academicJournals

Vol. 15(49), pp. 2787-2793, 7 December 2016
DOI: 10.5897/AJB2016.15707
Article Number: AFED90361939
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Allelopathic effect of aqueous extract of fresh leaf castor beans (*Ricinus communis* L.) applied to the beginning stage of soy (*Glycine max* L.) and safflower (*Carthamus tinctorius* L.)

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Received 29 September, 2016; Accepted 17 November, 2016

Allelopathy defines the production of specific biomolecules (allelochemical) by a plant that can induce positive or negative impacts on another culture. The crop of castor beans (*Ricinus communis* L.) is being economically valued and receiving attention, mainly by the biodiesel production, castor oil and animal feeding. In this study, the allelopathic effect of aqueous extract from fresh leaves of castor beans (*Ricinus communis* L.) applied to the beginning stage of soy (*Glycine max* L.) and of safflower (*Carthamus tinctorius* L.) was investigated. An experimental was setup with five blocks and four replications, considering that each block refers to a concentration of extract of castor beans fresh leaf (0, 25, 50, 75 and 100%); the one without castor beans leaves was taken as 'witness'. The statistic treatment was done using analysis of variance (ANOVA) and Tukey test with an error rate of 5%. The result showed evidences that there are allelopathic effects among the extract of castor beans fresh leaf and the beginning stage of soy and safflower only in the root part of the seedling.

Key words: *Ricinus communis L., Glycine max* L., allelopaty.

INTRODUCTION

Allelopathy term defines the production of specific biomolecules (allelochemicals) by a plant that can induce positive or negative impacts on other cultures (Babula et al., 2009). There are many ways for a plant to release its allelopathic components: By volatilization, by soil through roots and leaves, that after they have fallen, they

decompose and release allelopathic substances (Rizvi et al., 1992).

Safflower (*Carthamus tinctorius* L.) is an oleaginous of multiple purposes and it has been cultivated for many centuries in the worldwide, mainly in Iran, Argentina, Kazakhstan, Mexico and in Brazil in the last years (FAOSTAT, 2013; Silva, 2013). In the ancient times, it was used as pigment and tea, but only in the last century its oil started being used for food and industrial purposes, due to its high level of quality (Ahmed et al., 2007; Sehgal and Raina, 2005). It is rich in unsaturated fatty acids with the linoleic acid proportion of about 78% in relation to the total fatty acids. It has red and orange pigments extracted from its flowers (Davood et al., 2013). In Brazil it has been receiving emphasis as second harvest crop, and in 2013 the Brazilian sunflower and safflower oil exportation reached a participation of 0.05% in the world market (Guidorizzi, 2016; MARA, 2013).

Soy is one of the main responsible for the concept of agribusiness and cultivation of grain. In world level, it represents the main oilseed consumed and produced. Its importance is justified by the fact that it may be used for animal consumption and for human consumption. The soy complex has a chain of processes which are the base for the Brazilian economy, which transforms the raw product in the most diverse uses, as animal feed, oil derived (Furtuoso and Guilhoto, 2013).

According to FAO (2016), soy (*Glycine max* L.) will continue being the crop that produces more oil and protein until 2025, at least, because of its great world demand of products derived of this culture. However the projections show that Brazil will overcome the United States in the production ranking because of its productivity and available area. In the same publishing, FAO states that the increasing demand for grains rich in protein and oil has been the main reason of the expansion of oleaginous production in the past years.

As the biodiesel and castor oil production, the castor beans (*Ricinus communis* L.) cultivation is being economic valued and receiving attention from the producers (Costa et al., 2014). According to CONAB (2016) the estimation is that Brazil will raise its production, planted area and productivity in the next years. According to Santos et al. (2011), another reason of interest for castor beans is the possibility that it is a culture for familiar crop because it is easily adaptable and resistant to the climate and soil conditions.

Recent studies done by Kpanja et al. (2016) concluded that if the castor beans pie is cooked for 30 min on steam it can complement or even substitute the peanut pie to the level of 50% in the diets of broiler chicken, showing new purposes for castor beans products and stimulate the interest for castor beans crop.

