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Research Paper

GERMINATION OF MAIZE, COWPEA AND MELON UNDER SEED EXTRACTS OF *ZIZIPHUS JOAZEIRO*

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Abstract

Species of Brazilian northeast can have all elopathic effects on germination and affect development of crops. The allelopathic potential of *Ziziphus joazeiro* seed extracts was studied on seedlings' growth and germination of maize, melon and cowpea. The experimental design was completely randomized with five treatments (extract concentration of 1.0, 0.5, 0.25, 0.125 and 0%) with four replications with twenty seeds. The aqueous seed extract of *Ziziphus joazeiro* did not affect the emergence percentage (85 to 96%), emergence rate index (3.7 to 4.1) and shoot length (8.2 to 8.6 cm) of maize seedlings, but reduced the percentage of normal seedlings, dry weight and root length of seedlings. There was negative effect of extract of *Ziziphus joazeiro* on shoot length and root length of cowpea. The allelopathic activity index showed that the highest concentrations of the extracts adversely affected the germination of maize, cowpea and melon. The melon was more sensitive to the action of the extracts.
Key words: *Allelopathy, bioassay, Zea mays, Cucumis melo, Vigna unguiculata.*

INTRODUCTION

Allelopathy has chemical and ecological nature when competing organisms produce chemicals that inhibit the growth of members of their own species or other species[1].Suitable manipulation of the allelopathy towards improvement of crop productivity and environmental protection through eco-friendly control of weeds, pests, crop diseases and synthesis of novel agrochemicals based on natural products have gained attention of the scientists engaged in allelopathy research [2].

The woody plants of the "Caatinga" (Biome of Brazilian northeast) may exhibit inhibitory potential on germination, seedling growth and development of crops. The aqueous extract of *Croton sonderianus* Mull. Arg. and Mimosa tenuiflora Willd branches affected the vigor of *Vigna unguiculata* L. Walp seedlings[3]. The allelopathic effect was verified on germination of lettuce in different extracts of *Erythrina velutina* Willd organs[4].Seed extracts of *Ziziphus joazeiro* Mart. had allelopathic activity on lettuce germination [5]and aqueous extract of leaves of *Schinuste rebinthifolius* Raddi. Influenced lettuce seed germination [6].

Ziziphus joazeiro Mart. is a typical species of northeastern Brazilian caatingaused in ornamentation, in folk medicine as soap and toothpaste, in addition to its importance in protecting the banks of watercourses [7]. The bark is effective in removing microbial plaque and pharmacological studies demonstrated the antifungal activity, antibacterial and antioxidant [8].
This study aimed to evaluate allelopathic activity of aqueous extracts from *Ziziphus joazeiro* seeds in the emergence, growth and early development of maize, cowpea and melon.

