of *C. viscosa* extracts on the germination percentage in the cultivation of *C. annuum* variety Verano 1

Treatments	*C. annuum* variety Verano 1		
	Fifth day	Tenth day	Last day
Control	34.78 %	44,93 %	78.26 %
RD1	26.09 %	44,93 %	73.91 %
RD2	27.54 %	46,38 %	72.46 %
RD3	15.94 %	30,43 %	63.77 %
TD1	17.39 %	47.83 %	71.01 %
TD2	24.64 %	37.68 %	59.42 %
TD3	23.19 %	40.58 %	65.22 %
HD1	23.19 %	44,93 %	68.11 %
HD2	26.09 %	44.93 %	73.91 %
HD3	13.04 %	49.27 %	65.22 %
FD1	21.74 %	44.93 %	71.01 %
FD2	18.84 %	39.13 %	66.67 %
FD3	26.09 %	49.27 %	78.26 %
FrD1	27.54 %	46.38 %	75.36 %
FrD2	34.78 %	37.68 %	78.26 %
FrD3	21.74 %	47.83 %	65.22 %
PED1	24.64 %	47.83 %	65.22 %
PED2	27.54 %	40.58 %	82.61 %
PED3	20.29 %	49.57 %	73.91 %

In the Española-16 variety (Table 3), the results obtained were different. This is said because on the fifth day after the evaluations were carried out; all the treatments where plant extract was applied exceeded or equaled the control in percentage of germinated seeds, except for concentrations 1 and 3 of flowers. From the tenth day, all the extracts at their different concentrations, except flower concentration 1, are capable of exceeding the control in the percentage of germination behavior that is maintained until the end of the evaluations carried out. As in the Verano 1 variety, most of the percentages obtained are not related to the certificate for the seeds used (75%), being much lower in the case of the control. However, when the concentrations 1 and 3 of root and stem, 1 of leaf, 2 of fruits and whole plant are applied, a germination superior, inclusive, to the certificate for the seeds of this variety is obtained.

Germination is one of the vital physiological processes on which the future of the plant and all the productive aspects related to it largely depend. It is for this reason that all the necessary conditions must be guaranteed to favor the fact that this process develops in the best possible way. Everything seems to indicate that the studied plant extracts favor these conditions since the two indicators evaluated up to here were favored by different extracts at different concentrations. Up to this point, making a general analysis, it is considered that the different concentrations of the extracts of root, stem, leaf and concentrations 2 and 3 of the whole plant can be used in these varieties of *C. annuum* with beneficial effects on germination. It can be said that the recommended plant extracts tend to have a beneficial allelopathic effect, which gives it potential to obtain biostimulants of plant origin. On the other hand, if the low quality of the seeds is considered, despite being certified, these extracts seem to have the added value of improving the qualitative aspects of the propagation material related to its viability and germination power.

Table 3
Effect of *C. viscosa* extracts on the germination percentage in the cultivation of *C. annuum* variety Española-16

Treatments	*C. annuum* variety Española-16		
	Fifth day	Tenth day	Last day
Control	11.11 %	42.22 %	55.55 %
RD1	20.00 %	66.67 %	75.55 %
RD2	22.22 %	68.89 %	68.89 %
RD3	42.22 %	80.00 %	82.22 %
TD1	40.00 %	80.00 %	84.44 %
TD2	31.11 %	64.44 %	66.67 %
TD3	26.67 %	73.33 %	82.22 %
HD1	22.22 %	77.78 %	77.78 %
HD2	26.67 %	55.55 %	62.22 %
HD3	22.22 %	66.67 %	66.67 %
FD1	6.67 %	35.55 %	53.33 %
FD2	31.11 %	64.44 %	68.89 %
FD3	6.67 %	60.00 %	66.67 %
FrD1	20.00 %	60.00 %	64.44 %
FrD2	11.11 %	73.33 %	80.00 %
FrD3	13.33 %	64.44 %	64.44 %
PED1	17.78 %	55.55 %	64.44 %
PED2	28.89 %	73.33 %	77.78 %
PED3	31.11 %	60.00 %	64.44 %

These results are important because according to Morales et al. (2017), the germination capacity and vigor are some of the attributes that affect the quality of the seed. It depends on this that germination, as a physiological process, develops from the embryo the essential structures for the formation of a normal plant.

On the other hand, Giardini et al. (2018) pointed out that germination studies are the first step for the large-scale use of secondary products from a donor plant over other recipients. In these studies, it can be demonstrated whether the products influence the final germination potential over time or on the germination speed.

