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# Allelopathic effects of aqueous extract of *Brassica napus* on germination of seeds of *Phaseolus vulgaris*

## ABSTRACT

Secondary compounds produced by plants are considered an alternative method of weed suppression but can cause negative effects on crops in succession, especially in a no-tillage system, due to the degradation of crop residues with allelopathic potential. The objective of this work was to analyze the influence of foliar aqueous extracts of *Brassica napus* on the germination and initial development of seedlings of *Phaseolus vulgaris*. The extract was prepared as a stock 10 % weight/volume solution, and diluted into treatments of relative concentrations of 100 % (i.e. 10 % w/v stock), 75 %, 50 %, 25 % and 0 % (untreated control consisting of distilled water), in a completely randomized design. The seeds of *Phaseolus vulgaris* were moistened with the differing concentrated extracts and kept in a germination chamber at 25 °C, with a photoperiod of 12 h for nine days. The variables evaluated were: percentage germinating, first count of germination and germination velocity index, as well the root and hypocotyl length, and fresh and dry mass of the seedlings. The aqueous leaf extracts of *Brassica napus* did not influence the germination of *Phaseolus vulgaris* seeds, but did induce the growth of abnormal seedlings by inhibition of secondary roots and reduced prominence of the primary root.

**Key words:** allelochemicals, development of seedlings, crop succession

## Efeitos alelopáticos dos extratos aquosos de *Brassica napus* na germinação de sementes de *Phaseolus vulgaris*

## RESUMO

Os compostos secundários produzidos pelas plantas são considerados uma alternativa para a supressão de plantas daninhas, porém podem ocasionar efeitos negativos sobre culturas em sucessão, principalmente no plantio direto, a partir da degradação dos restos culturais com potenciais alelopáticos. Objetivou-se, com este trabalho, analisar a influência dos extratos aquosos foliares da *Brassica napus* sobre a germinação e o desenvolvimento inicial de plântulas de *Phaseolus vulgaris*. O extrato foi obtido a partir da relação 10% massa/volume, constituindo os tratamentos conforme concentrações de 100; 75; 50; 25 e 0% (testemunha - apenas água destilada), em delineamento inteiramente casualizado. As sementes de *Phaseolus vulgaris* foram umedecidas com as soluções dos extratos e mantidas em câmara de germinação, na temperatura de 25 °C, com fotoperíodo de 12 h, durante nove dias. As variáveis avaliadas foram: porcentagem, primeira contagem e índice de velocidade de germinação, tal como, também, comprimento de raiz e hipocótilo, massa fresca e seca de plântulas. Os extratos aquosos foliares de *Brassica napus* não influenciam a germinação das sementes de *Phaseolus vulgaris* mas induzem a produção de plântulas anormais pela inibição das raízes secundárias, além da pequena proeminência das primárias.

**Palavras-chave:** aleloquímicos, desenvolvimento de plântulas, raiz

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## INTRODUCTION

Species which belong to Brassicaceae family, for example canola (*Brassica napus*), are widely used in crop rotation designs, but those plants produce secondary metabolites called glucosinolates which are stored in the vacuoles and are released as straw decomposition during crop senescence. Studies indicated that even at low concentrations those compounds can delay seed germination, or even prevent it completely at high concentrations, as for example seen on *Glycine max* (Rizzardi et al., 2008b) and *Bidens pilosa* (Petersen et al., 2001).

Since agriculture is undergoing an important transformation in productive processes, it is crucial to reduce the use of pesticides because of both economic and environmental aspects. Thus, some alternatives, which aim at reducing or replacing the use of chemical management, have arisen, such as, for example, use of plants with allelopathic potential in crop rotation design in the cultivation area (Carvalho, 2004). In addition, according to this study, the use of those plants may help in controlling invading plants by the action of allelopathic compounds whilst minimizing the use of herbicides; however, there are few studies highlighting allelopathic effects among successively cultivated major economic crops.

The use of herbicides can be reduced when plant with allelopathic potential are cultivated because they can directly affect proteins needed in the germination of the seeds, depending on the specificity of the molecules (Hadacek, 2002). Nevertheless, allelopathic effects caused by residue decomposition and subsequent release of the compounds in the soil may affect negatively the development of weeds, but also the development of successive commercial crops. Studies carried out by Santos et al. (1991) showed evidence that yield, final populations of plants and insertion height of the first soybean pod were harmed when *Brassica napus* was used as predecessor crop. Similarly, Haddadchi & Gerivani (2009) found that *Brassica napus* caused a reduction in the germination, length and dry matter of *Glycine max* seedlings.

Species belonging to the Brassicaceae family produce secondary metabolites with allelopathic potentials called glucosinolates, present at high contents in *Brassica napus*, mainly in the leaves and roots (Uremis et al., 2009; Yasumoto et al., 2010). These compounds are degraded by the myrosinase enzyme, and are metabolized into a great variety of molecules with allelochemical potential, particularly isothiocyanate and nitrile (Halkier & Gershenzon, 2006).

