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Influence of *Ilex paraguariensis* aqueous extract on safflower growth and germination *in vitro*

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Allelopathy is a natural botanical effect in which certain crops release inhibitory substances which affect growth and development of other crops to the environment. The ability of plants to exhibit allelopathy vary among species. In Brazil, the allelopathic studies are restricted to common plants, such as corn, soy, wheat, and safflower. Given these factors, there are still many cultures as *Ilex paraguariensis* that are forgotten, even though, they play an important role in the regional cultivation. The aim of this study was to evaluate the effect of cold water extract of *I. paraguensis* leaves on germination rates, seeds early growth and speed of germination of *Carthamus tinctorius* L. sprayed with aqueous extract of mate. The extract production process consists in grinding mate leaves along with cold water and then filtering it to remove the particles. Concentrations of 0 (control), 25, 50, 75 and 100% of the extract were used to induce the treatment, considering 200 g of extracted adult fresh leaves per liter of cold water. The seeds were planted in individual tubes containing specific soil for cultivation and they were all sprayed manually every 3 days using the volume of 500 ml in appropriate concentrations. The experimental design was completely randomized as it follows: 5 treatments, 4 repetitions, and 1 safflower genotype. To carry out the analysis, the emergency data were collected until the thirtieth day. Considering the statistical analysis, there was no change resulting in allelopathic effect in the following rates: germination rate, first germination counting, average time of germination and percentage of germination, stem diameter, root length, and leaf dry mass, but it was observed that in the concentration T3 (50%), there was a significant increase in safflower growth in the following characteristics: leaf fresh mass, root fresh mass, leaf length, leaf area, and root dry mass.

Key words: Allelopathy, germination, influence, safflower, mate.

INTRODUCTION

Safflower, *Carthamus tinctorius,* is an annual crop that belongs to the family Astaraceae and it is native to Africa and Asia (Abud et al., 2010). Its flower has a red dye called cartamina, which was widely used in antiquity for

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the manufacture of pigments for fabrics. It was widely used in cooking because of the yellow pigment of some species (Arslan, 2007). Currently, in Brazil, it is a high economic value crop, because of its versatility and use for production of high quality vegetable oil. The seed contains about 30% of oil which can be extracted, thus it can contribute significantly to the production of biodiesel and silage (Bradley, 1999; Siddiqui et al., 2006).

It is widespread throughout the world medical and industrial importance (Velasco et al., 2005). Petals, leaves, and seeds have medicinal values, but the spread of this crop is due to its high oil content, which serves for the biodiesel industry (Dwivedi, 2005; Carneiro, 2012). It is considered as an energy crop or as a source of renewable energy (Kafra, 1998; Feizi et al., 2010).

The allelopathic effect can be set by the process of allelochemicals production due to the presence of one plant and it is produced by exudation, which may have improved the process with the presence of microorganisms in the soil. This effect becomes important in the suppression of unwanted crops, thus, it becomes a tool for future studied for application in several crops (Jabran et al., 2015).

In the plant, allelochemicals are derived from the secondary metabolism of the plant and are released into the environment via root in soil or volatile substances in the air via leaves (Kaffka, 1998; Rickli et al., 2011).

Allelochemicals are substances released by plant metabolism and they can affect the local environment. These toxins that are emitted around the crop decrease the intraspecific and interspecific competition among species, creating advantages for the plant which produces toxins. The inhibition on the growth and development of plants is an interesting knowledge for agriculture, which seeks ways to control pests and inhibit the growth of unwanted species (Brass, 2009).

Mate (*Ilex paraguariensis*) is a conducive crop in subtropical climates and it is widely used in Southern Brazil, Argentina, Paraguay and Chile. It is present as a cultural link between these countries and its production is concentrated in Argentina, which produces 750 thousand tons per year, and in Brazil, the production reaches 80 thousand tons per year (Fiedler et al., 2008). Mate substrate extraction process produces a series of components such as theobromine, caffeine, and derivatives and these substances can have biological, pharmaceutical and industrial effects (Isolabella et al., 2010).