This paperwork aims to verify if the aqueous extract of castor beans fresh leaves has influence on soy and safflower germination and development in its beginning stage, when this fluid is applied with certain frequency comparing to seedling that did not receive this treatment.

MATERIALS AND METHODS

Study location

This study was conducted at Universidade Estadual do Oeste do Paraná[State University from the West of Paraná], UNIOESTE, campus from Cascavel, Paraná, Brazil, with geographic coordinates 24° 57' 21"S and 53° 27' 19"W and average height of 781 m above sea level. The seedling in the greenhouse was done in the campus and the analysis at Laboratório de Física dos Solos [Soil Physics Lab]. The experiment with soy was conducted with Nidera 5909 variety and with a safflower genotype made available by the Instituto Agronômico do Paraná [Agronomic Institute from Paraná] (IAPAR).

Experimental design

Fresh castor beans leaves were collected from UNIOESTE campus with the geographic coordinates of 24º 57'21"S and 53º 27'19"W and average altitude of 781 m. The collected materials were triturated with electric blender and sieved for the extract preparation. For the seedling, 10 seeds were disposed in a spread way, each treatment had 4 repetitions, totalizing 40 seeds per treatment. The germinating of 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 deepness of the seeding was constant in all the treatments, being 1.5 cm.

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 grams of leaf per each liter of cold water, the concentrations were represented by the terms T1, T2, T3, T4 and T5. For the irrigation, 500 ml of water was used, divided into the concentrations. The extract production process consists in grinding mate leaves along with water and then filtered to remove the particles.

Evaluation of allelopathic effects

Germination evaluations were done daily after the installation of the experiment; it started on the first day and ended on the fifteenth day. It is necessary to consider that the stabilization was reached on the thirteenth day on safflower and twentieth day on soy and we considered a germinated seed the one to show 2 mm of protrusion root (MARA, 2009).

There was a difference in relation to the evaluated germination, which was: First germination counting (PCG) –done on the ninth day after the experiment installation (MARA, 2009).

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Table 1. Tukey trust break of parameters evaluated in soy.

GSI (A)			FGC (B)		
Factors	**Averages**	**Group**	**Factors**	**Averages**	**Group**
T1	0.62(0.58)	a	T1	1.50(1.00)	a
T2	0.51(0.29)	a	T2	1.75(0.96)	a
T3	1.05(0.73)	a	T3	1.75(0.50)	a
T4	0.67(0.33)	a	T4	2.25(0.96)	a
T5	0.67(0.27)	a	T5	2.25(0.50)	a
AGT (C)			**G (D)**		
Factors	**Averages**	**Group**	**Factors**	**Averages**	**Group**
T1	10.17(1.37)	a	T1	58.33(31.91)	a
T2	9.92(0.69)	a	T2	58.33(31.91)	a
T3	9.08(1.99)	a	T3	66.67(27.22)	a
T4	10.63(0.64)	a	T4	91.67(16.67)	a
T5	9.92(0.99)	a	T5	75.00(16.67)	a

Values followed by the same letter in the same column do not differ among themselves by the Tukey test in 5% of significance; values in brackets show the standard deviation. (A), Germination speed index; (B), first germination count; (C), average germination time; (D), percentage of germination.

The equation proposed by Maguire (1962) was used in the Germination speed index (GSI). Where G1, G2, ...,Gn are the numbers of calculated seeding from the first, second to the last counting, N1, N2, ..., Nn are the numbers of days of seeding to the first, second to the last counting.

$$GSI = \frac{G1}{N1} + \frac{G2}{N2} + \cdots + \frac{Gn}{Nn}$$

Percentage of germination (G) where N is the number of germinated seed and A is the total number of put seeds (MARA, 1992).

$$G = \frac{N}{A} * 100$$

The average germination time (AGT) was obtained through daily counting of germinated seed to the fifteenth day after the seeding and calculated with the equation proposed by Labouriau (1983), being the expressed results in days. In which ni is the number of germinated seed in the break among each counting and ti is the time that passed in the beginning and i-th counting.

$$AGT = \frac{\Sigma\, ni.ti}{\Sigma\, ni}$$

For the other variables only the 6 more homogeneous seedlings of each treatment were used, these are the variable: radicle length (RL) and aerial part length (CA) - measured with a caliper; leaf fresh mass (LFM); root fresh mass (RFM), leaf dry mass (LDM) and radicle dry mass (RDM) and - the seedling were dried in a greenhouse of forced air circulation on 65°C for 72 h, until it reached a constant weight.