In accordance with what was reported by González et al. (2018) when the germination percentage in a study exceeds 90% it is said that the product is not phytotoxic. If it is considered that the seeds used presented low germination percentages in the control and that both products and concentrations used exceed or resemble this, it cannot be said that there is a phytotoxic effect. Contrary to the above, these results show potentialities in *C. viscosa* for the search for alternative biostimulant products.

The importance of these results is reinforced by the fact that the germination of many *Solanaceae* seems to show susceptibility to the action of natural products of plant origin. Valcheva et al. (2019) reported, for example, that tomato seeds (*Solanum lycopersicum* L.) when treated with plant extracts from different plant species tended to inhibit germination with all concentrations, especially the highest.

Other factors that directly depend on germination are those related to the emergence of the embryonic root and stem. In this sense, the results obtained also show a beneficial effect when plant extracts are applied. In the case of the Verano 1 variety, all the concentrations of the different extracts, with values between 34.6 and 69.8 mm, managed to exceed the radicle length obtained in the control (29.53 mm) with statistically significant differences (Table 4). The best results are reported when the highest concentration of the root extract (69.67 mm) and the lowest of the stem (69.8 mm) were applied without statistical differences between them.

The result achieved when applying concentration 1 of the root extract can be considered promising, exceeding the control by more than 30 mm, although below the concentrations referred to above. The values obtained with concentrations 2 of root, stem and leaf are considered good, in addition to concentration 1 of the fruit extract with which the control is exceeded by 30 mm without differences between them. On the other hand, except in the root, flower and whole plant extracts, in the rest there is a decrease in the length of the radicle when the highest concentration is applied, although the values achieved exceed the control.

In relation to the length of the hypocotyl, all the extracts, with values between 25.2 and 50.4 mm, are also capable of exceeding the results obtained in the control (24.67 mm). Only the values reached when the flower concentration 1 and the fruit concentrations 1 and 2 were applied are like the control without difference between them. The length of the hypocotyl is more favored when the lowest concentration of the root extract is used, differing significantly from the control when more than 25 mm in length is achieved. The root extract concentration 3 can be considered as promising, where a length of 44.73 mm less than the lowest concentration of the product of this same organ is reached, but greater than the control by more than 20 mm.

Table 4
Effect of *C. viscosa* extracts on radicle length (mm) and hypocotyl (mm) in the cultivation of *C. annuum* variety Verano 1

Treatments	*C. annuum* variety Verano 1	
	Radicle length (mm) Mean ± DS	Hypocotyl length (mm) Mean ± SD
Control	29.53±0,75 i	24.67±0.87 l
RD1	60.73±0.99 e	50.40±0.80 a
RD2	59.80±0.98 e	34.53±0.88 fg
RD3	69.67±0.94 c	44.73±0.44 b
TD1	69.80±0.98 a	32.33±0.79 h
TD2	59.80±0.98 b	35.40±0.88 e
TD3	34.60±0.71 h	40.40±0.80 c
HD1	45.13±0.62 f	34.73±0.77 fg
HD2	59.53±0.96 b	34.60±0.88 fg
HD3	55.53±0.88 c	34.67±0.79 fg
FD1	45.53±0.88 f	25.20±0.65 kl
FD2	39.93±0.85 g	29.60±0.71 j
FD3	35.53±0.88 c	34.93±0.68 ef
FrD1	59.53±0.96 b	25.20±0.65 kl
FrD2	54.40±0.80 d	25.40±0.49 k
FrD3	50.40±0.95 e	40.73±0.99 c
PED1	50.93±0.99 e	34.20±0.98 g
PED2	50.47±0.88 e	37.40±0.80 d
PED3	55.20±0.98 c	30.40±0.80 i

Means followed by equal letters do not differ statistically (Fisher's LSD $p \leq 0.05$).

The values obtained when the stem and fruit extracts are applied at their highest concentration without statistical difference between them but superior to the control are also considered good. In the case of the length of the hypocotyl, the same effect related to the increase in concentrations that was manifested in the case of the radicle is not observed, since only when the concentration 3 of whole plant is applied is a decrease in the values obtained, although always superior to control. For this variety of pepper, the extracts with the best potential are those obtained from the root and stem, although the concentrations with the best influence on both indicators are 1 and 3 of the root extracts.

