AILANTHUS ALTISSIMA SPECIES INVASION ON BIODIVERSITY CAUSED BY POTENTIAL ALLELOPATHY

C. BOSTAN, F. BORLEA, Corina MIHOC, Mihaela SELESAN

Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Calea Aradului Street, no. 119, Timişoara, Romania
Corresponding author:bostancristian2000@yahoo.com

Abstract: *This paper aims to highlight the direct and indirect allelopathic influences showed by species Ailanthus altissima, through morphological and biological investigations. An important factor in the success of Ailanthus species invasion is the release of allelopathic compounds that influence negatively the vegetation in habitats where it installs. Recent studies show that interactions between invasive species and native species based on allelopathy may be one of the mechanisms underlying the remarkable success of some of the most aggressive invasive plants. Ailanthus contains toxic allelopathic compounds to many woody and herbaceous plants, toxic compound called ailantona, identified and classified as the most effective phytotoxic component. Several studies have shown that extracts of Ailanthus inhibit germination and growth of several species of angiosperm and gymnosperm plants, in biotests and greenhouse experiments. Our studies included a laboratory experience in which we studied the germination of seeds treated with watery extracts obtained from Ailanthus species and behaviour of species under the influence of the same extracts treated containing allelopathic compounds. Based on research conducted in the laboratory, by studying the degree of externalization of the main morphological features, it was revealed that seed germination of Sinapis alba and Brassica napus treated extracts was 0%, compared with the control Sinapis alba 83% and Brassica napus 96,66%. Treated seeds were inhibited regardless of the concentration of watery extracts they have been treated with (Variant 1 - Standard substances; Variant 2 - obtained by dilution 1:1; Variant 3 - obtained by dilution 1:2.). The average length of hypocotyl and Brassica napus roots for the control was 3.36 cm and respectively 3.25 cm, and the hypocotyl of Sinapis alba root was 2.95 cm and 3.2 cm. Experiments carried out demonstrate that the allelopathtic potential of the species is very powerful and can influence negatively the neighbouring species. Given the current climate, the species Ailanthus altissima is favoured over native species. This fact combined with the allelopathtic effects demonstrated also by experiments carried out in the current paper may lead to future expansion of this species with major impacts on biodiversity.*

Key words: *Ailanthus altissima, allelopathy, germination, leguminous species.*

INTRODUCTION

The purpose of the present work is to analyse the allelopathic effect of aqueous extracts prepared from the plant *Ailanthus altissima* on the germination of seeds and growth of seedlings of *Sinapis alba* and *Brassica napus*.

Biological invasions are recognized as one of the most important causes of ecosystem degradation and biodiversity loss worldwide (VITOUSEKET et al., 1996; MACK et al., 2000).

Allelopathy theory associated with the success of some invasive plants is currently supported by a number of publications.

Since *Ailanthus altissima* is very widespread in Romania, there needs to be an efficient management for the control of the invasive process of species which is very prolific, it changes the chemical composition of the soil by releasing substances and it affects human health via pollen and induced contact dermatitis.

An important factor in the success of *Ailanthus species* invasion is the release of compounds that influence the surrounding vegetation. These potentially allelopathic effects

were the subject of most experimental studies regarding the *Ailanthus*. Besides his prolific reproduction, the biochemical substances present in all tissues of the species demonstrates that allelopathy is another mechanism that confers invasiveness.

HEISEY (1996) showed that the toxic compound called ailantona was identified and classified as the most effective phytotoxic component, that by a concentration of 0.7ml / l causes a 50% inhibition in the growth of root system (JESSICA S. THOMPSON, 2008).

In greenhouse tests, ailantona exerted an inhibitory effect on the activity of several grass species. Ailantona is released into the soil, but its effects are short-lived, probably due to microbial activity.

In field experiments, LAWRENCE (1991) has shown that allelopathic substances affect neighboring plants. Information on autotoxicity are contradictory, some studies have detected low growth of *Ailanthus* seedlings watered with leaf extract of the species, while HEISEY (1996) did not detect any damage caused by the application of ailantone seedlings.

Several studies have shown that extracts of *Ailanthus* (roots, leaves, trunk) inhibits germination and growth of several plant species in biotests and greenhouse experiments (MERGEN 1959; VOIGT & MERGEN, 1962; Heisey, 1990 A, B; DE FEO et al., 2003, quoted by LORENA GÓMEZ-APARICIO and CHARLES D. CANHAM, 2008).

Previous phytochemical studies have shown the presence of quassinoids alkaloids, lipids and fatty acids in *Ailanthus* (OGURA et al., 1977).