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 are 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 the 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 carried out with the Action Stat 3.1 software, using analysis of variance ($p < 0.05$) and Tukey test.

RESULTS AND DISCUSSION

Soy is a worldwide important culture and high economic value; therefore, many researchers study this culture. The allelopathic effect that other plants may cause on soy is relevant because the organic material left by winter crops can harm or benefit the development of the plant. In the familiar agriculture it is even possible to induce aqueous extracts with allelopathic potential on purpose to better develop the plant to avoid the progress of weed (Nóbrega et al., 2009).

As Table 1 shows the germination speed index (A), the first germination count (B), germination average time (C) and percent of germination (D) of soy did not suffer significant changes on influence of aqueous extract of castor beans. Rickli et al. (2011) noticed that the aqueous extract from Neem (*Azadirachta indica*) leaves did not show allelopathic effects when applied to the soy seedling in relation to germination, but the germination

Table 2. Tukey trust break of the parameters evaluated in soy.

LFM (A)			RFM (B)		
Factors	**Averages**	**Group**	**Factors**	**Averages**	**Group**
T1	1.54(0.32)	a	T1	0.61(0.12)	a
T2	1.45(0.30)	a	T2	0.51(0.16)	a
T3	1.06(0.52)	a	T3	0.43(0.20)	a
T4	1.48(0.26)	a	T4	0.77(0.12)	a
T5	1.23(0.15)	a	T5	0.53(0.18)	a
SD (C)			**APL (D)**		
Factors	**Averages**	**Group**	**Factors**	**Averages**	**Group**
T1	1.58(0.07)	a	T1	16.91(2.45)	a
T2	1.77(0.29)	a	T2	16.59(2.16)	a
T3	1.71(0.58)	a	T3	13.93(6.41)	a
T4	2.27(0.46)	a	T4	15.48(1.21)	a
T5	1.88(0.42)	a	T5	14.19(0.45)	a

Values followed by the same letter in the same column do not differ among themselves by the Tukey test in 5% of significance; Values in brackets show the standard deviation. (A), Leaf fresh mass; (B), root fresh mass; (C), stem diameter; (D), aerial part length.

Table 3. Tukey trust breaks of the evaluated parameters in soy.

RL (A)			LA (B)		
Factors	**Averages**	**Group**	**Factors**	**Averages**	**Group**
T1	8.26(1.11)	ab	T1	47.82(9.82)	a
T2	8.72(1.29)	b	T2	44.76(9.20)	a
T3	11.58(4.00)	a	T3	32.80(15.95)	a
T4	9.60(0.32)	ab	T4	45.89(7.99)	a
T5	8.92(0.92)	ab	T5	38.03(4.67)	a
LDM (C)			**RDM (D)**		
Factors	**Averages**	**Group**	**Factors**	**Averages**	**Group**
T1	0.16(0.03)	a	T1	0.05(0.02)	b
T2	0.16(0.02)	a	T2	0.05(0.01)	ab
T3	0.12(0.04)	a	T3	0.05(0.02)	ab
T4	0.16(0.02)	a	T4	0.08(0.02)	a
T5	0.15(0.01)	a	T5	0.06(0.02)	ab

Values followed by the same letter in the same column do not differ among themselves by the Tukey test in 5% of significance; Values in brackets show the standard deviation. (A), Radicular length; (B), leaf area; (C), leaf dry mass; (D), radicle dry mass.

average time and germination average speed were considerable affected by the concentration of 40%, where the average time increased. These authors suggest that these negative influences on the germination average time and germination average speed could harm the agriculture's harvest by changing the uniformity in the production.

