ALLELOPATHIC EFFECT OF *Tephrosia cinerea* L. (Pers.) PLANT EXTRACTS IN LETTUCE

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ABSTRACT

Allelopathy has been conceptualized as the direct or indirect effect of a plant onto another one through secondary metabolites, or allelochemicals spread in the environment, with the power to interfere in seed germination and increase or inhibit the development of neighboring plants. The objective of this study was to analyze the possible allelopathic effects of aqueous extracts of *Tephrosia cinerea* in the germination of lettuce (*Lactuca sativa* L.) seeds. The experiment has been carried out at the Laboratório de Nutrição Mineral de Plantas (Laboratory of Plant Mineral Nutrition)/UAEF/UFCG in Patos (state of Paraíba). We collected *T. cinerea* organisms, having separated the stem, leaves, roots fractions and the aerial part of the plant + roots, having prepared the extract at 20%. The experimental delineation that we adopted was entirely casualized, with five treatments and four repetitions (without extract, leaf extract, root extract, stem extract, aerial part extract + roots). The seeding was held in aluminum trays, with autoclaved sand as the substrate, setting 25 seed per tray. The calculation of the number of germinated seeds was made in a daily-basis, and, after a week of seeding, the next step was the deactivation, with the evaluations being performed regarding the length of the radicle and of the hypocotyl, as well as the height of the seedlings. The data collected in this research allows us to conclude that: the extracts obtained from *T. cinerea* plants has a negative impact on the germination of lettuce seedlings. Now, when it comes to the height of lettuce seedlings, it is affected by the use of leaf, stem and aerial part extracts + *T. cinerea* roots. It is crucial to make efforts in order to determine the existing constituents in the several parts of *T. cinerea* plants.

Keywords: Allelopathy; Neighboring Plant; Germination

RESUMO

EFEITO ALELOPÁTICO DE EXTRATO DE PLANTAS DE TEPHROSIA CINEREA L. (PERS.) EM ALFACE (LACTUCA SATIVA L.)

A alelopatia tem sido conceituada como o efeito direto ou indireto de uma planta sobre outra por meio de metabólitos secundários ou aleloquímicos liberados no ambiente, podendo interferir na germinação de sementes e aumentar ou inibir o desenvolvimento de plantas vizinhas. O objetivo deste trabalho foi estudar os possíveis efeitos alelopáticos de extratos aquosos de *Tephrosia cinerea* na germinação de sementes de alface (*Lactuca sativa* L.). O experimento foi conduzido no Laboratório de Nutrição Mineral de Plantas/UAEF/UFCG, em Patos (PB). Indivíduos de *T. cinerea* foram coletados, separando as frações caule, folhas, raízes e parte aérea + raízes, preparando-se o extrato a 20%. O delineamento experimental utilizado foi inteiramente casualizado, com cinco tratamentos e quatro repetições (sem extrato, extrato de folhas, extrato de raízes, extrato de caule, extrato de parte aérea + raízes). A semeadura foi realizada em bandejas de alumínio tendo como substrato areia autoclavada, dispondo-se 25 sementes por bandeja. A contagem do número de sementes germinadas foi realizada diariamente e, após sete dias da semeadura, seguiu-se a desativação, sendo feita avaliações no tocante de comprimento da radícula e do hipocótilo, assim como da altura da plântula. Os dados obtidos no presente estudo permitem concluir que: os extratos obtidos de plantas de *T. cinerea* exercem efeito negativo na germinação das plântulas de alface. A altura das plântulas de alface é afetada pelo uso do extrato de folhas, caule e parte aérea + raízes de *T. cinerea*. Há necessidade, urgente, de esforços no sentido de se determinar os constituintes existentes nas diversas partes das plantas de *T. cinerea*.

Palavras-chave: alelopatia, planta invasora, germinação

Recebido para publicação em 12/04/2016. Aprovado em 24/05/2016.

