Allelopathic Action of the N ger (*Guizotia abyssinica* Cass.) on Soybean (*Glycine max* (L.) Merrill)

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Abstract

Soybean (*Glycine max* (L.) Merrill) is an agricultural crop with a large increase in production in the last three decades and is an essential component in the manufacture of animal and human food. Understanding the interactions between crop and other plant species used as green manure that can improve yield and so reduce environmental damage. Forages can release secondary metabolites in the environment that influence in a beneficial or harmful way to other plants, characterizing the allelopathy process. The N ger (Guizotia abyssinica Cass.) is used in agricultural systems because it releases allelochemicals, especially flavonoids. Therefore, this study is aimed to evaluate the allelopathic effects of the Niger straw on the germination and initial growth of soybean seedlings. The treatments consisted of aqueous extracts of niger stems, leaves and roots in different concentrations (0; 25; 50; 75 and 100%). For each treatment, five replications were performed, each composed by a 25 seeds distributed in gearbox kept in incubator chamber for 7 days at 25 °C and 12 hours photo period. The experimental design was completely randomized and the data were evaluated by analysis of variance and the means of treatments compared by Tukey's test at 5% significance. The results show a reduction in germination and initial growth of soybean seedlings submitted to aqueous extract of niger stem and roots. In contrast, the aqueous extract of the Niger leaves increased the length of soybean seedlings. The allelopathic effects of the Niger probably occur due to the presence of flavonoids in the tissues of this plant species.

Keywords: allelopathy, green manure, flavonoids



1. Introduction

The Soybean (*Glycine max* (L.) Merrill.) is among the main crops consumed and produced worldwide, behind only corn, wheat and rice (Hirakuri and Lazzarotto, 2014). Among the seeds, soy is the most important source of oil and proteins (Hartman et al., 2011), is widely used by agribusiness, chemical and food industry. It is a crop with a important value in the world economy, responsible for most of the oil seed production (Wilson, 2008). The large consumption and the new genetic materials developed, to justify the widespread cultivation of soybean and enable their's consumption worldwide, especially in the United States, Brazil, Argentina and China (Campos, 2011; Nishinari et al., 2014, Rocha et al., 2018).

With the progressive increase in world soybean production it became necessary to find alternative cultivation, such as the implementation of a no-tillage system which can increase soil productivity in a sustainable and economical way by promoting improvements in physical, chemical and biological attributes, helping to protect the soil from erosion and irradiation, besides conserving moisture and providing nutrient cycling, which motivates farmers to use green manure in crop succession and rotation systems (Carvalho et al. 2014; Simonetti et al., 2019).

This planting techniques were pointed out as a system capable of contributing to sustainability by maintaining the straw of the previous crop, so the organic matter maintained can release allelochemicals that act as herbicides, reducing agrochemical costs and has an environmental impact (Darolt, 2000; Fontan éti et al., 2004; Monquero et al., 2009). Knowledge of the allelopathic effects of various substances is important to understand interactions between plant species in both natural and agricultural ecosystems (Jabran et al., 2015).

Allelopathy is defined as any, direct or indirect, beneficial or harmful, effect of a plant or microorganisms on other organisms by producing chemical compounds that are released into the environment. (IAS, 2015; Santos, 2012; Linares et al., 2006). Such substances are found distributed in varying concentrations in different parts of the plant and during its life cycle. They can be found in all tissues, including leaves, flowers, fruits, roots, rhizomes, stems and seeds (Almeida et al., 2008) and are released into the environment by volatilization, leaching and decomposition (Blum, 2011).

When these substances are in insufficient quantities, allelopathic effects may be observed on germination, growth and/or development of plants and microorganisms. (Barbosa et al., 2018; Battistus et al., 2011). Effects may be intra- and interspecific and may affect both the donor and recipient organisms (Oliveira, 2009).

The Niger (*Guizotia abyssinica* Cass.) is an annual herbaceous plant originally from Ethiopia. It belongs to the Asteraceae family and can reach up to 2m in height, being the only cultivable species of the genus *Guizotia* (Getinet and Sharma, 1996; Gordin et al., 2014). The seeds contain 30% oil and are used for commercial biodiesel production (Sarin et al., 2009). Niger is also used as green manure for soil by increasing organic matter and its seeds can be used as human food (Carneiro et al., 2008; Seegeler, 1983). It is adapted to different soil



types and grows in temperate and tropical regions (Bottega et al., 2013)

This plant is rich in flavonoids, class of secondary metabolites known for allelopathic potential (Taiz et al., 2017). Kuo et al. (2007) identified nine flavonoids isolated from the aerial parts: (–)-liquiritigenin, (–)-7,3',4'-trihydroxyflavanone, (–)-7,8,3',4'-tetrahydroxylflavanone, 7,3',4'-trihydroxyflavone, quercetin, isoliquiritigenin, butein, okanin, and 3,2',4'-trihydroxy-4,3'-dimethoxychalcone. Therefore, it is a potential donor of phytotoxins capable of acting on the natural inhibition of agricultural pests, especially on weeds (Carneiro et al, 2008). However, little is known about the allelopathic effects of the Niger, especially on agricultural crops, and there are no reports on soy studies.