The leaf fresh mass (A), root fresh mass (B), stem diameter (C) and Aerial part length (D) of soy did not suffer significant change related to the development when compared to a witness (T1) as shown in Table 2. However, some authors point that the allopathic compounds may show different results not only by concentration, but also by chemical composition (Richardson and Williamson, 1988), and then it was possible to have different results with residues from other castor beans parts and applying aqueous extract of dry leaves.

Results in Table 3 indicate that leaf area and leaf dry mass of soy were not influenced by the introduction of aqueous extract of castor beans leaf. On the other hand, the radicle length and the soy radicle dry mass suffered positive changes when the castor beans aqueous extract was applied. A similar result was found in a work where the radicle increasing growth of soy was observed under the influence of aqueous extract of pine (*Pinus elliottii*) and millet (*Pennisetum glaucum*) (Faria et al., 2009).

Silva et al. (2015) by applying salvia (*Salvia officinalis)*

Table 4. Tukey trust break of evaluated parameters of safflower.

GSI (A)			FGC (B)		
Factors	**Averages**	**Group**	**Factors**	**Averages**	**Group**
T1	3.48(0.48)	a	T1	3.75(0.50)	a
T2	3.84(0.53)	a	T2	3.25(0.50)	a
T3	3.25(2.36)	a	T3	3.50(2.65)	a
T4	1.98(1.22)	a	T4	2.00(1.41)	a
T5	2.88(1.73)	a	T5	3.00(1.41)	a
AGT (C)			**G (D)**		
Factors	**Averages**	**Group**	**Factors**	**Averages**	**Group**
T1	8.04(1.14)	a	T1	57.50(9.57)	a
T2	8.11(0.30)	a	T2	60.00(8.16)	a
T3	7.87(1.50)	a	T3	50.00(33.67)	a
T4	8.74(1.96)	a	T4	37.50(9.57)	a
T5	6.43(1.01)	a	T5	32.50(12.53)	a

Values followed by the same letter in the same column do not differ among themselves by the Tukey test in 5% of significance; Values in brackets show the standard deviation. (A), Germination speed index; (B), first germination count; (C), average germination time; (D), percentage of germination.

leaf extract noticed that there is a stimulation of soy growth, mainly of the radicular part and there was no difference in the germination percentage in relation to the witness with this interference. Nunes et al. (2014) in studies about allelopathic activity of extract from plants covering soy, cucumber (*Cucumis sativus*) and lettuce (*Lactuca sativa*) related that *Crotalaria* is the crop that the extract suffered more positively than other crops, but the linseed extract (*Linum usitatissimum*) showed opposite behavior.

A study done by Peron et al. (2014) obtained that aqueous extract of fresh leaves and dry tobacco (*Nicotiana tabacum* L.) showed a marked inhibitory effect in germination and initial growth of soy seedling. It was also observed that the extracts done with tobacco dry leaves showed more significant effects on germination and speed rate of soy seeds germination, while the extracts made with fresh leaves, as in this work, had more interference in the length root and in the fresh and dry biomass.

With safflower the interferences done with extract concentrations 25, 50, 75 and 100% in relation to germination, considering the averages submitted to analysis of variance and compared by Tukey test with an error rate of 5%. In this study, it was observed that there was no significant difference of development among the seedlings that received the aqueous extract and those which received only water as shown in Table 4.

It can be further noted from Table 4 that trust break to each variable analyzed on germination, germination speed index (A), first germination count (B), average germination time (C) and percentage of germination (D) showed significant changes all the breaks belong to group A.

The aerial part length was the only parameter analyzed that had significant change as item D shows in Table 5. In this case, the treatment T3 the length was the biggest in relation to the other treatments and treatment T2 was significantly smaller than the other treatments.

About the safflower development (*Carthamus tinctorius* L.) in the other parameters of radicular and leaf development there was no positive or negative effect in the treatment with aqueous extract in relation to the witness either, considering the same statistic treatment done with the germination variables. Tables 5 and 6 show the trust break graphics on 95%.

