

*The influence of *Azadirachta indica* extract on the initial development of lettuce, cabbage and chive*

*Influência do extrato de *Azadirachta indica* no desenvolvimento inicial de alface, couve e cebolinha*

Dinamara Sousa Brito¹
Geslanny Oliveira Sousa¹,
Leticia Karen Oliveira Carvalho¹,
Alinne da Silva²,
Francisco de Assys Romero da Mota Sousa³,
Thyago Araújo Gurjão^{3,4}
Aline Carla de Medeiros⁴
Patricio Borges Maracaja^{4,5}

ABSTRACT: The allelopathic effects can be listed based on their frequency of occurrence, and the main effect is the capacity of suppress the germination of concurrent species. The main objective of this paper was to verify the allelopathic potential of the neem leaf aqueous extract on the germination of lettuce, cabbage and chive plants. The bioassay was conducted in a lab of the Science and Technology Federal Institute of Tocantins - campus Araguatins. The green leaves of neem were grinded in a blender with distilled water and then sieved, and extract solution was stored. The statistical design was completely randomized, with 5 treatments (0, 20, 50, 75 and 100%) and 4 repetitions. The data was submitted to variance analysis and mean comparison through the Scott Knott test with a 5% level of probability, using the software R. The neem leaves aqueous extract has shown higher allelopathic potentialities, affecting the germination speed rate in all the studied species, in the concentrations of 0 and 25%. It has also affected the roots development, mainly in the lettuce and chive plants. However, regarding the fresh matter and dry matter there was not any affect on the aqueous extract.

Keywords: allelopathy, *Allium schoenoprasum*, *Brassica oleracea*, germination, *Lactuca sativa*.

RESUMO: Os efeitos alelopáticos podem ser listados com base em sua frequência de ocorrência, e o efeito principal é a capacidade de suprimir a germinação de espécies concorrentes. O objetivo principal deste trabalho foi verificar o potencial alelopático do extrato aquoso da folha de nim na germinação de plantas de alface, couve e cebolinha. O bioensaio foi realizado em um laboratório do Instituto Federal de Ciência e Tecnologia do Tocantins - campus Araguatins. As folhas verdes de nim foram trituradas em liquidificador com água destilada e, em seguida, peneiradas, e a solução do extrato armazenada. O delineamento estatístico foi inteiramente casualizado, com 5 tratamentos (0, 20, 50, 75 e 100%) e 4 repetições. Os dados foram submetidos à análise de variância e comparação de médias através do teste de Scott-Knott com nível de 5% de probabilidade, utilizando o software R. O extrato aquoso de folhas de nim apresentou maiores potencialidades alelopáticas, afetando a velocidade de germinação das sementes em todas as espécies estudadas, nas concentrações de 0 e 25%. Também afetou o desenvolvimento das raízes, principalmente nas plantas de alface e cebolinha. No entanto, em relação à matéria fresca e seca não houve efeito no extrato aquoso.

Palavras-chave: alelopatia, *Allium schoenoprasum*, *Brassica oleracea*, germinação, *Lactuca sativa*.

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¹Instituto Federal do Tocantins, Campus Araguatins. E-mail: dinamarasbrito@gmail.com; geslannyoliveiral@gmail.com; kaarenleticia32@gmail.com.

²Universidade Estadual da Região Tocantina do Maranhão, UEMASUL. E-mail: alinnesilva@uemasul.edu.br

³Faculdade Rebouças de Campina Grande, FRCG - Campina Grande - Brasil. E-mail: assis.agronomia@gmail.com; thyagogurjaovp@gmail.com

⁴PPGSA – Programa de Pós Graduação em Gestão em Sistemas Agroindustrial CCTA-UFCG – Pombal – PB – Brasil – alinecarla.edu@gmail.com; patriciomaracaja@gmail.com

⁵ Instituto Nacional do Semiárido Campina Grande – PB – Brasil Patricio.maracaja@insa.gov.br

INTRODUCTION

The practice of the conventional agriculture with the current food production technologies have been contributing to aggravate the problems related to natural resources. The main responsible for that situation is the exacerbated use of pesticides and phosphate fertilizers (BEN HASSEN, e EL BILALI, 2022). These practices can affect the human health and the environment, contributing with innumerable natural imbalances. According to Anvisa (2012), among the pesticides the most used ones are the insecticides (12%), herbicides (45%), fungicides (14%) and others (29%) represented by other pesticide categories.