A number of secondary metabolites have been identified in *Ailanthus*, including the β-carbolines, canthin-6-one alkaloids, coumarins, and quassinoids. The most phytotoxic of these is the ailanthona water-soluble quassinoide (HEISEY, 1996). The highest levels of toxicity are present in the roots of *Ailanthus*; an average level of toxicity is found in leaflets, cob, young plant shoots and low levels of toxicity are present in wood, trunk bark and small inflorescences. Seasonal changes are also observed on the leaves with decreased toxicities with age and which correspond to a decrease in the content of nutrients (HEISEY, 1990; LAWRENCE et al., 1991). This finding provides evidence that those phytotoxic compounds can act as an obstacle to feeding. *Ailanthus* root has been shown to reduce both the emergence of seedlings and development of seedlings of several species (HEISEY 1990; LAWRENCE et al., 1991).

MATERIAL AND METHODS

Our studies included a laboratory experience in which we studied the germination of seeds treated with extracts obtained from *Ailanthus* and the behaviour of species treated under the influence of the same extracts. The experiments were installed in the laboratory of the subject "Plant Physiology" of the USAMVB Timisoara, Romania.

The plant material used in biotests consisted of *Ailanthus altissima* leaves harvested in May and *Ailanthus* bark, as well as seeds of *Sinapis alba* and *Brassica napus.*

The allelopathic effect of aqueous extracts prepared was tested on species of dicotyledonous plants *(Sinapis alba* and *Brassica napus)* considered receivers of phytotoxic substances present in *Ailanthus altissima.*

Preparation of material

The watery extracts were prepared as follows: 250 g of plant material was broken into pieces and mixed with 500 ml of distilled water for 30 minutes; The mixture thus obtained was allowed to rest for 48 hours after which it was filtered. The extracts were kept in the dark at 4° C. The solutions obtained were regarded as the reference. The extracts were prepared in

different concentrations: Variant 1 - Standard substances; Variant 2 - obtained by dilution 1:1; Variant 3 - obtained by dilution 1:2.

The seeds used in biotests were verified to meet all the quality standards needed for germination and seedling generation. The surface of the seed was sterilized in 5% sodium hypochlorite to prevent fungal contamination after which the seeds were rinsed with deionized water.

Founding the experiments

In each Petri dish, after a prior washing with deionized water, was placed a disk of filter paper having the same size as the bottom of the Petri dish with a diameter of 12 cm, then 100 seeds were placed in three replications. After placing the seeds, they were covered with a patch of filter paper and added extract (liquid) or distilled water for the witness. The moisture content of the filter paper was maintained by adding in each Petri dish 12 ml of liquid, in two applications (6 ml + 6 ml).

The prepared Petri dishes were placed in germinator at a temperature of 25 ^{0}C during the day and to 20^0 C at night. The light used for seed germination was cold light emitted by fluorescent tubes for 10 days. Light has been used for a period of 16 hours, in the remaining 8 hours it was dark.

The seed germination and plant growth biotest

Germination was assessed by counting the germinated grains. Germination was reported as the germinating percentage of 100 seeds prepared for germination for each species.

The increase in length of embryonic roots and stems was determined by using the biometric.

Statistical processing by analysis of variance

The significance of individual differences from witness was calculated through the method of limit differences, represented by values 0 - significant, 00 - distinctly significant and 000 - very significant. Calculation and interpretation of data obtained from the measurements and the measurements mentioned above were processed by statistical analysis methods described by (CEPOIU, 1968).

RESULTS AND DISCUSION

VOIGT and MERGEN found in 1962 a seasonal variation in the toxicity of *Ailanthus* leaf extract, the inhibitory effects were less obvious in the extract obtained in October, compared to young leaves. In a more detailed analysis, HEISEY (1990) found that the highest toxicity occurs in young leaves emerging, it is decreasing with age.

Germination experiments showed that the toxicity of the species is highest in the first two years of life.

Based on bibliographic sources, in this work we tested the effect given by extracts of *Ailanthus altissima* on seed germination under study (mustard and rapeseed).

Germination of seeds for the species *Sinapis alba*, in the case of the witness was of 83%. By applying high concentrated watery extract, the germination capacity of mustard seeds was inhibited completely. In dilution 1 the inhibitory effect of the extract was just as high (0% germination). The same phenomenon has been recorded in the case of treating mustard seeds with a weaker extract concentration, obtained through dilution 1:2.