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INTRODUCTION

Allelopathy has been described as a process by which products of a secondary metabolism from a specific plant are spread, preventing germination and the development of other plants that are relatively close (OLIVEIRA, 2016). When those compounds present any inhibitory property, it may affect the germination of seeds of several species, and their sensitivity varies according to the concentration that is applied (SEVERINO, 2007).

Some studies have demonstrated the effects of aqueous extracts over the germination of seeds from different species (SOUZA-FILHO, 2009; ROSADO et al., 2009). In most of the species, the allelopathic effect is more evident when leaf extracts are used (SOUZA, et al., 2007).

The resistance or tolerance to secondary metabolites is a species-specific characteristic, with the presence of those more sensitive and referred as indicator plants of allelopathic activity, like *Lactuca sativa* L. (lettuce), *Lycopersicon esculentum* Miller (tomato) and *Cucumis sativus* L. (cucumber). In order to be indicated as a testing plant, the specie must present a fast and uniform germination and a degree of sensitivity that permit to express the results under low concentrations of allelopathic substances (SOUZA, et al., 2007).

Tephrosia cinerea, known as "blue false indigo", is not a very palatable plant, it is invasive, but, during the dry season, when there are not other fodder plants available, ovine animals eat them in large quantities, and the ingestion of the fresh plant, during 60-80 days, is toxic for the animal, causing a disease known as "water belly" due to the fact that the abdominal cavity presents great amounts of liquid, which leads to the increase of the abdominal volume. That liquid is also found in the thoracic and pericardial cavities. However, the most relevant injury can be seen in the animal's liver. Animals affected may present clinical signs weeks or months before they die, and there is not a treatment available. Although, if they are removed from the pastures, they may restore their health (SANTOS et al., 2007).

The recognition of flavonoids in different species of the genus *Tephrosia* and the antioxidizing activity attributed to the metabolites abovementioned appear as an incentive for the conduction of studies regarding the chemical constituents and the analysis of the antioxidizing activity, as well as the allelopathic power of *T. cinerea*, a species of great incidence in the Brazilian semiarid biome, not to mention the wide applicability in people's lives, yet lacking of validation studies (SANTOS et al., 2011).

There are few information on the allelopathic effects of *T. cinerea* components in cultivated species.

Faced with such fact, we aimed, in this paper, to study the potential allelopathic effects of aqueous extracts from the germination specie aforementioned in lettuce seed, in addition to the height of the radicle and the hypocotyl.

MATERIALS AND METHODS

The experiment was carried out in nylon screenhouse at the Laboratory of Plant Mineral Nutrition from the Federal University of Campina Grande (UFCG), Campus of Patos (State of Paraíba).

In order to conduct the experiments, samples of *T. cinerea* were collected from a nearby area to the unit of Patos/UFCG, from which the collected material was separated according to the following fractions: leaves, stem, roots and aerial part + roots (Figure 1).

The lettuce seeds (*Lactuca sativa* var. *Crespa Cinderela*), with a germination rate of 98%, were purchased in a local commercial store.

To prepare the extracts, each fraction was mixed with deionized water to obtain an extract with 20% of concentration, from the homogenization in a blender for approximately 30 minutes. Afterwards, the material was filtered with a strainer and accommodated in dark glass bottles containing one litter, wrapped up in tin foil and kept under room temperature until it was ready to be used.

The experimental design that we employed was entirely of the casual type, comprising five treatments and four repetitions. The treatments consisted of: (1) no application of *Tephrosia* extract; (2) application of leaf extract of *Tephrosia*; (3) application of stem extract of *Tephrosia* plants; (4) application of root extract of *Tephrosia* plants; and (5) application of an extract obtained from the aerial parts + roots of *Tephrosia* plants.