The study of the allelopathic effect of straw used for mulching on cultivated species is generally done with bioassays that use aqueous or alcoholic extracts in germination chambers tests, evaluating germination percentage, root elongation and fresh and dry biomass of roots and shoot (De Carvalho, et., 2014).

Thus, this study is aimed to evaluate the effects of *Guizotia abyssinica* Cass. extracts on seed germination and initial root and shoot growth, as well as fresh and dried biomass of soybean seedling.

2. Material and Methods

2.1 Vegetable Material

The Niger (*Guizotia abyssinica* Cass.) production was conducted at the experimental farm of the University Center of Maring á - UNICESUMAR, Maring á Paran á, Brazil, altitude of 550 meters, Eutrophic Red Latosol soil and subtropical climate.

The collection was done after 3 months and before flowering, selecting plants without physical injuries. No treatment or control of pests and diseases was performed.

The soybean variety (*Glycine max* (L.) Merrill.) used was BRS 216, which was provided by the Brazilian Agricultural Research Corporation – EMBRAPA/Soybean, Londrina, Paran á Brazil.

2.2 Obtaining Aqueous Extracts of the Niger

Roots, stems and leaves of the Niger were separated, packed in paper bags and kept in a drying oven until constant mass, at 50 $^{\circ}$ C.

After the drying period, 3g of each vegetable part was weighed and and distilled water was added in the proportion of 3g of dry mass of roots, stems or leaves to 100 mL of the distilled water, crushed in a blender and kept at rest for 5 minutes.

Subsequently, the material was filtered and the obtained extract was considered as 100% aqueous extract for each plant part. This extract was diluted with distilled water to obtain the other concentrations (25, 50 and 75%). The control (0%) consisted only a distilled water.

2.3 Experimental Conduction



The soybean seeds were dipped in 2% sodium hypochlorite solution (NaClO) for 2 minutes and washed thoroughly with distilled water.

Twenty-five soybean seeds were distributed in gerbox (11 x 11 x 3,5 cm) containing two sheets of autoclaved germination paper and 14 mL of the aqueous extract were added at different concentrations (0, 25, 50, 75 and 100%). After sowing, the gerboxes were conditioned in a B.O.D. germination chamber at 25 $^{\circ}$ C with a photoperiod of 12 hours for seven days.

After the incubation period, germination and initial growth of soybean were evaluated.

2.4 Germination

Germinated seeds were checked on the seventh day and then the germination percentage was determined. The seeds with 2 mm of root protrusion were considered germinated. (Hartmann et al., 2001)

The percentage of germination (PG) was obtained by representing the percentage of seeds germinated in relation to the total number of seeds per gearbox under the determined experimental conditions (Ferreira and Borguetti, 2004), given by:

 $PG = (\Sigma ni . N^{-1}). 100$

where: $\sum ni = total$ number of germinated seeds; $N^{-1} = number$ of seeds arranged to germinate.

2.5 Initial Growth

To analyze the initial growth of soybean seedlings, the root and shoot length and their biomass, fresh and dry, were evaluated.

Shoot length was determined between apex and root-shoot junction, while the length of the main root corresponded to the distance between this region and the root end, both measured with a millimeter ruler. Only seedlings with development capacity were measured (Brasil, 2009).

Subsequently, root and shoot fresh biomass of soybean seedlings were obtained by weighing in analytical balance. The samples were then packed in paper bags and kept in a drying oven until constant mass, at 50 $^{\circ}$ C, to obtain the value of dry biomass (Borella and Pastorini, 2009).

2.6 Statistical Analysis

The experimental design was completely randomized with five replications of each treatment. Data were evaluated by analysis of variance and means between treatments compared by Tukey's test at 5% significance, using the statistical program Sisvar® (Ferreira, 2014).

3. Results and Discussion

After treatment with aqueous extract from the Niger stem, there were reductions in soybean germination and initial growth (Table 1). However, the germination percentage of soybean seeds was inhibited (9.5%) only at 50% concentration of this extract. According to Ferreira and Borghetti (2004), germination is less responsive to allelochemicals than to growth.



Several studies indicate that soybean seed germination is poorly sensitive to the effects of allelopathic compounds. Freitas et al. (2010) reported that *Pinus* extracts did not influence soybean seed germination as well as Nunes et al. (2014) did not observe changes in the germination of this crop after seeds were submitted to extracts of canola (*Brassica napus* L.), crambe (*Crambe abyssinica* Hochst), sunn hemp (*Crotalaria juncea* L.), linseed (*Linum usitatissimum* L.) and forage turnip (*Raphanus sativus* L.). The results of Silva et al. (2015) also corroborate those obtained in this research, where extracts of *Salvia officinalis* L. had no effects on soybean germination.

