ALLELOPATHIC POTENTIAL OF *Asarum europaeum* TOWARD *Lycopersicon esculentum*

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Abstract. *Asarum europaeum* L. contains water-soluble substances which manifest allelopathic potential. Aqueous extracts from leaves and stems of *Asarum europaeum* were assayed to determine their allelopathic effects on *Lycopersicon esculentum* and *Zea mays* seeds germination and early seedling growth. The germination of the investigated seeds was found to be inhibited with increasing of the *Asarum europaeum* L. extract concentration. Moreover, the active substances extracted from leaves were found to be more inhibitory on the seeds germination in comparison with those extracted from stems.

Keywords: Allelopathy, *Asarum europaeum*, leaves, rhizomes, growth inhibitor

INTRODUCTION

Allelopathy [14] is the chemical interaction between plants, including stimulatory as well as inhibitory influences. Allelopathy plays an important role in both natural and agro-ecosystems and has potential in integrated weed management. Plants, including different species of algae [26], contain thousands of natural products, but not all are supposed as having allelopathic effects [2, 15]. Allelochemicals are low molecular weight compounds excreted from plants during the processes of secondary metabolism [1, 16] and they can accumulate in plants, soils and other organisms. These compounds vary in chemical composition, concentration and localization in plant tissues and from plant to plant with changes in both biotic and abiotic conditions [8].

Asarum europaeum, commonly known as Asarabacca, European Wild Ginger, Haselwort, and Wild Spikenard, is a species of wild ginger with single axillary dull purple flowers, lying on the ground. It is widespread across Europe, ranging from southern Finland and northern Russia down to southern France, Italy and the Republic of Macedonia. It is also grown extensively outside its natural habitat as ornamental plant. It is sometimes harvested for use as a spice or as source of flavors [19]. In the forest ecosystems, *Asarum* species demonstrate a high competitiveness compared to other grass species due to morphological characters, but also potential allelopathy. The species of the *Asarum* genus contain a high variety of chemical compounds including flavonoids such chalcone, flavonols, anthocyanides, methylisoeugenol, α-asarone (19.2%), α-asarone and methyleugenol.

Iwashina et al. [10] have extracted and isolated from the *Asarum* genus two new chalcone glycosides, chalcononaringenin 2`,4`-di-Oglucoside and chalcononaringenin 2`-O-glucoside-4`-O-gentiobioside, from the leaves of *A. canadense* with seven known flavonol glycosides, quercetin 3-O-galactoside, quercetin 3-O-robinobioside, quercetin 3-O-galactoside-7-O-rhamnoside, kaempferol 3-O-galactoside, kaempferol 3-O-glucoside, kaempferol 3-Ogalactoside- 7-O-rhamnoside and isorhamnetin 3-O-hamnosylgalactoside [10]. Flavonoids are generally considered to inhibit germination and cell growth [3], thus their allelochemical release early in the season could affect other species at susceptible life stages (e.g., germinating seeds and young seedlings) [24]. In addition to temporal and seasonal variation in allelochemical production, differences may exist among genotypes, populations, or plants of different ages.

On the other hand, it was demonstrated that the essential oils from *Asarum* have antimicrobial activity [20]. In previous investigations, this essential oil was found to possess the promising antifungal activity against a variety of plant pathogens [13, 22, 23, 25].

This article aims to identify the allelopathical effect of *Asarum europaeum* and moreover to compare the inhibitory effect of the different vegetative organ extract on the germination and seedling growth of *Lycopersicon esculentum* and *Zea mays* .

MATERIALS AND METHODS

Obtaining of the extracts from rhizomes and leaves of *Asarum europaeum*

In March 2010, about a hundred samples of *Asarum* were randomly taken from *Fagetum* and *Carpinetum* forests near Baia Mare and alongside the Somes river. The plant tissues were clipped by hand 1 cm above the soil and directly oven-dried at 60^0C for 5 days. Forty grams of dried leaves, respectively rhizomes, were extracted by soaking in 1 l-distilled water at 24^0C for 24 h in a stirrer Heidolpf Unimax 1010 to give a concentration of 40 g dry tissue /L. The extract was filtered using an Laboport vacuum pump. Each stock extract was diluted appropriately with sterile distilled water 1:1, 1:2 and 1:3 (v:v). Distilled water was used as reference.