The planted herbal area has increased in recent years and the system that has been most widely adopted is the consortium with other crops, particularly annual crops, such as mate harvest, require a wide spacing between plants and smaller crops to adapt well when intercropped planting of mate (Medrado et al., 2000).

The yerba mate intercropping system with other crops such as safflower, enables increased income of farmers and environmental point of view can contribute to the recovery of degraded marginal land. This type of consortium can also reduce the number of pests because the increased diversity of the local flora will increase natural enemies (Pasinato and Arthur, 2012).

Factors such as climate, soil and mate cultivation period can influence the production of chemical components, which consequently have adverse effects. It can be observed that *I. paraguariensis* contains large amounts of chlorogenic acid (derived from caffeine), and this by-product is used in the industry because of its properties that affect metabolism (Marques et al., 2009).

In Brazil, the allelopathic studies are restricted to common plants such as corn, soy, wheat and safflower. Given these factors, there are still many cultures as *I. paraguariensis* that are forgotten, even though, they play an important role in the regional cultivation. Mate itself has several chemical compounds that are used in medicine, but their adverse effects are not studied or are still being discovered. The allelopathic effect of mate has to be studied in the way that may be harmful to commonly planted crops like safflower, since between them there is an intercrop system. Thus, the aim of this study is to assess the growing and development in safflower germination submitted to mate leaves extract, in order to verify the allelopathic effects.

MATERIALS AND METHODS

Source of plant materials and study location

Mate adult and fresh leaves were used to assess the allelopathic effect on *C. tinctorius* L. seeds. Mate leaves were collected in the city of Cascavel, Paraná, Brazil, with the geographic coordinates of 24° 57'21"S and 53° 27'19"W and average altitude of 781 m. The safflower seeds were obtained from Instituto Agronômico do Paraná (IAPAR). The experiments took place in April and May 2016 (30 days) at the campus of Universidade Estadual do Oeste do Paraná (UNIOESTE) in a greenhouse made of polyethylene (free from any weather phenomenon) in a humid subtropical climate.

Preparation of extract

The extract was manufactured in concentrations of 0 (control), 25, 50, 75 and 100% of extract to induce the treatment, considering 200 g of leaf per each liter of cold water. For the irrigation, 500 ml of water was used and divided into the concentrations. The extract production process consists of grinding mate leaves along with water and then filtered to remove the particles.

Evaluation of allelopathic effects

The germinating of safflower seeds occurred in tubes of polyvinyl chloride (PVC) with diameter of 200 mm and height of 15 cm, housed in trays with sufficient diameter heights to accommodate the PVC pipes. The tubes were filled with the same type of soil up to a height of 14 cm. For the crop planting, 10 seeds per tube were organized, dispersed so that the seeds could germinate and develop without any interference.

The samples were placed in a greenhouse at the campus of Universidade Estadual do Oeste do Paraná (UNIOESTE), in such a

way that they were exposed to sunlight intermittently during the daytime. The experiment was performed in quadruplicate for each concentration and to maintain soil moisture, it was carried out the maintenance of water and extract manually, by means of a surface irrigation since the installation of the experiment. Surface irrigation was performed every three days and started on the first day of the experiment, all containing 500 ml per tube following the considered concentrations.

The experimental design was completely randomized as it follows: 5 treatments, 4 repetitions and 1 safflower genotype (IAPAR). To carry out the analysis, the emergency data were collected until the thirtieth day. The data analyzed were: germination speed rate, first germination count, germination average time, germination percentage, as well as fresh and dry mass of plants, root and plant length, stem diameter and leaf area.

Germination speed rate (GSI): Which was proposed by Maguire(1962) where E1, E2 ... En: number of normal seedlings recorded in the first, second and final counting. N1, N2 ... Nn: number of days after sowing, the first, second and last one.

$$GSI = \frac{E1}{N1} + \frac{E2}{N2} + \cdots \frac{En}{Nn}$$

First germination count (FGC): Counting the number of seeds with leaf protrusion and their respective days.