Given the chaotic circumstances of the indiscriminate use of pesticides comes up the necessity of using other resources such as biologic control to avoid that situation. More and more it is necessary to run a more sustainable and organic agriculture, this kind of production make use of alternative controls that cause less damage to the environment and to people, it can be listed as examples the plant extracts, plant oils and mixtures, which are viable products (SANTOS, 2018).

The plants have the capacity of producing chemicals that contribute to their survival and/or development of defense mechanisms (RICE, 1984). Such chemicals are bioactive metabolites (allelochemicals) originated from the secondary metabolism, which are released by various parts of the plant or through the decomposition of leaves and stems or yet from direct exudation in the soil by the roots (HERNÁNDEZ-TERRONES *et al.* 2007; ARAÚJO TRINDADE, *et al.* 2007).

In this context, one of the plant extracts very used is the neem's (*Azadirachta indica*), from the Meliaceae family, it is found abundantly at the dry area of India. Neem, when used for controlling insects (phytosanitary), can affect negatively on the some species production. The neem plant has a potential toxic effect in its fruits, leaves, seeds, and can be used in the control of many species of insects, viruses, fungus and bacteria in many crops (VIANA; PRATES; RIBEIRO, 2006; MESQUITA, 2010). According to Martinez (2002), the *Azadirachta indica* has an active ingredient, which is capable of cause many physiological alterations in weeds, such as interruption of the development, reduction of the fertility and

fecundity, being able to compromise the germination (NERI, 2006; LINHARES, 2011; CHOWDHURY, 2023).

Therefore, the objective of this paper was to verify the allelopathic potential of the aqueous extract of the leaves of neem in a initial phase of lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea*) and chive (*Allium schoenoprasum*) crops.

MATERIAL AND METHODS

The bioassay was conducted in the Laboratory of Biology of the Technology and Science Federal Institute (IFTO), campus Araguatins, Brazil. With a statistical design completely randomized and 4 repetitions, the treatments constituted of 5 concentrations (0, 25, 50, 75 and 100%) of the aqueous extract of neem, where 0% is only distilled water.

The neem leaves used to prepare the extract were collected from plants inside the campus. To prepare the aqueous extract the leaves were cleaned and washed with water, dried, and later they were grinded in a blender with distilled water. The stock solution was concentrated in 200 g in 10 mL⁻¹ (p/v). The stock solution was stored for 24 h in covered conical flasks and put in the refrigerator. The filtration and preparation of the other concentrations of the treatments were also performed.

Germitest paper was used for conducting the test, it was soaked with the solutions with the listed concentrations, rising in 2.5 times its dry weight. Seeds from commercial origin were used, in a number of 50 seeds of each species (lettuce, cabbage and chive) per experimental unity, selected according to its physical qualities. The seeds were distributed between 3 moisten sheets of Germitest paper, constituting one experimental unity and maintained in a B.O.D. incubator in 25 °C and with photoperiod of 12 hours of light. For such procedures, the methodology of RickII et al. (2011). In order to avoid loss of water through evaporation, the sample must be kept in a high humidity environment, in order to reduce the need for rewetting the substrate after sowing. This high relative humidity can be provided by placing a sufficient amount of water in the germinator's vat, or by keeping the substrates in closed containers, such as bags or plastic boxes. Daily evaluations were carried out, starting the day after sowing, being considered as germinated seed that which presented at least 2 mm of

radicle (HADAS, 1986). For defining the percentage of germination the cycle of each species was respected (lettuce 7 - days, cabbage - 10 days and chive - 14 days).

The germination rate was calculated according to the Labouriau & Valadares's equation (1976).

$$GR (\%) = \frac{N}{A} * 100$$

Where N = number of germinated seeds at the end of the test; A = number of seeds put on the beginning of the trial.

The germination speed rate (GSR) was calculated according to the formula proposed by Maguire (1962).

$$GSR = \frac{G1}{N1} + \frac{G2}{N2} + \dots \frac{Gn}{Nn}$$

G1, G2, Gn = number of seeds in the first, in the second and in the last count.

N1, N2, Nn = number of days since sowing of the first, second and last count.

The radicle length was obtained since the apex of the main root until the base of the plant. The fresh matter was measured by weighting the entire plants, and for the dry matter the plants were put in paper sacks and placed in a heating chamber in 65°C during 72 h and then weighted again.

The data was subjected to analysis of variance, for mean comparison the test Scott-Knott was performed with 5% of probability level, using the software R (package *ExpDes.pt*).