The influence of the allelopathic effect does not occur only in developing plants but also on the subsequent crops (Reigosa et al., 1999). Choesin & Boerner (1991), when reporting specifically on the allelopathic potential of *Brassica napus* on successively cultivated plants, reported that the main negative effect on the subsequent crop was the reduction in the availability of soil nutrients, specially sulfur, due to degradation of glucosinolates present in the crop remains of *Brassica napus*.

Thus, the objective of this work was to evaluate the effects and allelopathic potential of an aqueous leaf extract of *Brassica napus* on the emergence and initial development of *Phaseolus vulgaris* seedlings.

## MATERIAL AND METHODS

This study was carried out in the Research and Chemical Analysis Laboratory (Laboratório de Pesquisa e Análise Química - LAPAQ) at Santa Maria Federal University (Universidade Federal de Santa Maria), Campus Frederico Westphalen - RS, in August 2010. Extracts of *Brassica napus* were obtained by collecting leaves of cultivar Hyola 43 plants at full flowering, and followed by cleaning, asepsis and drying (performed in an oven at 60 °C for 24 hours). Afterwards, the samples were ground in a Willy® knife-type mill, their mass was estimated and, using distilled water as solvent, a 10 % mass/volume stock solution (100 g of dry plant in 1 L of distilled water) were prepared. Homogenization was carried out in a Turax® agitator, the supernatant was collected and diluted with distilled water, resulting in the following concentrations: 100 %, 75 %, 50 %, 25 % and 0 % (control consisting of only distilled water).

Asepsis of seeds of *Phaseolus vulgaris* was performed by use sodium hypochlorite and water before sowing, following which seeds were distributed onto three germitest paper sheets moistened with distilled water (zero) and solutions of *Brassica napus* extract equivalent to 2.5 times the mass of dried paper. The paper sheets were placed in a gerbox and transferred to a germination chamber with a controlled temperature of 25 °C and 12 h photoperiods for nine days (Brasil, 2009).

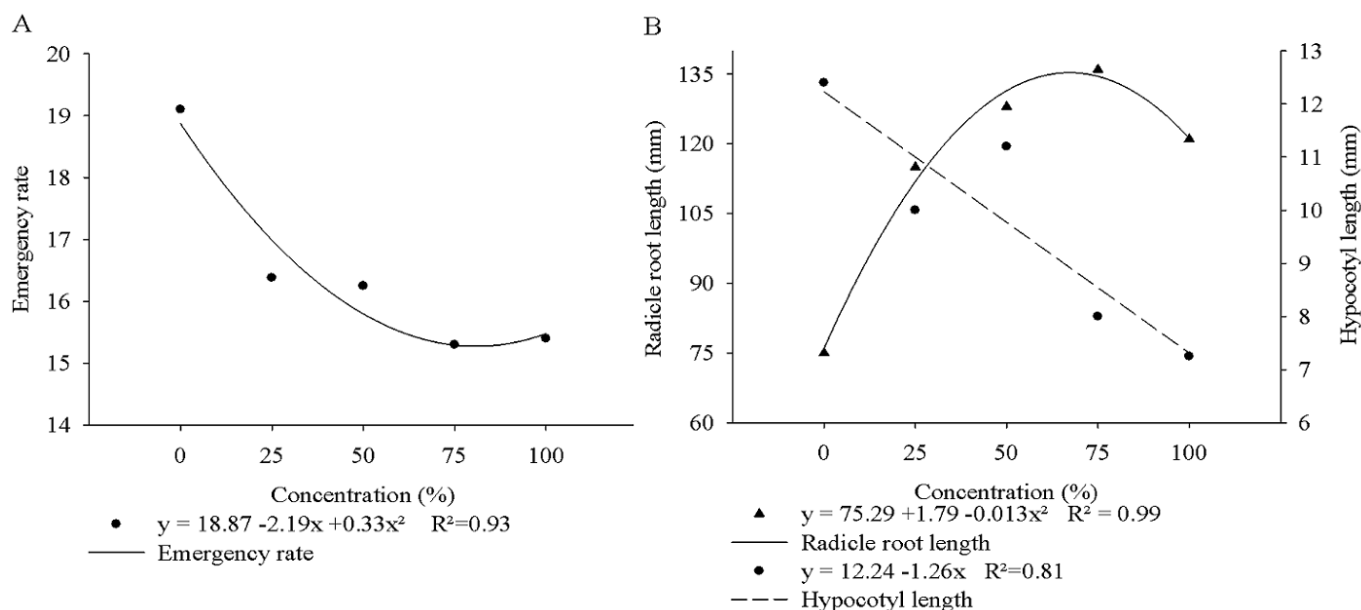
Counting of sprouted seeds was performed daily, therefore determining the germination speed (GS) of seeds according to Maguire (1962), by adopting root protrusion with at least 2 mm of length as germination criterion. The first count and final germination percentage were performed on the fifth and ninth day after sowing, respectively (Brasil, 2009). After the final period of evaluation, root and hypocotyl length of 50 % of the seedlings of each experimental unit were measured using a digital pachymeter. Following which, the average fresh mass of seedlings was measured and the plants were then placed in an oven at 65 °C for 24 h for evaluation of average dry mass (Brasil, 2009).