Average germination time (AGT): According to Labouriau et al. (1976), where ni is the number of seeds germinated in each counting interval; ti is the time elapsed between the start of germination and the i-th counting.

$$AGT = \frac{\sum ni\ ti}{\sum ni}$$

Germination percentage (G): Proposed by Siddiqet et al. (2007).

$$G = \frac{\text{nº of germinated seeds}}{\text{total of seeds}} \text{ x } 100$$

Fresh mass: Weighing of the plant newly collected in precision scale; in this case, we split the top part (plant) and bottom part (root). Dry mass: Plant weighing, top part (plant) and bottom part (root) after drying in an oven at 65°C after 72 h. Plant and root length: Measurement in cm from the plant apex to the base (plant) and subsequent measurement from the root beginning to the longest part using scalimeter. Stem diameter: Measurement of plant stem diameter with a digital caliper, considering the extent slightly below the first leaf protuberance. Leaf area: Holding of the measurement of each leaf in greater length and greater width to calculate the area.

Statistical analysis

The data obtained from the collection of information were analyzed with analysis of variance (ANOVA) to evaluate whether there is any evidence that the sample of the populations of plants differ. This analysis of variance leads to a conclusion that there is evidence that the group of the concentration differ, indicating whether there is a need in investigating which of them is different. This is where the Tukey multiple comparison test is used. The Tukey test compares the difference between each pair of samples with appropriate adjustment for the multiple testing. The test uses tables and comparative letters in columns, meaning equal letters do not differ themselves and columns with different letters have differences in level of 95% confidence.

The results are presented as a matrix showing the result for each pair, either as a P-value ($p < 0.05$), which shows the confidence interval of 95%. The Tukey multiple comparison test and the analysis of variance assumes that the data from different groups come from populations where the observations have a normal distribution and the standard deviation is the same for each group.

Data were statistically analyzed and were carrried out with the Action Stat 3.1 software, using analysis of variance ($p < 0.05$) and Tukey test.

RESULTS AND DISCUSSION

The different aqueous extract concentrations of *I. paraguariensis* did not cause any effects on the safflower samples in relation to the Germination Rate (1A), First Germination Counting (1B), Germination Average Time (1C) and Percentage of Germination (1D), because all the letters in the same column of concentrations are the same, therefore when considering a significance level at 5%, it can be said that the hypothesis of equality between the average levels is the sameas shown in Table 1.

Evaluating the germination of maize by the mate fruit extract in different concentrations, it was observed that the germination and emergence of the maize seedlings were not affected, and it was also found that the analysis was not performed only with the crop leaf (Miro et al., 1998).

The concentrations of *I. paraguariensis* extract did not cause allelopathic effects on safflower growth. The stem diameter, root length and leaf dry mass showed no effect; in other words, after the analysis of variance, it was found out that $p>0.05$, thus, the samples were all similar, not rejecting the null hypothesis (equality between samples).

A sample variance was observed between the control sample T1 (0% of extract) and T3 (50% of extract) in leaf fresh mass, root fresh mass, leaf length, leaf area, and root dry mass, thus noticing that there is rejection of the null hypothesis with $p < 0.05$. By Tukey test (Figure 2), it was observed that the comparison between the T1 and T3 samples were outside the ranges, with an average higher than the control.

Mate extract may contain many different chemical compounds, among them there are caffeine, chlorogenic acid and rutin, products which can cause effects when applied to different metabolisms (Blum-Silva et al., 2016).

There was a considerable increase in relation to the safflower growth comparing the averages of T1 samples (control) with samples of T3 in Table 2, as follows: 97.92% for leaf fresh mass (2A); 82,31% of root fresh mass (2B); 29.27% leaf length (2C); 97.92% leaf area (2D); and 154.49% to root dry mass (2E), proving the positive variance by Tukey test, where mate extract concentrations strengthened the growth in these analysed characteristics.