RESULTS AND DISCUSSION

The total fresh weight of lettuce seedlings had a significant effect while the cabbage and chive did not show significance (Table 1). The seedlings submitted to the control (0%) and to the smaller concentration (25%) of aqueous neem extract have presented the biggest total fresh weight. In the concentrations from 50% up, the neem extract showed to decrease the average fresh weight of 53% compared to the control (Figure 1). Santos (2018) evaluated the development of lettuce seedlings submitted to doses of neem extract and observed that a dose of 0.5 mL L⁻¹ promoted an increase of 14% in its fresh weight, however, up from this concentration it could be noticed a decrease in this rate.

Tabela 1 - The significance levels (P values) for total fresh weight (TFW), total dry weight (TDW), root length (RL), germination seed rate (GSR) and germination rate (GR) of lettuce, cabbage and chive seedling subjected to different concentrations of neem extract.

	Variables				
	TFW	TDW	RL	GSR	GR (%)
	(g seedling ⁻¹)	(g seedling ⁻¹)	(cm)		
Lettuce	*	*	ns	*	ns
Cabbage	ns	ns	ns	*	*
Chive	ns	*	*	*	ns

ns = not significant; * p<0.05; ** p<0.01; *** p<0.001.

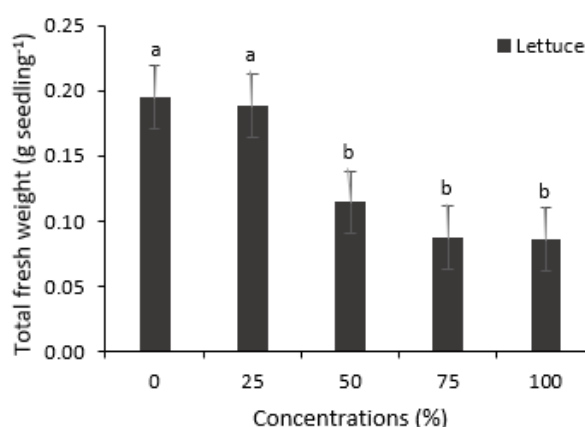


Figure 1 - Total fresh weight of lettuce seedling subjected to different concentrations of neem extract. Means followed by the same letter not differ by Scott Knott test (p<0.05). Each column represents the mean of 50 seedlings.

Among the crops studied, only the chive presented a significant effect (p<0.05) for total dry weight of the plantlets. The control (0%) was the treatment that obtained the biggest dry weight (0.020 g seedling⁻¹ ± 0.46), the percentages of 25 and 50%, and 75 and 100% did not differ between each other (Figure 2). In the study with juazeiro plant extract, Oliveira (2014) observed that as far as the extract concentration increased, the plants weight decreased. It can have been caused due to the allelopathic effect of the extract used, so as the neem aqueous extract can have caused an allelopathic effect in the chive seeds, interfering on its total dry weight.

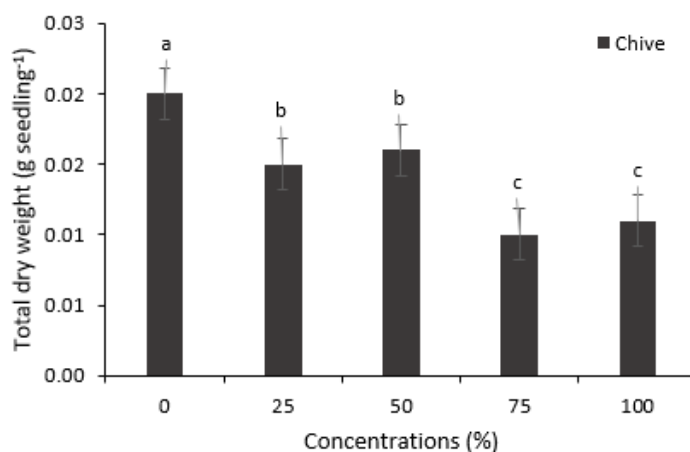


Figure 2 - Total dry weight of chive seedling subjected to different concentrations of neem extract. Means followed by the same letter not differ by Scott Knott test ($p < 0.05$). Each column represents the mean of 50 seedlings.

The lettuce and the chive presented significant effect for the root length (Table 1). It was observed that all the concentrations of neem extract used reduced significantly the seedling root length. Especially for the concentration of 75% of extract, that in both species has presented the smallest growth (Figure 3). Similar results were obtained by Rickli *et al.* (2011) who noticed in their study that 20% of aqueous extract of fresh neem leaves affected the length of roots in lettuce, corn, beans and 40% affected the root length of beggar ticks.