MATERIALS AND METHODS

The experiments were conducted in Universidade Federal Rural do Semi-Árido(UFERSA), in Mossoró. The material used in the bioassays was maize Bandeirante BL(*Zea mays* L.), melon Iracema (*Cucumis melo* L.) and cowpea Gurgueia BR 17 (*Vigna unguiculata* L.).
The maize emergence percentages were 97.5%, 100% in melon and bean 91.25%, respectively.
In the production of *Ziziphus joazeiro* seed extracts, the seeds harvested in May 2010 of adult trees in the field of UFERSA were used. The seeds were stored for 20 months in cold camera (14°C temperature and 45% RH). For the hydro alcoholic extract of the seeds, a solution at 70% concentration was prepared (70% ethanol and 30% distilled water). Seven hundred grams of seed – previously dried at 50ºC for 48h and then crushed – were used.
The material was placed in frosted glass containers with capacity of 1L and it was added an initial volume containing 800 ml of hydro alcoholic solution. The material was submitted to three extractions and exposed to constant agitation to potentiate the solvent action. In each extraction of material, it was done a filtering, and the liquid part was placed for evaporate, resulting in a doughy mass.
This mixture was placed in a beaker that was placed on a porcelain sheet attached to a container containing water that was superimposed on a magnetic stirrer at 60°C, in order to promote the gradual drying of the liquid to obtain a dry weight of extract (66g, final weight of seeds). Then the vessel containing the mass was stored under a temperature of 7ºC until ready to use them for the production of aqueous extracts.
In the preparation of the crude extract, twenty grams of the dry mass extract in balance of accuracy were weighed, and 2000 milliliters of water were added, resulting in a concentration of 1%, that is, for every gram of solute (dry mass extract) 100 ml of water were used.
The pH and electrical conductivity of the extracts were determined and subsequently calculated the values of osmotic potential[9].After that, the crude extracts were stored until the time of bioassays assembly.
It is known that different concentrations may produce different morphological and physiological responses of plants. Therefore, in this study, we sought to methods in the literature that present the dosages the closest to natural conditions. Hence we selected five concentrations (1, 0.5, 0.25, 0.125 and 0%) and the dilutions were obtained from the higher concentration.
The experimental design was completely randomized with four replications of 20 seeds. Each extract at five concentrations (1, 0.5, 0.25, 0.125 and 0%) constituted one experiment, which tested a possible allelopathy activity in the growth and species development of maize, melon and cowpea. Each plot was composed of plastic box (depth 17 cm, width 9.5 cm and height 4.3 cm), sterilized with alcohol, containing 400g of washed sand and sterilized [10].
Each experimental unit was moistened with 50 ml of extract and the plastic boxes were placed in germinator under temperature of 25°C and a photoperiod of 24 h for seven days.
The beginning of seedling emergence was considered with the issuance of the coleoptile (maize) and cotyledons (cowpea and melon). The counting of the emergence of seeds was daily. Seedling assessments occurred on the seventh day after sowing, classifying them as normal or abnormal according [10].The measurement of shoot and root of all normal seedlings was made with a ruler and it was determined the dry mass of normal seedlings[10].
The variables studied were emergence percentage (PE), emergence rate index (IVE), percentage of normal seedlings (PN), root length (distance in mm from the lap until the apex meristem) and shoot (distance in mm from the lap until the apex meristem), number of roots (maize). The emergence rate index was calculated from a formula adaptation $IVG = G_1/N_1 + G_2/N_2 + ... +$

G_n/N_n, where G_1, G_2, G_n = number of germinated seeds counted in the first, second and last counts, and N_1, N_2, N_n = number of days from sowing to first, second and last counts[11].
The allelopathic effect index (RI) was calculated according to the formula RI = 1 - C/T (T ≥ C) or RI = T/C - 1 (T < C) where: C = speed of germination of the control and T = speed of germination of the treatment[12].The analysis of regression variance was performed by the statistical program ASSISTAT [13].

RESULTS AND DISCUSSION

The aqueous seed extract of *Z. Joazeiro* and their dilutions showed normal values of pH and osmotic potential(Mpa), so they are not responsible for possible changes in germination behavior and early seedling growth of maize, melon and cowpea(Table 1).

Table1.Physico-chemical characteristics of *Ziziphus joazeiro* Mart. seeds' aqueous extracts used in bioassays to evaluate the allelopathic potential of *Zea mays* L., *Cucumis melo* L. and *Vigna unguiculata* (L) Walp. seeds.

Concentration(%)	pH	Osmoticpotential (Mpa)
1	5.10	-0.01131
0.5	4.75	-0.00584
0.25	4.93	-0.00292
0.125	5.81	0.00000
0	5.11	0.00000