All other mate extract concentrations (25, 50, 75 and

Table 1. Tukey confidence interval: Germination Index (A), First Germination Count (B), Average Germination Time (C) Percentage of Germination (D).

GSI (A)			FGC (B)		
Factor	**Averages**	**Group**	**Factor**	**Averages**	**Group**
T1	1.70(0.13)	a	T1	6.5 (1.0)	a
T2	1.38 (0.61)	a	T2	6.75 (2.06)	a
T3	1.41 (0.52)	a	T3	6.25 (2.22)	a
T4	1.14 (0.32)	a	T4	5.25 (1.71)	a
T5	1.42(0.52)	a	T5	6 (1.63)	a

AGT (C)			G (D)		
Factor	**Averages**	**Group**	**Factor**	**Averages**	**Group**
T1	16.66(1.73)	a	T1	65	a
T2	19.43 (2.21)	a	T2	67.5	a
T3	18.34 (1.1)	a	T3	62.5	a
T4	17.71786 (1.61)	a	T4	52.5	a
T5	17.67(2.21)	a	T5	60	a

Values followed by the same letter in the column do not differ itself by Tukey test at 5% significance; Values in parentheses represent standard deviation.

Table 2. Leaf fresh mass (A), root fresh mass (B), leaf length (C), leaf area (D), and root dry mass (E).

Leaf fresh mass (A)			Root fresh mass (B)			Leaf length (C)		
Factor	**Averages**	**Group**	**Factor**	**Averages**	**Group**	**Factor**	**Averages**	**Group**
T1	1.85 (0.70)	b	T1	0.049 (0.013)	b	T1	15.98 (1.58)	b
T2	2.04 (0.91)	ab	T2	0.064 (0.02)	ab	T2	17.53 (2.67)	ab
T3	3.67 (1.31)	a	T3	0.09 (0.02)	a	T3	20.66 (2.27)	a
T4	1.58 (0.20)	b	T4	0.087 (0.02)	a	T4	17.08 (1.63)	ab
T5	2.67 (1.28)	ab	T5	0.08 (0.016)	ab	T5	18.38 (2.70)	ab

Leaf area (D)			Root dry mass (E)		
Factor	**Averages**	**Group**	**Factor**	**Averages**	**Group**
T1	39.33 (15.04)	b	T1	0.0067 (0.002)	b
T2	43.43 (19.17)	ab	T2	0.0107(0.003)	b
T3	77.85 (27.87)	a	T3	0.017 (0.002)	a
T4	33.50447 (4.27)	b	T4	0.00968 (0.002)	b
T5	56.66014 (27.1)	ab	T5	0.0112 (0.004)	ab

Values followed by the same letter in the column do not differ itself by Tukey test at 5% significance; Values in parentheses represent standard deviation.

100%) showed no significant difference from the control sample (0%) in Table 2, because when considering a significance level at 5%, it can be stated that the hypothesis of equality between the average levels is the same.

It can be seen that the confidence intervals in Figure 1 has only the concentration of 50% extract different from all others. The observation is made in the confidence intervals, where there is a comparison with the control sample T1. It is observed that the confidence interval T1-T3 (comparison sample T1 to T3) does not pass within the point of origin (zero), so it can be stated that the hypothesis of equality between the averages is not the same. Although, it is observed that the confidence interval obtained values higher than zero, so there was a positive development of safflower to extract.

Conclusion

It can be concluded that the different mate extract concentrations (25, 50, 75 and 100%), which were used to induce the treatment, did not cause any allelopathic effects on safflower development and germination.