We used a complete random experimental design with five concentrations of aqueous extract in four replicates; each experimental unit consisted of 100 seeds, distributed in four gerbox and the results were submitted to analyses of variance and polynomial analysis.

## RESULTS AND DISCUSSION

There was a significant difference among treatments for germination speed, root and hypocotyl length. However, the germinating percentage of seeds of *Phaseolus vulgaris* was not affected by the increasing concentrations of *Brassica napus* in the first nor final counting. Santana et al. (2006), mentioned that although the final germination percentage may not be significantly affected by the action of allelochemicals, germination patterns may be modified, as they found differences in the speed and synchrony of germination of seeds exposed to such compounds.

We observed that the germination speed was significantly affected by the different concentrations of extracts, with the



**Figure 1.** Mean values of emergency rate (A) and radicle root length and hypocotyl length of *Phaseolus vulgaris* seedlings (B) as a function of leaf extract concentration of *Brassica napus*. Frederico Westphalen, RS, Brasil, 2010

**Figura 1.** Valores médios de índice de velocidade de germinação das sementes (A) e comprimento de raiz e hipocótilo de plântulas do *Phaseolus vulgaris* (B) em função de concentrações dos extratos foliares de *Brassica napus*. Frederico Westphalen, RS, Brasil, 2010

highest value being for control seeds (19.19), whereas, regardless of the concentration, seeds exposed to the other treatments resulted in negative effect on this variable (Figure 1A).

Those results are in agreement with the ones of Nunes et al. (2003), and Rizzardi et al. (2008b), who found a reduction in the emergence speed of *Glycine max* seedlings as the amount of *Sorghum bicolor* and *Brassica napus* straw increased. Germination is less sensitive to allelochemicals effects when compared to the consequences of those compounds on the growth and length of seedlings as well as germination speed (Ferreira & Aquila, 2000).

Thus, germination speed is a marker of vigor and seeds which germinate slowly are more prone to attack by predators and disease (Vidal & Trezzi, 2004). The differences show that that germination speed is a more sensitive marker to allelochemicals than germination percentage, as well as fresh and dry mass.

Regarding the final evaluations of the biometrics of *Phaseolus vulgaris*, the regression analyses showed significance for root length (Figure 1B), where increasing doses of extract show an increase in length of up to 80 % in relation to the roots of seedlings from control seeds. This result can be explained by the action of the leaf extract of *Brassica napus* in inhibiting growth of secondary roots of *Phaseolus vulgaris* seedlings.

The length of the hypocotyl responded negatively in a linear fashion as concentrations increased, especially at the concentrations of 75 % and 100 %. However, no difference was found in the fresh and dry mass of seedlings caused by the extracts, which is probably because the treatments that showed shorter root length resulted in longer hypocotyl length, and hence equivalent fresh and dry masses.

Therefore, the evaluated results do not completely converge with Leszczynski et al. (2009), where they state that *Brassica*

*napus* did not influenced crops and only compromised germination of seeds of weed species.

In a study relating to the allelopathic effects of *Brassica napus* on weeds, Rizzardi et al. (2008a) found that extracts interfered negatively on the length of the root of *Bidens pilosa*. Espinosa et al. (2008), when evaluating phenolic compounds of *E. gradis* and *E. urophylla*, found that allelopathic effects inhibited seed germination and the initial growth of *Phaseolus vulgaris* seedlings.

Although germination speed showed differentiation between the use of extracts at the first counting (carried out on the fifth day), there was no influence of the treatments on the germination percentage, because at this time the seeds were germinating despite there being a distinction in root protrusion. Therefore, it can be seen that seeds exposed to the extract of 100 % concentration showed a remarkable inhibition of secondary root formation, in addition to a reduction in prominence of the primary roots.

At the final counting, which was carried out on the ninth day, the maximal dose of extract showed the longest length of roots, although secondary roots were absent, whereas control seeds showed normal length roots with the presence of secondary roots.

Similarly, Cruz-Ortega et al. (1998) treated seeds of *Phaseolus vulgaris* with allelochemicals produced by *Sicyos deppei* and found abnormalities in the roots, which may have been caused by alterations in the genetic material, due to allelochemicals affecting with the synthesis of proteins and lipids, and in mitochondrial respiration.

These results partially corroborate with those obtained by Tokuta & Nóbrega (2002), who also found longer lengths of roots in soybean grown in the presence of extracts from *Brassica napus*, demonstrating that these compounds can also cause abnormalities in this legume. Jafariehyazdi & Javidfar

(2011), found that, besides germination percentage, length of the root and hypocotyl of sunflower seedlings from seeds exposed to extracts of *Brassica napus* were also affected.

## CONCLUSION

Aqueous extracts of *Brassica napus* did not influence germination of *Phaseolus vulgaris* seeds but did induce the production of abnormal seedlings.

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