The Española-16 variety seems to be less influenced by the effect of the treatments, the radicle length oscillated in a range between 20.46 and 45.47 mm including the control (Table 5), which are lower than those reached in the

Verano 1 variety. Despite this, all the extracts with their concentrations are capable of stimulating radicle length with significant differences compared to the control. In the latter, a length of 20.46 mm was reported; however, when the seeds were treated with concentration 3 of the stem extract (45.47 mm) and 2 of the whole plant (45.2 mm), it was possible to increase the size of the embryonic root more than double what was achieved in the control without differences between them. A similar result is obtained when the stem 2 concentration is applied and, although there are significant differences between this and the two previous ones, numerically the values are similar (44.8 mm).

Table 5
Effect of *C. viscosa* extracts on radicle length (mm) and hypocotyl (mm) in the cultivation of *C. annuum* variety Española-16

Treatments	*C. annuum* variety Española-16	
	Radicle length (mm) Mean ± DS	Hypocotyl length (mm) Mean ± SD
Control	20.47±0.91 k	24.20±0.86 k
RD1	29.73±0.70 h	36.40±0.91 c
RD2	34.60±0.83 ef	45.00±0.92 b
RD3	35.67±0.97 d	50.20±0.77 a
TD1	24.47±0.91 j	34.47±0.83 ef
TD2	44.80±0.77 b	34.80±0.77 def
TD3	45.47±0.99 a	34.93±0.26 de
HD1	35.13±0.52 de	25.53±0.99 i
HD2	34.47±0.91 f	29.60±0.83 h
HD3	30.40±0.83 g	35.40±0.83 d
FD1	25.00±0.52 i	24.67±0.89 jk
FD2	24.93±0.96 ij	29.67±0.89 h
FD3	30.40±0.83 g	29.67±0.89 h
FrD1	29.60±0.83 h	34.27±0.96 f
FrD2	24.93±0.96 ij	25.20±0.77 ij
FrD3	30.40±0.83 g	25.33±0.89 i
PED1	39.73±0.70 c	30.53±0.91 g
PED2	45.33±0.89 ab	35.13±0.99 d
PED3	39.67±0.89 c	35.20±0.77 d

Means followed by equal letters do not differ statistically (Fisher's LSD $p \leq 0.05$)

When applying concentrations 1 and 3 of the whole plant extract, values of 39.73 and 39.67 mm respectively are obtained without differences between them, with which it is possible to increase the length of the radicle by more than 19 mm, which is why these concentrations are they classify as promising. Concentration 2 of the leaf extract can also be considered good, as well as concentrations 2 and 3 of the root, which, although there are statistical differences between the latter, all exceed the control. The length of the radicle in this variety does not seem to be affected too much by increasing concentrations, since only when leaf and whole plant extracts are applied is a decrease in this indicator observed at the highest concentration, always showing better results than in the control.

In the case of hypocotyl, the results showed a greater range of values (24.2-50.2 mm) than those reached in the radicle. All the extracts and concentrations managed to overcome the control with significant differences, except for concentration 1 of the flower extract, which behaved similarly to this. The best results are obtained when the root extract is applied at its highest concentration (50.2 mm), significantly exceeding the control (24.2 mm), which implies an increase of 26 mm, more than double that, reported for the control. The results achieved with the root concentration 2 (45 mm) are considered promising because, although lower than those obtained at the highest concentration of this same organ, it significantly exceeds the control.

In the case of the values reported with the application of the three concentrations of the stem extract, the 2 and 3 of the whole plant, the 1 of the root and fruit, as well as the 3 of the leaves, they are considered good because the length of the stem embryonic is above 30 mm. The longitudinal growth of hypocotyl is not affected by the increase in concentrations as it increases or is maintained with their increase, except when the higher concentration of the fruit extract is applied. In general, the best effects on the length of both the radicle and the hypocotyl for the Española-16 variety are obtained with the application of extracts based on root, stem and whole plant using, in all cases, concentrations 2 and 3.

Achieving products that are able to stimulate both the radicle and the hypocotyl is important, since the subsequent development of the adult root and stem depends on the good development of these embryonic organs. These results also show that the effect that occurs in the germination parameters does not necessarily have to affect the output processes of the embryonic organs. Pélagie et al. (2016) when using extracts from different plant species, they obtained an inhibitory effect on the seeds of the treated plants, however, when they were divided, they led to a significant increase in the diameter of the seedlings. They pointed out that the aqueous extracts used were effective in stem growth although the results in root growth were lower.

For their part, Radwan et al. (2019) when applying the highest concentrations of aqueous extracts of *Calotropis procera* L. obtained a significant reduction in comparison with the control of the germination percentage, the radicle length and the hypocotyl of the treated seeds. The results obtained in the present study do not coincide with what was stated above, since in most cases, with increasing concentrations, inhibitory effects are not obtained.