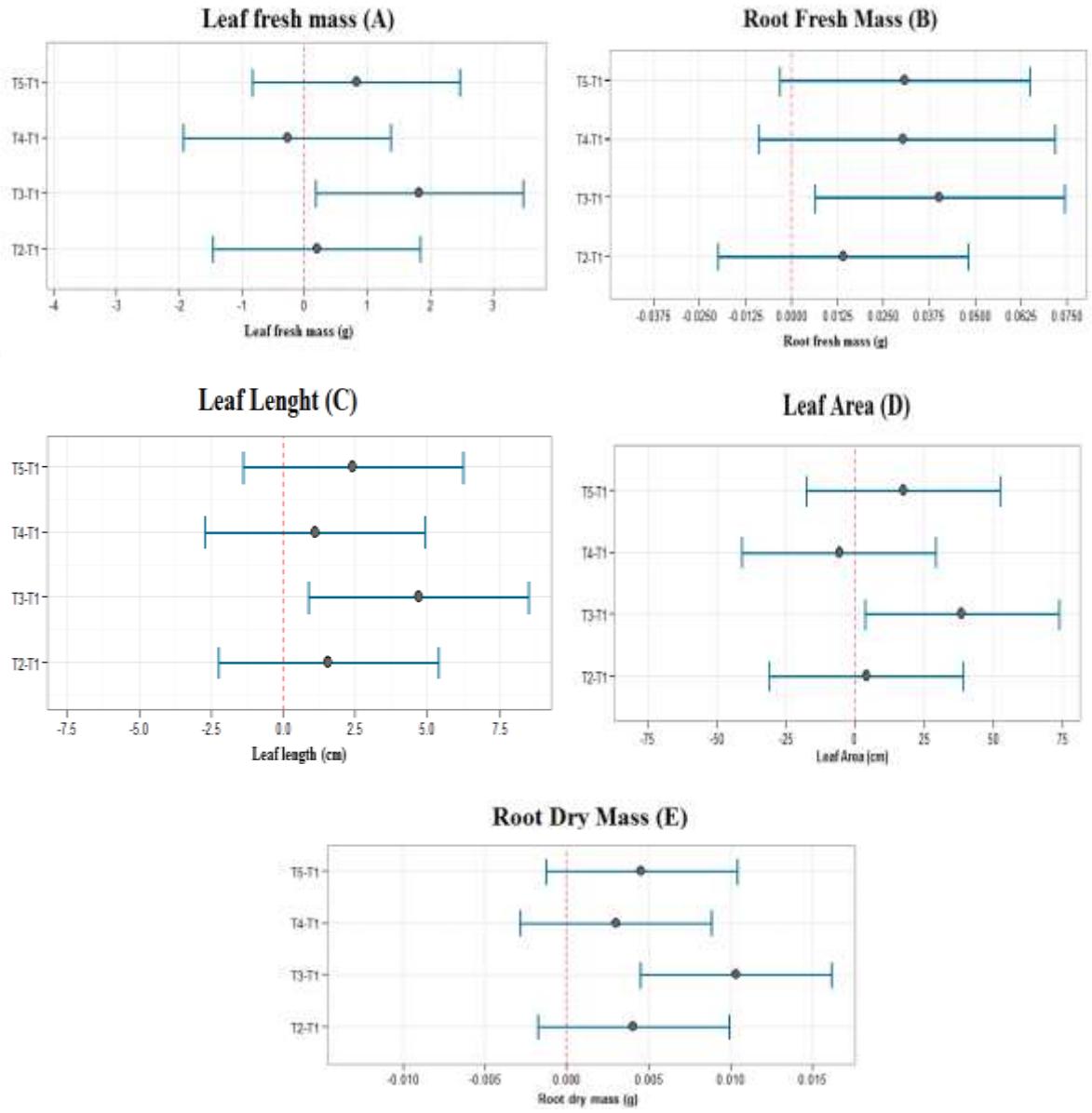

Figure 1. Leaf fresh mass (A), root fresh mass (B), leaf length (C), leaf area (D), and root dry mass (E).

However, given the 50% concentration of the extract, there was a potentiation in the samples average compared to the control sample T1, observing this variance in the following features: leaf fresh mass, root fresh mass, leaf length, leaf area, and root dry mass.

Conflict of interests

The authors have not declared any conflict of interests.

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