It can be observed that the treatments with lower concentrations of the neem aqueous extract have presented results superior to the treatments with higher quantity. According to Borges *et al.* (2011), elevated concentrations of extracts cause effect over cellular division in seeds, leading to a reduction of its mitotic rates and a consequent downtime of root growth.

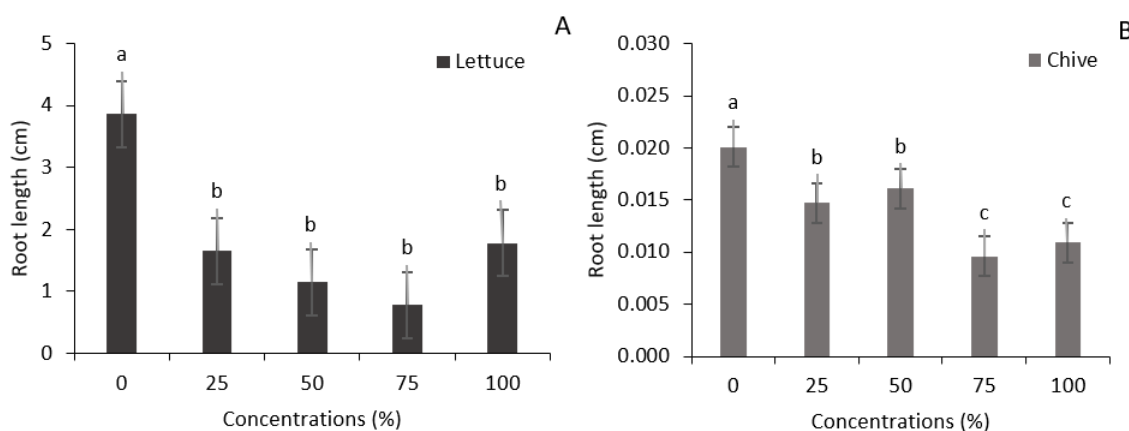


Figure 3 - Root length of lettuce (A) and chive (B) seedlings subjected to different concentrations of neem extract. Means followed by the same letter do not differ by Scott Knott test ($p < 0.05$). Each column represents the mean of 50 seedlings.

The germination speed rate (GSR) to the concentrations of 0 and 25% were superior, presenting statistical difference ($p < 0.05$), compared to the treatments of 50%, 75% and 100% for all the studied species (Figure 4). The concentration of 75% of fresh leaves aqueous extract decreased widely the GSR of seeds in lettuce. Generally, above the concentration of 50% the reduction in this rate could be noticed in any crop. Considering the allelopathic effect of neem, referring to the GSR, it is important to highlight the importance of this stage, because it is on this moment that the conditions of growth and development of the seedling can be spotted, that is why any barrier for germination must be observed with attention and care.

Gusman, Vieira e Vestena (2012), have analyzed the effect of various aqueous extracts in the development of greenery species, including cabbage, and they observed that decrease of GSR in all the tested species with all the aqueous extracts in concentrations up from 10%.