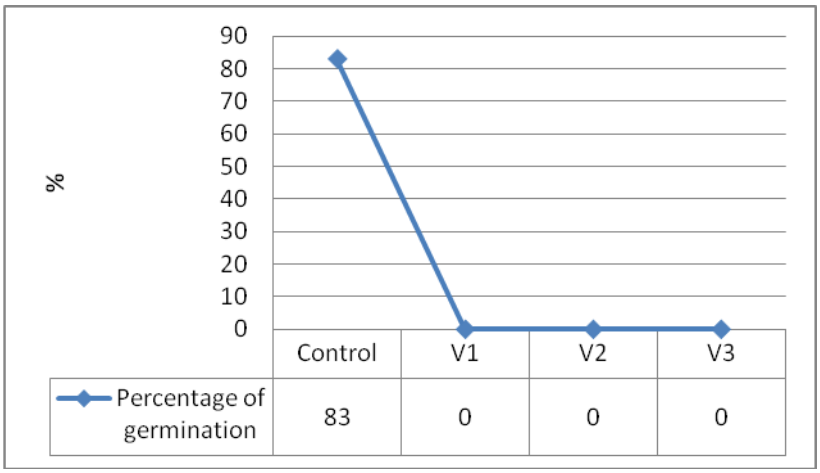

Figure 1: The percentage of germination of seeds *Sinapis alba*

The differences in the germination capacity are significantly negative between seeds treated extract (regardless of dilution applied) and seeds treated with distilled water as in the case of the witness, concluding that the allelopathic potential of species *Ailanthus altissima* is a strong one.

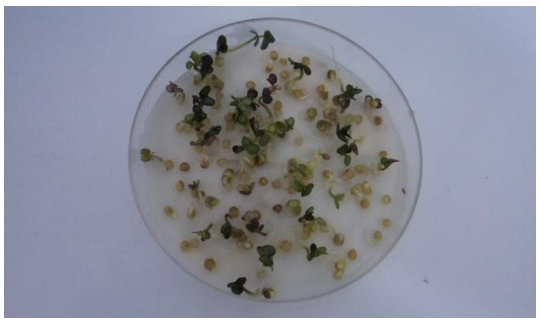

Figure 2: *Sinapis alba* seed germination in control

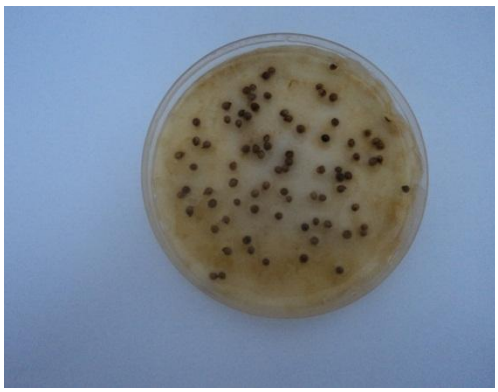

Figure 3: *Sinapis alba* seed germination variants treated with extracts

If the case of species *Brassica napus* seed germination in the witness was 96.66%. *Brassica napus* seed germination in all three extracts is completely inhibited. With the dilution of the extracts, with variants 2 and 3, the germination percentage of the rape seeds treated remains constant as in the concentrated variant, namely 0%. Watery extracts of leaves and bark had similar strong harmful effects on rape seeds.

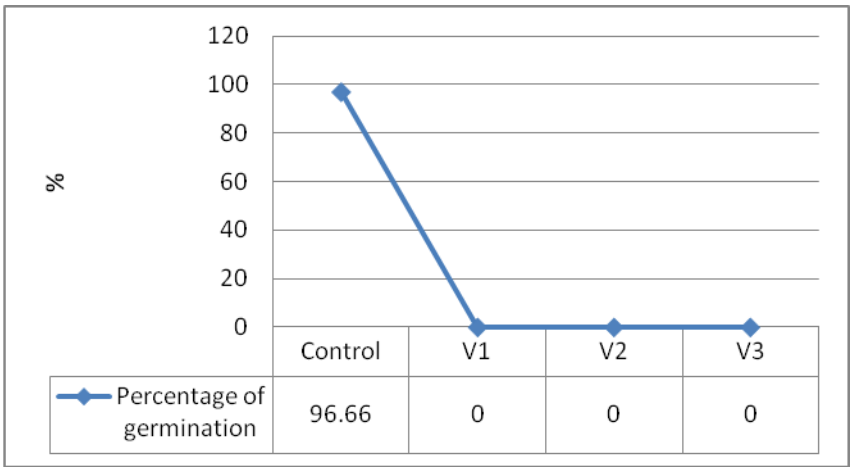

Figure 4: The percentage of germination of seeds *Brassica napus*

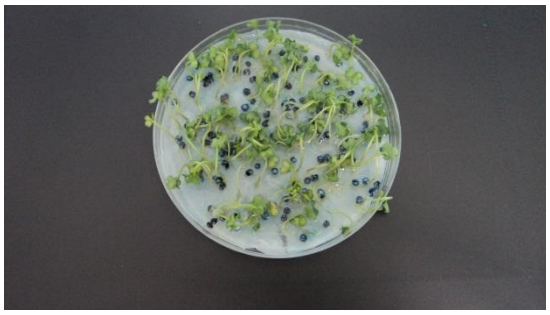

Figure 5: *Brassica napus* seed germination in control

Figure 6: *Brassica napus* seed germination variants treated with extracts

The increasing in the length of the embryonic roots and stems (hypocotyl) in the case of the witness *Brassica napus* have shown that the average hypocotyl length was 3.36 cm and the root length was recorded 3.25 cm (Fig. 7).