In the specific case of *C. annuum* Akgül & Akgül (2019), with the lowest application of extracts obtained from *Spirulina platensis* (Gomont) an earlier germination was obtained than with the rest of the solutions. However, the best results are obtained when a 25% solution is applied. This same concentration was the most effective for all the parameters evaluated (seed germination, root and stem length, fresh and dry matter, number of lateral roots). However, the beneficial impact was lost as the concentration of the extract increased. These authors reported that other *Solanaceae*, including other species of the genus *Capsicum*, also showed satisfactory results for these variables when applying products of natural origin.

These extracts generally show a similarity in the presence of different chemical families (Table 6). Most of them (five) were presented in the root and stem extracts. However, between these two extracts only four chemical families were common, since in the first the presence of quinones was reported and in the second the presence of resins. In the extract of leaves, the presence of four chemical families was evidenced that, unlike the previous ones, showed the presence of alkaloids in which it is similar to the whole plant extract in which only the presence of two chemical families was reported.
The presence of free amino acids was common to all the extracts, while for those of the root, stem and leaf, the presence of coumarins and phenolic compounds was common, as well as the flavonoids in the root and stem. In the case of the presence of free amino acids in the root, stem and leaf, it is considered abundant, while the alkaloids in the leaf are classified as moderately present. The rest of the chemical families are only considered as present. Taking into account the above, it is possible that the effect obtained with these extracts on the varieties of *C. annuum* studied is related to the presence of free amino acids.

Mali (2010) reported the presence of different types of coumarins in the phytochemical constitution of this plant species. Rajani et al. (2014) and Lakshmanan et al. (2018) in phytochemical studies carried out in *C. viscosa* reported the presence of saponins, terpenoids, steroids, tannins, flavonoids, phenols and alkaloids, although only the last three coincide with the present investigation. It is considered that this lack of similarity is since in the aforementioned investigations there are no reports of techniques for the determination of the other chemical families. Related to phenolic compounds, Nasseri et al. (2019) pointed out that are an important group of secondary metabolites in natural products, present a great variety of structures and include water-soluble functions within them quinones.

Table 6
Phytochemical composition of the extracts obtained with the best effect

Chemical families	Extracts			
	Root	Stem	Leaf	Whole plant
Saponins	-	-	-	-
Resins	-	+	-	-
Coumarins	+	+	+	-
Reducing sugars	-	-	-	-
Flavonoids	+	+	-	-
Phenolic compounds	+	+	+	-
Alkaloids	-	-	++	+
Mucilages	-	-	-	-
Free amino acids	+++	+++	+++	+
Quinones	+	-	-	-

Legend: + presence, ++ moderate, +++ abundant, - not found.

For their part, amino acids are the most abundant nitrogen species in xylem and phloem and considered the main carriers of this element (Okumoto & Pilot, 2011). This is since amino acids simultaneously contain amino and carboxyl groups given the need in the living cell for a constant supply of them, for protein synthesis and other physiological processes such as growth (Wolf et al., 2016). These authors point out that their functions include activating the synthesis of chlorophyll, which is why they influence the growth and development of plants; hence the free amino acids are of great application as invigorating and stimulating plants in critical periods.
López (2014) pointed out that the action of amino acids on the plant organism has always focused on their action to help them overcome situations of stress and high metabolic activity. Early research focused on the effects of amino acids on growth regulation. Within the eight categories of biostimulant substances that are currently recognized, according to Du Jardin (2015) are

free amino acids and other nitrogenous substances. As referred by Avilés (2017) there are reports in the literature where it is stated that amino acid concentrates are used in agriculture.

4. Conclusions

Cleome viscosa is promising for obtaining natural products with biostimulant effect on the varieties of pepper (*Capsicum annuum*) Verano 1 and Española-16, obtaining a better effect when the extracts of root, stem, leaf and whole plant are applied. In the extracts obtained with the best results, differences were verified in the richness of chemical families, showing the root and stem the best composition, being the presence of free amino acids a common factor in all of them.

Acknowledgment

To all the members of the student scientific group on Environmental Management of Agricultural Ecosystems, especially Lic. Jesús Díaz Labrada, Osniel Fuentes Miranda, Pedro Rafael Ríos Castellanos, Ing. Daliena Fernández Baños and Ing. Manuel Cobas Magdariaga. To Vismar Vargas Batiz for reviewing the english version of the manuscript.

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