We made use of aluminum trays (9.8" x 4.7" x 1.5"), having as a substrate autoclaved sand. In the seeding process, we placed 25 lettuce seeds per tray (experimental unit) and, subsequently, we

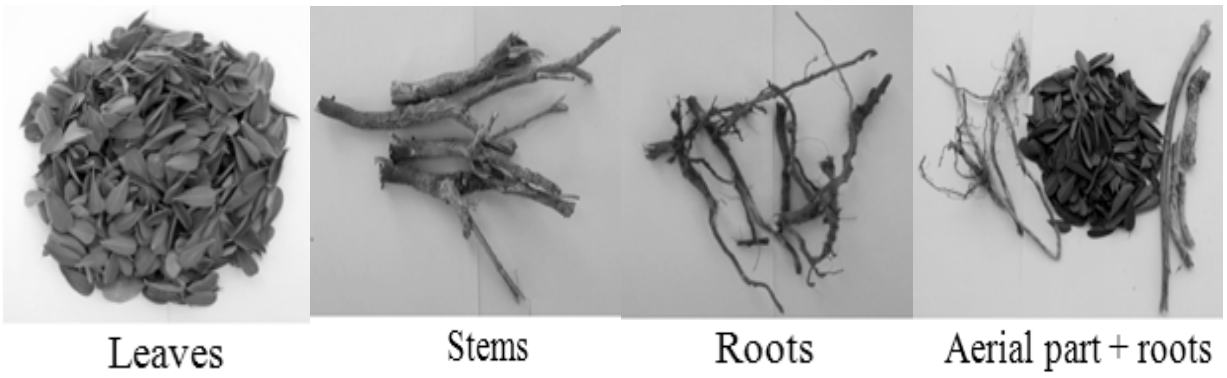

Figure 1. *T. cinerea* material separated in fractions for the preparation of the extracts.

applied the extracts referred to each treatment.

The germination started three days after the seedling, and, a week later, the experiment was discontinued. After the discontinuation of the experiment, the following procedures were carried out: measurement of the height of the radicle and hypocotyl, germination rate, speed index and average germination time.

The count of the number of germinated seeds was performed in a daily basis, being considered the germinated seed that presented a primary root with approximately 0.39" of height. The seed count test was based on the principle that the samples which showed a higher rate of average seedlings are the most vigorous (BRASIL, 1992).

Regarding the speed of germination index, the evaluations of the lettuce seedlings were done in a daily basis, at the same time, starting from the day when the first average seedlings bloomed, having being evaluated until the seventh day after the seeding phase. The speed index of germination was calculated through daily data about the amount of average seedlings, making use of the formula proposed by Krzyzanowski et al. (1999).

$$ISGI = \frac{G1}{N1} + \frac{G2}{N2} ... + \frac{Gn}{Nn} \quad (1)$$

where,

SGI = speed of germination index;
G1, G2, Gn = amount of average seedlings calculated in the first, second and last count; and
N1, N2, Nn = amount of days with which the seeding took place in the first, second and last count.

The average time of germination was obtained through daily counts of germinated seeds until the seventh day after sowing and calculated by the following formula proposed by Labouriau (1983), being the results expressed in days.

$$AGT = \Sigma (ni \, . \, ti) / \Sigma ni \quad (2)$$

where,

AGT = Average germination time (days);
ni = Number of seeds germinated in each count interval between; and
ti = Time elapsed between the onset of germination and the ith counting.

Immediately after deactivation of the experiment, were collected lettuce seedlings of each replicate, separately and placed in petri dishes with water to remove the excess sand and following the measurement of the radicle was performed using a caliper digital. Concurrently, they evaluated the length of hypocotyl and the height of the seedling, also with the aid of a digital caliper.

Data were subjected to analysis of variance and means compared by Tukey test at 5% probability. We used the ASSISTAT program (version 7.0).

RESULTS AND DISCUSSION

Data relating to lettuce seed germination subjected to extracts from different parts of *T. cinerea* are shown in Table 1. Consider that the following data were transformed into square root of x plus 1.