Processing of test plants seeds

The seeds of the species tested, namely tomato (*Lycopersicon esculentum* L.) and maize (*Zea mays* L.), were used for germination assays. The seeds were sterilized with 5% sodium hypochlorite for 10 min and five times rinsed with distilled water. In each experiment 100 seedlings were used and the experiments were repeated three times.

Bioassay

To evaluate the allelopathic effect to water soluble extracts, 50 seeds of tomato and separately maize, placed in a plate, containing two layers of filter paper moistened with 10 ml of aqueous extract of rhizomes and leaves, respectively, in different dilutions, as Figure 1 suggests. The plates were maintained, for 7 days, in a Sanyo environmental test chamber (temperature 23^{0}C, illumination 135 micromoli photon/m^2/s and humidity 82%). The experiments were replicated three times.

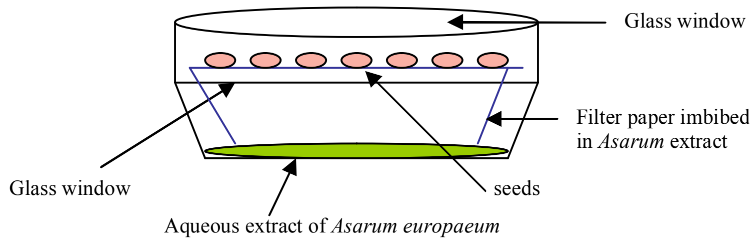

Fig. 1. Scheme of the germination experiments.

RESULTS

Asarum europaeum is a species peculiar to the *Querco-Fagetea* association, populating most of the forests of this genus, adding up into facieses and appearing as a competitor that visibly contributes to the extinction of the other species. One of the reasons is the low height of this species, its repent and reniform shapes covering high areas. The allelopathic influence that such species exercise on the germination process of other plants, as well as on those plants growing process could be another explanation. This species is known for the ability to preserve green leaves during winter and the rejuvenation of those leaves occurs only in spring, relatively at the same time with the germination of the other species on the same soil. Because of these reasons we have tested the allelopathic capacity of rhizomes and separately the capacity of the *Asarum europaeum* leaves on a reference sample of plants. We have considered for our experiments both a dicotyledonuos and a monocotyledonous species. In order to test the germination capacity we have performed separate experiments. We found a germination capacity in the 80% - 100% range for both species

Table 1. Parameters of germination and seedling growth of tomato seeds in the presence of rhizomes and leaves extracts of *Asarum europaeum*.

	Time (days)							
	3	4	5	6	7	15	32	39
	Germination (number of germinated seeds) (%)					Plant growth (cm)		
Rhizomes extract dilution (v:v)								
1 :1	4	11	31	42	57	0,7	3	6
1 :2	6	15	38	45	52	0,8	4	7
1 :3	12	18	36	41	55	1,3	5,5	8
Leaves extract dilution (v:v)								
1 :1	0	2	13	25	41	0,2	3	5
1 :2	7	16	35	41	56	0,3	5	6
water	50	64	67	69	75	1,5	7	11

Table 2. Parameters of germination and seedling growth of maize seeds in the presence of rhizomes and leaves extracts of *Asarum europaeum*.

	Time (days)					
	3	8	15	20	32	39
	Germination (number of germinated seeds) (%)			Plant growth (cm)		
Rhizomes extract dilution (v:v)						
1 :1	60	70	100	0,8	3,2	5
1 :2	80	86	100	0,9	5	6,2
1 :3	82	94	100	1,5	7,3	8,4
Leaves extract dilution (v:v)						
undilluted	60	76	83			
1 :1	36	38	60	1:1	4	11
1 :2	72	80	82	1:2	6	15
water	92	100	100	1:3	12	18

DISCUSSION

The results indicated that aqueous extract of *Asarum europaeum* plants, like other species (*Botriochloa laguroides var. laguroides* (D.C.) Herter) [18], contain indeed growth inhibitors that are capable of reducing growth of tomato and maize. The reduction in seedling length may be attributed to the reduced rate of cell division an cell elongation due to the presence of the allelochemicals [11, 18].