Figure 7: Growth and development to *Brassica napus* seedlings

The data presented in table 1 it can be noticed the inhibitory effect of extracts of *Ailanthus altissima* on the growth of rape seedlings.

In variant 1, variant in which the concentration of the allelopathic substances embodiment is the largest, the effect was completely inhibitor was so that the root and hypocotyl at treated seed did not increase at all, regardless of the extract applied.

In variant 2, variant in which an extract with a dilution of a 1:1 was used, the growth and development of the root and hypocotyl was totally inhibited in both the bark extract and the leaves extract.

The third variant is similar to the first and second variants, variant having a dilution of 1:2, where the concentration of allelopathic compounds present in the species of *Ailanthus* is lower.

In *Sinapis alba* the same trend is observed as with in *Brassica napus*. The increase in hypocotyl and the root are both influenced by the watery extracts containing alkaloids in all the studied variants (Table 2).

If we were to compare the witness and the three variants treating the studied seeds it can be observed that the extracts applied led to a total inhibition of germination, growth and development of the species treated, the difference being very significantly negative.

The allelopathic efficiency in *Ailanthus* extracts did not vary according to dilution, which demonstrates that the allelopathic potential of the species is very strong one and may influence negatively the neighbouring species.

Tabel 1

Influence of *Ailanthus altissima* extracts of *Brassica napus* species

Variants		**Control *Brassica napus***		
	$\bar{x} \pm sx$	$s\%$	u	Semnification
Hypocotyl	3.36±0.066	6.305		
Radicle	3.25±0.094	9.202		
		Bark extract		
Hypocotyl				
V1	0±0	-	-50.149	000
V2	0±0	-	-50.149	000
V3	0±0	-	-50.149	000
Radicle				
V1	0±0	-	-34.364	000
V2	0±0	-	-34.364	000
V3	0±0	-	-34.364	000
		Control *Brassica napus*		
Hypocotyl	3.36±0.066	6.305		
Radicle	3.25±0.094	9.202		
		Leaf extract		
Hypocotyl				
V1	0±0	-	-50.149	000
V2	0±0	-	-50.149	000
V3	0±0	-	-50.149	000
Radicle				
V1	0±0	-	-34.364	000
V2	0±0	-	-34.364	000
V3	0±0	-	-34.364	000
	DL 5%=2,26 (cm)	DL 1%=3,25 (cm)	DL 0,1%=4,78 (cm)	

Tabel 2

Influence of *Ailanthus altissima* extracts of *Sinapis alba* species

Variants	$\bar{x} \pm sx$	s%	u	Semnification
		Control *Sinapis alba*		
Hypocotyl	2.95±0.106	11.439		
Rădăciniţa	3.2±0.103	10.206		
		Bark extract		
Hypocotyl				
V1	0±0	-	-27.642	000
V2	0±0	-	-27.642	000
V3	0±0	-	-27.642	000
Radicle				
V1	0±0	-	-30.983	000
V2	0±0	-	-30.983	000
V3	0±0	-	-30.983	000
		Control *Sinapis alba*		
Hypocotyl	2.95±0.106	11.439		
Radicle	3.2±0.103	10.206		
		Leaf extract		
Hypocotyl				
V1	0±0	-	-27.642	000
V2	0±0	-	-27.642	000
V3	0±0	-	-27.642	000
Radicle				
V1	0±0	-	-30.983	000
V2	0±0	-	-30.983	000
V3	0±0	-	-30.983	000
	DL 5%=2,26 (cm)	DL 1%=3,25 (cm)	DL 0,1%=4,78 (cm)	

CONCLUSIONS

Based on research carried out in laboratory conditions, by studying the degree of externalization of the main morphological characters as well as by determining the influence of the allelopathic compounds present in *Ailanthus altissima* on leguminous species studied, the following conclusions have been drawn:

- germinating the seed of *Sinapis alba* and *Brassica napus* was inhibited by extracts containing allelopatic substances regardless of the concentration with which they had been treated; with all the extracts the effect was totally inhibitory;

- extracts negatively influence the growth of the treated seedlings, the phytotoxic effect of extracts was manifested by inhibiting their growth;
- extracts become active irrespective of the dilution used, the inhibiting effect being complete in all variants;
- differences between witnesses and species treated with leaves and bark extracts were highly significant negative;

Given the current climate conditions, the species *Ailanthus altissima* is favoured over native species, this combined with the allelopathic effects demonstrated through experiments carried out in the current paper may lead in the future to the expansion of this species with major impacts on biodiversity.

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