Table 1. Effect of Tephrosia cinerea extract on germination (G), germination speed index (GSI) and mean germination time (MGT) of lettuce seeds

Treatments	G (%)	GSI (%)	MGT (days)
Without *Tephrosia* extract	6,920 a*	2,572 a	2,507 c
Leaf Extract	1,000 b	1,000 b	2,830 a
Stem Extract	1,000 b	1,000 b	2,830 a
Root Extract	1,290 b	1,300 b	2,695 b
Aerial part + root extract	1,000 b	1.000 b	2,830 a
lsd**	1,452	0,381	0,111
CV (%)	29,65	12,69	1,87

* Means followed by the same letter in the columns do not differ statistically from each other when compared by Tukey test ($p < 0,05$); ** least significant difference.

This finding observed in Table 1 gives indication that *Tephrosia* plants have some/some component(s) which may(m) exercise allelopathic effect on lettuce. Therefore, further studies need to be performed in order to determine what those components contained in extracts from various parts of the plant that are inhibiting the growth of lettuce plants. This same observation applies to other plants.

Similar results to those observed in this study were found by Cantanhede Filho et al. (2013) to apply the extracts of *T. cinerea* sheets obtained with ethyl acetate and methanol showed high inhibitory potential for seed germination of two invasive species of grasslands, *Mimosa pudica* and *Senna obtusifolia*. According to the authors, the ethyl acetate extract showed inhibition percentage of 90.3% for species *Mimosa pudica* and 80.6% for *Senna obtusifolia*. But the methanol extract had inhibition percentage of 80.64% and 32.23% for *Mimosa pudica* to *Senna obtusifolia*, respectively. However, these authors also found that when it received the extract from *Tephrosia* leaves cinerea using hexane extract, this presented the inhibition percentage of 48.4% for *Mimosa pudica* and a stimulus of 3.3% for the species *Senna obtusifolia*.

When viewing the Table 2, it appears that the obtained extract of *Tephrosia* roots shows significant and positive effect on the growth of lettuce plants radicle. However, there was no difference in the treatment in which did not apply the *Tephrosia* extract. However, it is interesting to note that the extract obtained from the aerial parts as a whole or of its components in isolation, caused a reduction in the growth of the primary root of lettuce plants.

If this is so, mister becomes conducting research with extracts obtained from the aerial part of the plant *Tephrosia* in order to identify which components would be providing this deleterious effect on radicle, not just for lettuce but also to other plants.

Regarding the hypocotyl, it is observed that the various *Tephrosia* extracts applied at sowing did not produce significant effects on the hypocotyl length of lettuce ($p < 0,05$). The root system of plants is the most sensitive allelochemicals action because his stretching depends on cell divisions, which, if inhibited, compromise their normal development. Malformation in lettuce seedlings were also observed by Nery (2008) to study the allelopathic effect of turnip extract, which affected 87% of the analyzed plants with small-sized hypocotyl and oxidized and blackened roots.

Table 2. Effects of *Tephrosia cinerea* extract application on root growth (RG), shoot height (SH) and growth of hypocotyl (GH) of lettuce seedlings

Treatments	RG	SH	GH
	----------	-------- cm --------	----------
Without *Tephrosia* extract	0,775 ab*	1,175 a	0,775 a
Leaf Extract	0,700 b	0,700 b	0,700 a
Stem Extract	0,700 b	0,700 b	0,700 a
Root Extract	1,125 a	1,150 a	1,175 a
Aerial part + root extract	0,700 b	0,700 b	0,700 a
lsd**	0,3624	0,3898	0,503
CV (%)	20,79	20,16	28,42

* Means followed by the same letter in the columns do not differ statistically from each other when compared by Tukey test ($p < 0.05$); ** least significant difference.

CONCLUSION

- The extracts of *T. cinerea* plants exert negative effect on germination of lettuce seedlings. Since the height of the lettuce seedlings was affect by the use of the leaf extract, stem and shoot + roots of *T. cinerea*. There is, urgent need, efforts in order to determine the existing constituents in different parts of plants *T. cinerea*.

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