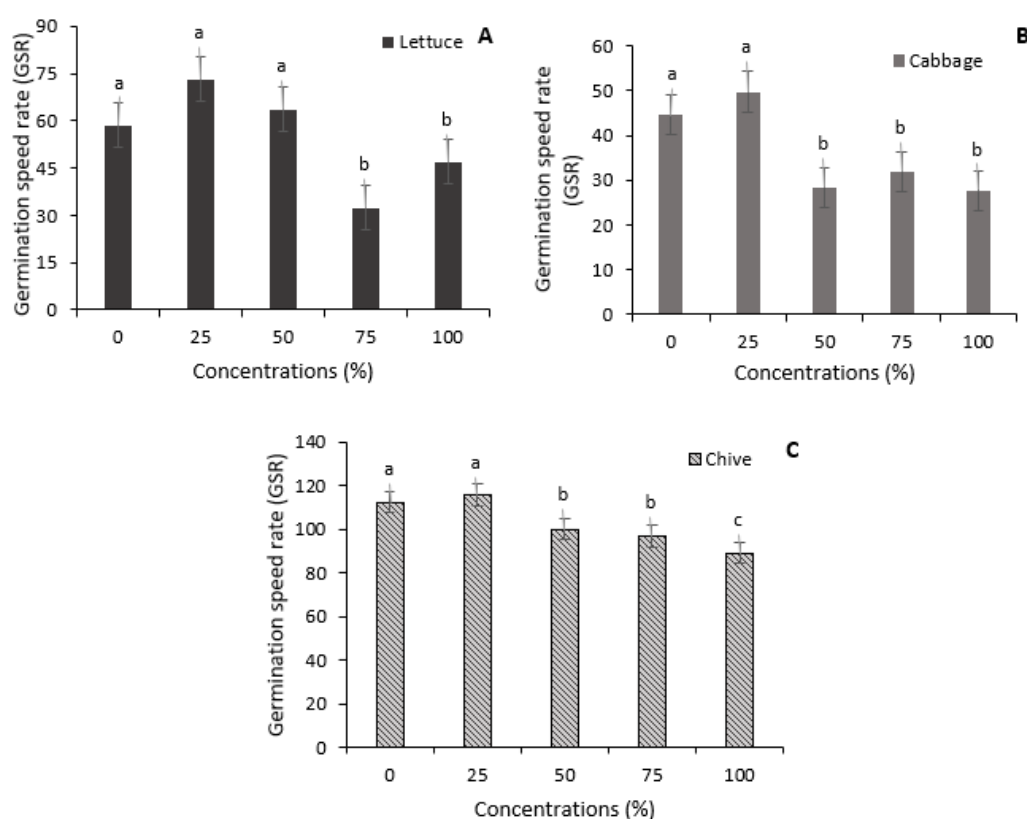


Figure 4 - Germination speed rate of lettuce (A), cabbage (B) and chive (C) seedling subjected to different concentrations of neem extract. Means followed by the same letter do not differ by Scott Knott test ($p < 0.05$). Each column represents the mean of 50 seedlings.

The percentage of germination had significant effect ($p < 0.05$) only for cabbage. In concentrations up from 50% of neem extract, there was a decrease in the germination rate, on

average 30% lower when compared with the control (Figure 5). So, it can be said that the neem aqueous extract presents phytotoxic action to the development of cabbage seedlings.

For chive the germination had values between 99.5 and 100%, all the treatments were statistically alike. This results indicate that the neem fresh leaves aqueous extract did not interfered on the germination of chive seeds, no matter the percentage of concentration used. Similar results were obtained for Corlett *et al.* (2015) in a study with different doses of neem essential oil in seeds of beans, not obtaining allelopathic effect in the germination of the seeds. In the other hand, Medeiros *et al.* (2007), evaluated the effect of fresh and dry neem leaves powder over the development of cowpea seeds, and observed that both have powders promoted a reduction on the percentage of seeds germination, presenting a significant difference in relation to the control treatment (with no neem powder).

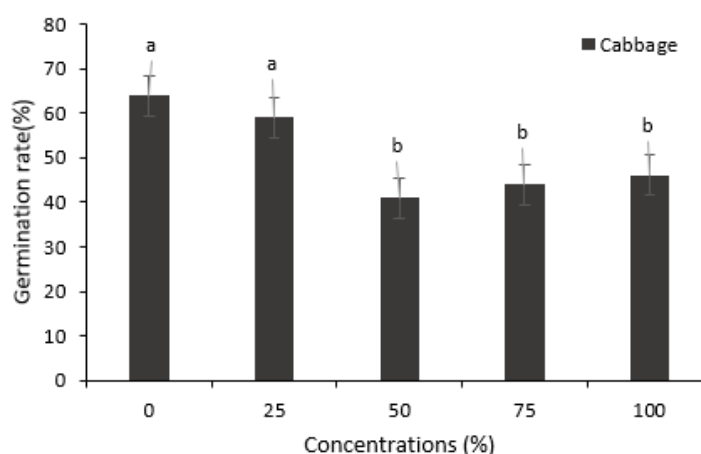


Figure 5 - Cabbage seedling germination rate subjected to different concentrations of neem extract. Means followed by the same letter do not differ by Scott Knott test ($p < 0.05$). Each column represents the mean of 50 seedlings.

CONCLUSION

The aqueous extract of neem leaves has affect the germination power and the initial development of the seed of the cultivated species evaluated. For the lettuce, there was a significant effect of the treatments for the germination speed rate (GSR), fresh matter (FM) and root length (RL) and there was not significance for germination (G) and dry matter (DM). Differently of what was observed for chive, where there was significant effect of the treatments for the percentage of germination (G) and the germination speed rate (GSR), and there was not

significance for fresh matter (FM), dry matter (DM) and root length (RL). For cabbage, there was significant effect for the treatments for the percentage of germination (G) and for the germination speed rate (GSR), and there was not significance for fresh matter (FM), dry matter (DM) and root length (RL). For the other tested species there was a decrease on the GSR in all the treatments.

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