***Ziziphus joazeiro* seed extract – the activity on maize**

The analysis of regression variance was not significant for the various models for the characteristics emergence percentage (85 to 96%), emergence rate index (3,7 to 4,1), shoot length (8,2 to 8,6cm) and number of roots(5,3 to 5,7) of maize seedlings, but it affected the percentage of normal seedlings, dry weight and seedlings' root length.
As the concentration of the extract increased there was a reduction in the percentage of normal seedlings (Fig. 1A), as well as of the seedling dry weight (Fig. 1B) and root length (Fig. 1C).
In other study with different concentrations of the *Ziziphus joazeiro seed*s' crude extract, there was phytotoxic effect at higher concentrations (75 and 100%) for the percentage of lettuce germination and percentage of normal seedlings[1].
Maize proved quite susceptible at the extracts of *Mimosa tenuifolia*, which had a negative influence on all variables [14]and it was found that increasing doses of extract of *Pinus* spp. caused decrease in radicle and hypocotyls of maize [15].The tolerance or resistance to compounds may be specific; some species are more sensitive than other, such as lettuce and tomato [16].
The allelopathy effect index (RI) indicates stimulation when shows positive values and negative values indicate inhibition when compared to the control. On the data (Fig. 1D),it is possible to observe that the highest concentrations of the extract of *Z. joazeiro* inhibited the germination of maize. Similar results were observed by [17]in extracts of *Schinus molle* L. on germination and early growth of *Raphanus sativus* L. and the aqueous extract *Tremamic rantha* leaves on seed germination and early seedling growth of radish[18].

***Z. joazeiro* seed extract-the activity on cowpea**

The variance analysis of the regression was significant for shoot length(linear model) and root length of cowpea(linear and quadratic model) and the other characteristics did not differ significantly.
The length of shoot and root length of cow pea decreased with higher concentrations of the extract(Fig.2A and2B).Similarly to what happened with maize seeds in this study; there was inhibition of germination of cowpea seeds in the highest concentrations of *Z. joazeiro* seed extract indicated in Figure 2C by allelopathy effect index.

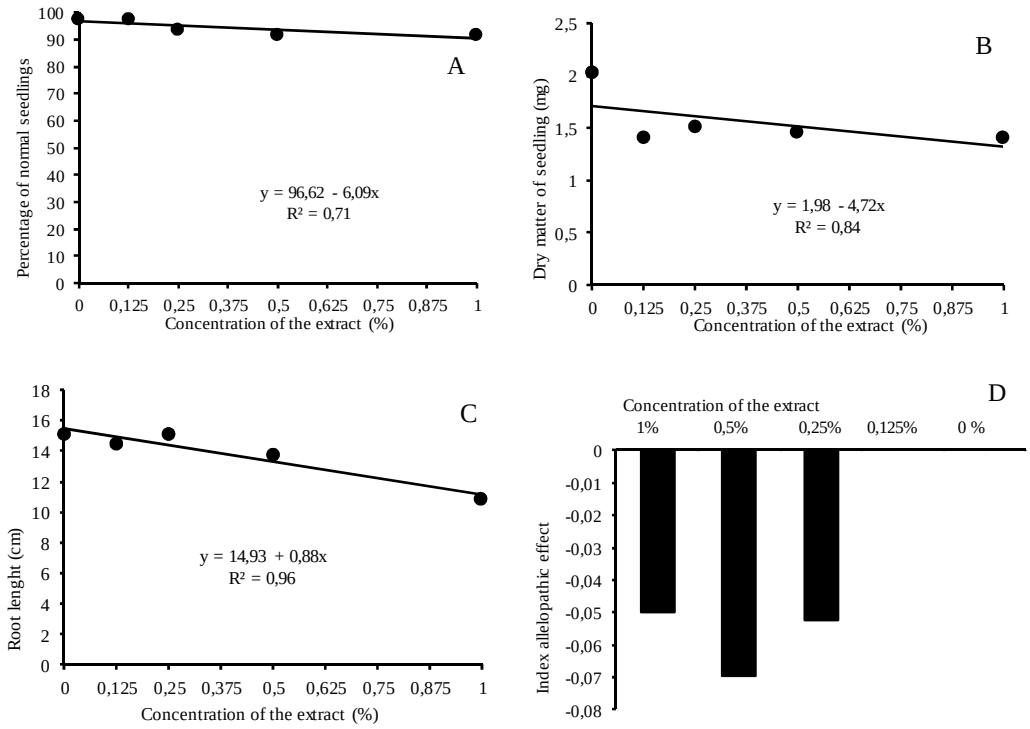

Figure 1.Percentageof normal seedlings (A), seedling dry weight(B), root length of maize (C) and allelopathic effect index(D) in the presence of different concentrations of *Ziziphus joazeiro* seed extract.