Table 1. Seed germination and initial growth of soybean seedlings treated with aqueous extract of niger stem at different concentrations (0, 25, 50, 75 and 100%). GP - Germination Percentage; RL - Root Length; SL - Shoot Length; FRB - Fresh Root Biomass; DRB - Dry Root Biomass; FSB - Fresh Shoot Biomass and DSB - Dry Shoot Biomass

Treatment (%)	GP (%)	RL (cm)	SL (cm)	FRB (mg)	DRB (mg)	FSB (mg)	DSB (mg)
0	21.00±0.45 a	4.91±0. 38 ab	3.24±0.34 a	2.17±0.41 a	0.23±0.02 a	1.77±0.32 a	0.19±0.02 a
25	21.60±0.68 a	5.80±0.40 a	2.11 ±0.17 b	1.26±0.13 b	0.18±0.01 ab	1.44±0.18 ab	0.22±0.01 a
50	19.00±0.55 b	4.39±0.27 b	1.96±0.34 b	0.93±0.11 b	0.15±0.01 b	0.91±0.07 b	0.19±0.01 a
75	20.00±0.35 ab	5.17±0.45 ab	1.99±0.14 b	1.22±0.21 b	0.17±0.01 b	1.11±0.08 b	0.23±0.01 a
100	21.20±0.22 a	4.20±0.06 b	1.53±0.11 b	0.87±0.058 b	0.15±0.01 b	0.95±0.04 b	0.21±0.01 a

Means followed by the same letter do not differ statistically from each other by the Tukey test at 5% significance. Data are represented as mean \pm standard error of mean (SEM)

Regarding the growth of soybean seedlings, the root and shoot length decreased, mainly in the highest concentration studied (100%), where there were decreases greater than 14 and 52%, respectively.

Biomass, another growth parameter, was also decreased after treatment with aqueous extract of the Niger stem, except the dry shoot biomass of soybean seedling, which was not altered. Fresh and dry root biomass presented larger reductions, at 100% concentration, where there were decreases of approximately 60 and 35%, respectively, when compared to the control experimente; while fresh shoot biomass was more inhibited (48.6%) in the treatment containing 50% of the niger stem aqueous extract (Table 1).

Allelochemicals in aqueous extracts promote reductions in seedling growth by decreasing cell division and elongation (Gomes et al., 2019; Javaid and Anjum, 2006). Despite few studies investigating the allelopathic effects of the Niger, recent studies indicate phytotoxic potential of this plant, probably due to the presence of flavonoids, an important class of polyphenols with strong biological activity.



The aqueous extract of the Niger leaves did not affect the seed germination percentage and the fresh and dry biomass of soybean root and shoot in none of the concentrations studied (Table 2).

Table 2. Seed germination and initial growth of soybean seedlings treated with aqueous extract of niger leaves in different concentrations (0, 25, 50, 75 and 100%). GP - Germination Percentage; RL - Root Length; SL - Shoot Length; FRB - Fresh Root Biomass; DRB - Dry Root Biomass; FSB - Fresh Shoot Biomass and DSB - Dry Shoot Biomass

Treatment	GP	RL	SL	FRB	DRB	FSB	DSB
(%)	(%)	(cm)	(cm)	(mg)	(mg)	(mg)	(mg)
0	22.60±1.03 a	3.96±0.26 b	4.00±0.22 b	1.24 ±0.24 a	0.25 ±0.02 a	2.50±0.41 a	0.30±0.03 a
25	21.20±0.80 a	4.93±0.42 ab	4.96±0.50 ab	1.00±0.19 a	0.20±0.02 a	2.60±0.24 a	0.28±0.02 a
50	21.20±1.20 a	5.66±0.48 a	5.56±0.39 a	1.11±0.09 a	0.21±0.01 a	2.65±0.26 a	0.29±0.02 a
75	22.60±0.76 a	5.77 ±0.42 a	5.48±0.26 a	1.22±0.09 a	0.25 ±0.02 a	2.70±0.19 a	0.30±0.02 a
100	20.00±0.79 a	5.33±0.10 a	4.00±0.28 b	1.25±0.21 a	0.26±0.02 a	1.71±0.26 a	0.24±0.01 a

Means followed by the same letter do not differ statistically from each other by the Tukey test at 5% significance. Data are represented as mean \pm standard error of mean (SEM)

However, the increases in root and shoot lengths of soybean seedlings treated with aqueous extract of the Niger leaves were identified (Table 2), reaching increases of 46% for root length to 75% of the extract and increments of 39% and 37% for shoot length in treatments with 50 and 75% extract, respectively.