According to Cuchiara et al. (2007) the decomposition of vegetal residuals is one of the most important sources of allelochemicals. In his work, in which he tried to verify the antiproliferative effect of aqueous extract of castor beans (*R. communis* L.) applied to lettuce (*Lactuca sativa* L.), he observed an opposite behavior with extracts done with fresh leaves and extracts done with dry leaves, where the last one showed the biggest allelopathic effect inhibitory in lettuce.

The allelochemial substances in a vegetal depend on the composition, concentration and localization in the plant. These compounds tend to be released in many ways, where ambient variables influence in dispersion (Cuchiara et al., 2007). Correlating with studies already shown, it was analyzed that the extract of castor beans fresh leaf does not have allelopathic effect of safflower plant, but using dry leaved the same result cannot be guaranteed.

Conclusion

The aqueous extract of castor beans positively influenced the development of soy seedlings. The interference

Table 5. Tukey trust break of safflower parameters evaluated.

LFM (A)			RFM (B)		
Factors	**Averages**	**Group**	**Factors**	**Averages**	**Group**
T1	3.76(0.82)	a	T1	0.15(0.03)	a
T2	3.57(1.19)	a	T2	0.14(0.07)	a
T3	4.14(152)	a	T3	0.14(0.05)	a
T4	4.10(1.28)	a	T4	0.16(0.04)	a
T5	3.53(1.49)	a	T5	0.08(0.03)	a
SD (C)			**APL (D)**		
Factors	**Averages**	**Group**	**Factors**	**Averages**	**Group**
T1	4.07(0.77)	a	T1	19.13(2.30)	ab
T2	3.50(0.50)	a	T2	15.61(3.29)	b
T3	3.54(0.44)	a	T3	21.30(2.73)	a
T4	3.24(0.60)	a	T4	19.83(1.75)	ab
T5	3.14(0.32)	a	T5	19.05(3.5)	ab

Values followed by the same letter in the same column do not differ among themselves by the Tukey test in 5% of significance; Values in brackets show the standard deviation. (A), leaf fresh mass; (B), root fresh mass; (C), stem diameter; (D), .aerial part length.

Table 6. Tukey trust break of safflower parameter evaluated.

RL (A)			LA (B)		
Factors	**Averages**	**Group**	**Factors**	**Averages**	**Group**
T1	13.77(3.34)	A	T1	77.26(16.76)	a
T2	15.61(3.29)	A	T2	73.23(24.48)	a
T3	15.98(5.61)	A	T3	84.93(31.29)	a
T4	14.68(4.07)	A	T4	84.19(26.21)	a
T5	12.62(3.70)	A	T5	72.39(30.58)	a
LDM (C)			**RDM (D)**		
Factors	**Averages**	**Group**	**Factors**	**Averages**	**Group**
T1	0.27(0.06)	A	T1	0.02(0.00)	a
T2	0.28(0.10)	A	T2	0.02(0.01)	a
T3	0.29(0.10)	A	T3	0.02(0.01)	a
T4	0.32(0.10)	A	T4	0.02(0.00)	a
T5	0.28(0.11)	A	T5	0.02(0.02)	a

Values followed by the same letter in the same column do not differ among themselves by the Tukey test in 5% of significance; Values in brackets show the standard deviation. (A), Radicular length; (B), leaf area; (C), leaf dry mass; (D), radicle dry mass.

happened mainly in the roots. In concentration T3 the intervention resulted in bigger radicular growth. But in concentration T4 there was the biggest dry mass radicular. Considering the obtained results by statistic treatments, it is concluded that extract of castor beans fresh leaf (*R. communis* L.) applied to the beginning stage of safflower (*C. tinctorius* L.) showed stimulant effects of leaf area with extract T3 (50%). It is suggested for further studies the elaboration of aqueous extract with castor beans dry leaves to verify the allopathic effect of it in soy and safflower crop.

Conflicts of Interests

The authors have not declared any conflict of interests.

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