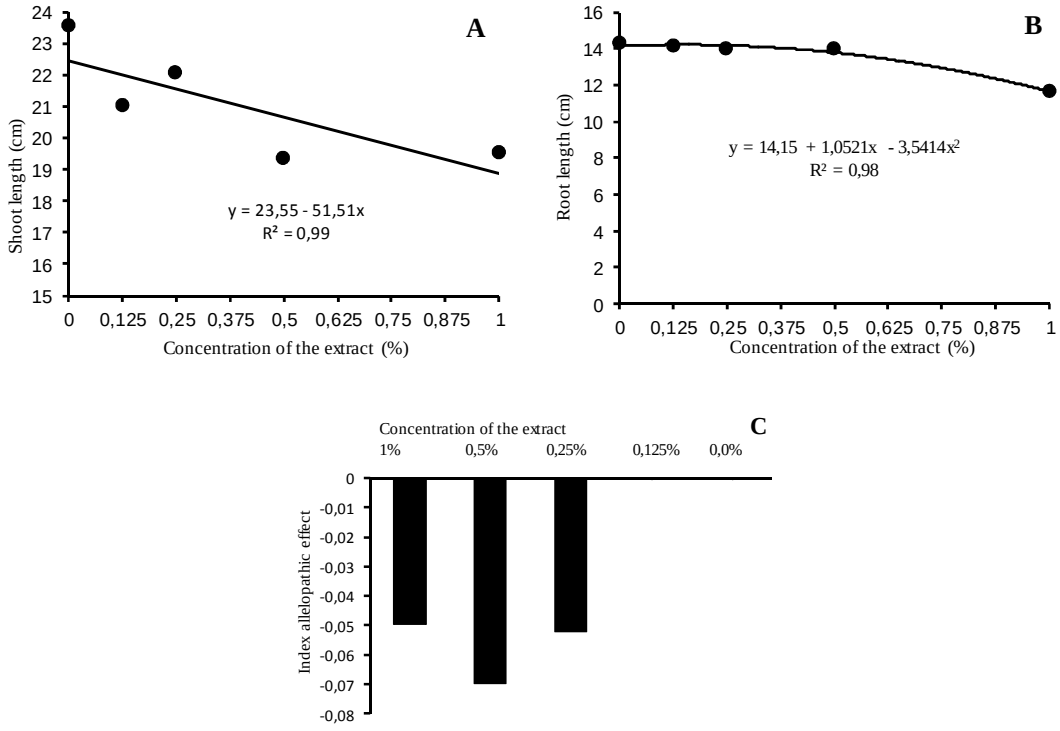

Figure 2. Shoot length (A), root length (B) and allelopathic effect index (C) of cowpea seedling in the presence of different concentrations of *Ziziphus joazeiro seed* extract.

Verifying the effect of plant extracts on the formation of roots and shoots of the target species is common in the studies of allelopathy. For example, there was allelopathy potential of *Crotalaria juncea* plant extracts on germination of maize and beans, the aerial part of maize suffered

interference from the extract concentration of 75%, but the lowest concentrations did not differ from the control[19].

***Z. joazeiro* seed extract-the activity on melon**

The variance analysis of the regression was significant for all traits studied with predominant explanation of the data by the linear model.