Allelopathic interaction depends on plant's part and extract concentration, as well as allelochemical stability and sensitivity among target species. This can be noted when comparing the results of the present study with those obtained by other researches that also evaluated the allelopathic effects of the Niger leaves. So, Gomes et al. (2017) verified inhibition of germination and root length of *Ipomoea grandifolia* (Dammer) O'Donell (morning glory) seedlings treated with ethanolic extract from the Niger leaves. In another study with the ethanolic extract from the Niger leaves, Gomes et al. (2019) observed germination and root length inhibition of ryegrass, as well as reduction of bittergrass germination; however, they observed increased root length of bittergrass.

The aqueous extract of niger roots, like leaves extract, did not cause changes in seed germination percentage. In contrast, seedling growth decreased significantly (Table 3). Inhibitions of up to 64.3% in root length of soybean seedlings at 50% of extract and up to 53.4% in shoot length at 100% of aqueous extract of the Niger roots were noted.



Table 3. Seed germination and initial growth of soybean seedlings treated with aqueous extract of niger roots at different concentrations (0, 25, 50, 75 and 100%). GP - Germination Percentage; RL - Root Length; SL - Shoot Length; FRB - Fresh Root Biomass; DRB - Dry Root Biomass; FSB - Fresh Shoot Biomass and DSB - Dry Shoot Biomass

Treatment	GP	RL	SL	FRB	DRB	FSB	DSB
(%)	(%)	(cm)	(cm)	(mg)	(mg)	(mg)	(mg)
0	21.00±0.32 a	9.02±0.75 a	5.72 ±0.30 a	2.82±0.26 a	0.22±0.01 a	3.52 ±0.258 a	0.31±0.01 a
25	20.60±0.40 a	3.42±0.35 b	3.56±0.14 b	1.69±0.09 b	0.17±0.01 b	2.35 ±0.139 b	0.25±0.01 b
50	18.40±0.58 a	3.27±0.54 b	3.37 ±0.36 b	1.85±0.14 b	0.18±0.01 a	2.16±0.15 b	0.23±0.01 b
75	19.60±0.57 a	3.47±0.24 b	3.42±0.20 b	1.68±0.06 b	0.19±0.01 a	1.90±0.19 b	0.23±0.01 b
100	19.00±0.61 a	4.71±0.45 b	2.66±0.18 b	1.76±0.10 b	0.20±0.01 a	1.50±0.06 c	0.194±0.00 b

Means followed by the same letter do not differ statistically from each other by the Tukey test at 5% significance. Data are represented as mean \pm standard error of mean (SEM)

The fresh root biomass, as well as fresh and dry shoots biomass of soybean seedling reduced significantly at all concentrations tested. A more significant reduction (57.4%) was observed in fresh shoot biomass with 100% aqueous extract of the Niger roots. Regarding dry root biomass, a significant reduction (22.7%) was observed only in treatments with 25% aqueous extract (Table 3).

A large number of abnormal seedlings with atrophied and necrotic primary roots were also observed when submitted to the aqueous extract of the Niger roots at the highest concentration analyzed (100%). In general, the roots are more sensitive to the substances present in the extracts when compared to the other seedling organs (Chon et al., 2000). This is due to the fact that the roots are in direct and prolonged contact with the allelochemicals present in the extract.

Gomes et al. (2017) also verified the phytotoxic effect of the Niger on morning glory (*Ipomoea grandifolia* (Dammer) O'Donell) and the authors suggested that the observed effects are related to the presence and activity of flavonoids, being the Niger a potential donor of these phytotoxins. According to Paszkowski and Kremer (1988), flavones, flavonoids and isoflavonoids inhibit germination and root growth of various angiosperm species.

Flavonoids may act to control plant hormones, such as allelopathic agents and enzyme inhibitors (Formagio et al., 2010) and may induce the production of reactive oxygen species and lead to loss of cell membrane selectivity (Tur et al. 2012). In addition, the effects of these compounds on plants include decreased oxygen uptake in mitochondria and, in chloroplasts, reduced electron transport and photosystem II efficiency (Moreland and NOVITZKY, 1988).



Few studies involving the identification of allelochemicals in the Niger are found. More research is needed on compounds in the Niger and their's mechanism of action.

4. Final Considerations

Although the aqueous extract of the Niger leaves increased the length of soybean seedlings, the aqueous extracts of the Niger roots and stem showed negative interference on soybean germination and initial growth. The phytotoxicity was more evident when the extract was used from roots of the Niger. Such effects possibly occurred due to the presence of flavonoids, promoting soybean susceptibility. Thus, the results suggest that the Niger should not be used in succession / rotation systems with soybean crop, however, further field studies are needed to confirm the data obtained in this research.

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