The melon crop was very sensitive to the extract and its dilutions. When the extract concentration increased, there was a reduction in the germination percentage (Fig. 3A), emergence rate index (Fig. 3B), percentage of normal seedlings (Fig. 3C) and seedling dry weight (Fig. 3D) of melon seedling. Abnormal seedlings showed primary roots atrophied absence of secondary roots and root necrosis, as well as structural differences in seedling size.

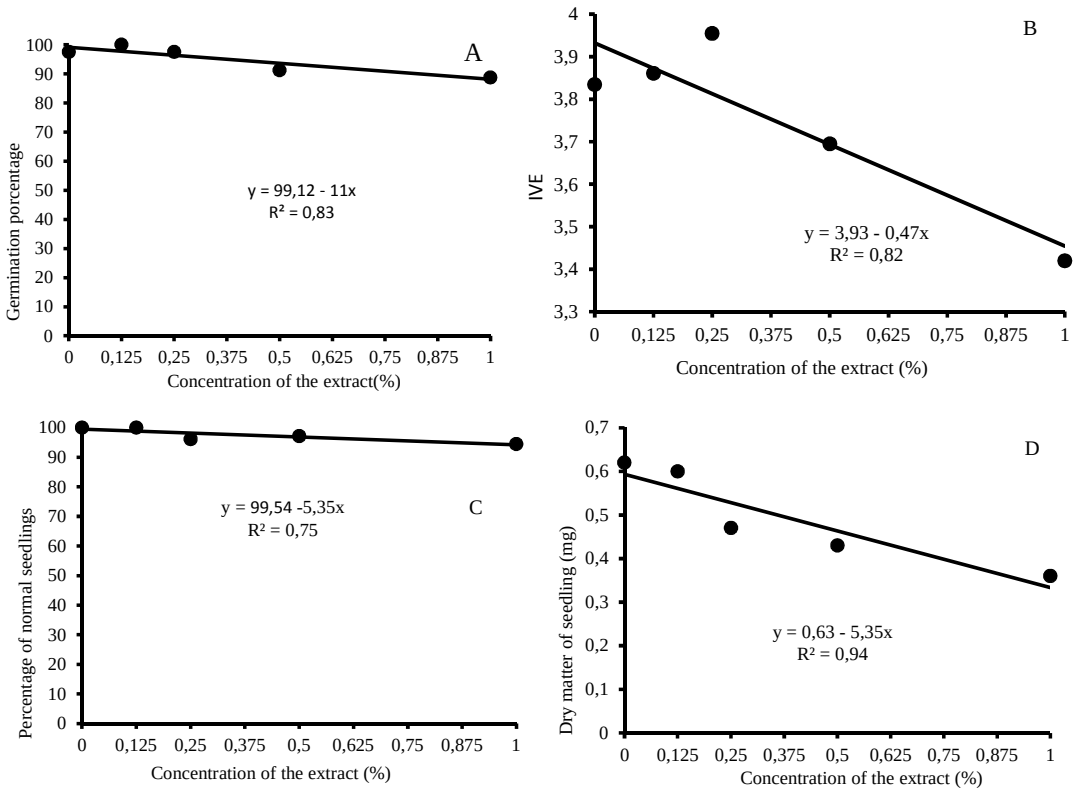

Figure 3. Germination percentage (A), germination speed index(B) normal seedlings percentage (C), seedling dry weight (D) of melon seedlings in the presence of different concentrations of *Ziziphus joazeiro* seed extract.

The shoot length (Fig. 4A), root length (Fig. 4B) and allelopathy effect index (Fig. 4C) were reduced when the seed extract concentration increased. The values of the allelopathy effect index (Fig. 4C) indicate inhibitory effect of higher extract concentrations (1% and 0.5%) on seed germination of melon, but the smaller concentrations acted as a stimulus to germination. RI values vary with the impact effect, whether positive or negative [20].

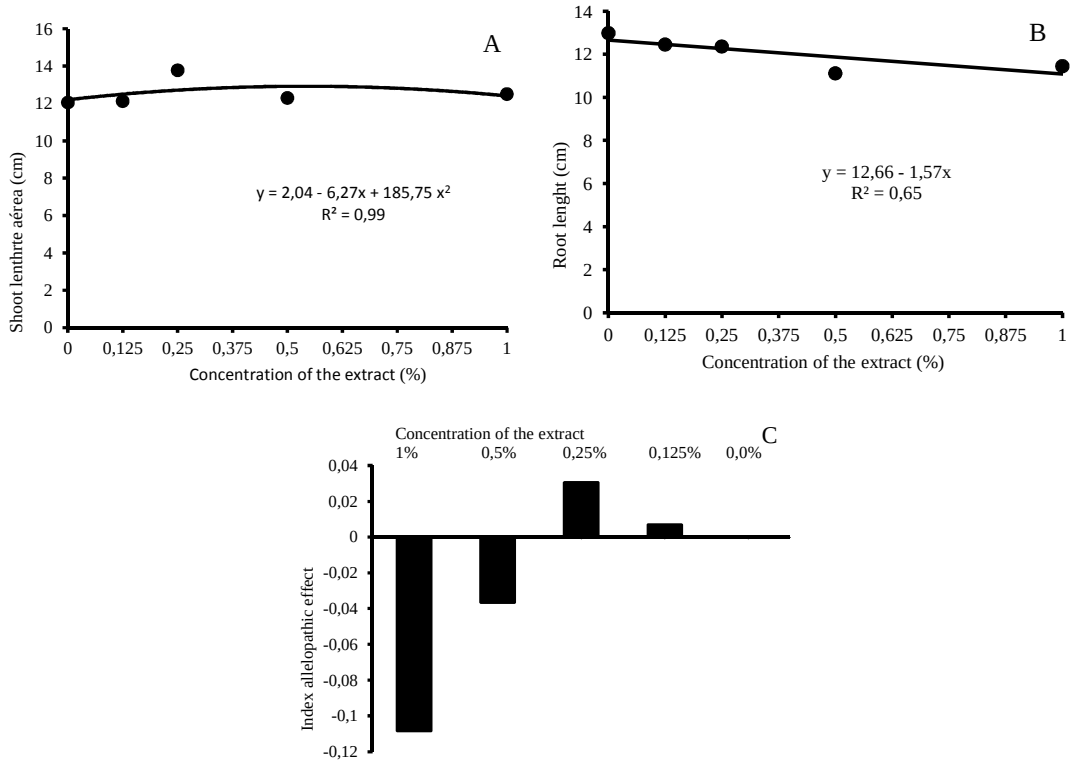

Figure 4. Germination percentage shoot length (A), root length (B) and allelopathic effect index (C) of melon seedlings in the presence of different concentrations of *Ziziphus joazeiro* seed extract.

The results of this study agreed with another study about roots' extracts of *Eucalyptus grandis* young plants when there were stimulatory effects on the radicle of radish and beans, at lower concentrations, and in the higher concentrations there were toxic effects with reduced radicle length of the tested plants[21].

In general, these effects are mostly seen in the hypocotyl and in the root. Thus, it was found that for hypocotyls of radish, the extracts stimulated the increase in length, being higher in all tested concentrations when compared to control[17].

A likely explanation for the germination behavior of species susceptible to the action of *Z. joazeiro* seed extract and its dilutions is that they have undergone change in the pattern of germination, when under allelopathy effect of some substance present in the extract. This effect may be on the permeability of membranes, the transcription and translation of DNA, the functioning of secondary messengers, respiration, for sequestration of oxygen, the conformation of enzymes and receptors or a combination of these factors [22].

CONCLUSIONS

The highest concentrations of *Ziziphus joazeiro* seed extract caused negative changes in root development patterns for all cultures tested, and negatively affect maize and melon seedling vigor. The melon was more sensitive to the action of the extracts that reduced germination percentage, emergence rate index, normal seedlings percentage, seedling dryweight, root length and shoot.

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