Details in table 1 and in fig. 2, 6 and 8 reveal for the rhizome extract a different allelopathic capacity as compared to that of leaves on the germination of la *Lycopersicon esculentum.* Both the rhizome extract and the extract of leaves, at various dilutions, inhibit the germination capacity with almost 50% as compared to the control sample tested in distilled water. This proves the unquestionable existence of an adaptive mechanism that the plant uses against other species. The allelopathic effect of the species tested shows up particularly as a mechanism that delays the germination of other species, in a way that debilitates the species in the neighborhood of *Asarum europaeum*. The extract of leaves (Fig. 2, the green line) has an inhibitory effect on germination which is obviously stronger than the rhizome extract. The inhibiting effect declines for both the leaves extract and the rhizome extract as the degree of dilution grows, and even for a 1:3 dilution ratio the number of seeds germinated is considerably below the level of the control sample. Both categories of extracts inhibit the germination of *Zea mays*, (Fig. 4) but while the rhizome only generates a delay, the *Asarum* leaves display an "aggressive" allelopathic effect which strongly inhibits the germination of maize seeds. The impact on seedlings is similar with the impact on germination. Leaves have a much stronger inhibitory impact than the rhizome.

We have statistically processed the results of the allelopathic effect on the germination material, on *Lycopersicon* and respectively on *Zea mays* seedlings (Fig. 10-13). Significant differences came out. The Euclidean similarity index comparing the response of the two species at different dilutions of the rhizome extract is less than one. This shows the existence of different mechanisms against the inhibitory effect (Fig. 10). *Zea mays* seeds are less sensitive to the allelopathic effect, and even if with a delay, their germination is almost complete, in contrast with the *Lycopersicon esculentum* seeds on which the inhibitory effect is visible in a much higher proportion. *Lycopersicon* has against *Asarum europaeum's* allelopathic activity a different level of resistance as compared to *Zea mays.* This comes out in (Fig. 11), showing the cluster analysis for germination, respectively the growth at various degrees of dilution. On one hand, there is very little similarity between the control samples tested at different degrees of dilution and the undiluted extract of sprouts. As the graph in figure 12 shows, the points that are the closest are

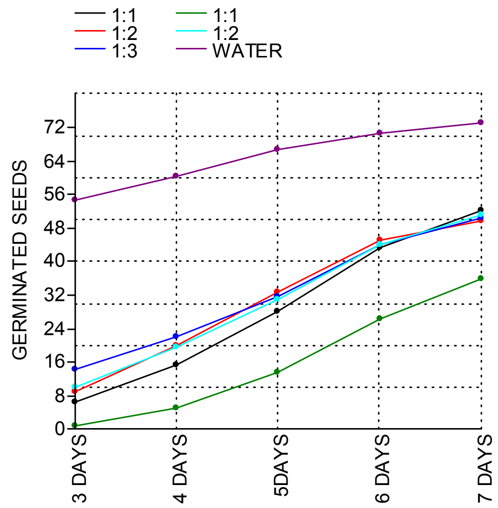

Fig. 2. No. of germinated seeds of *Lycopersicon esculentum*

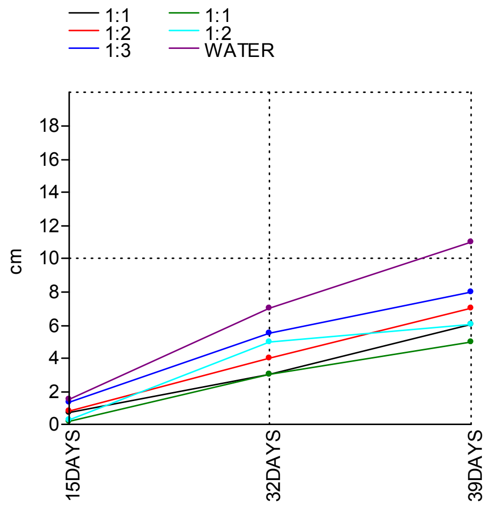

Fig. 3. *Lycopersicon esculentum* growth

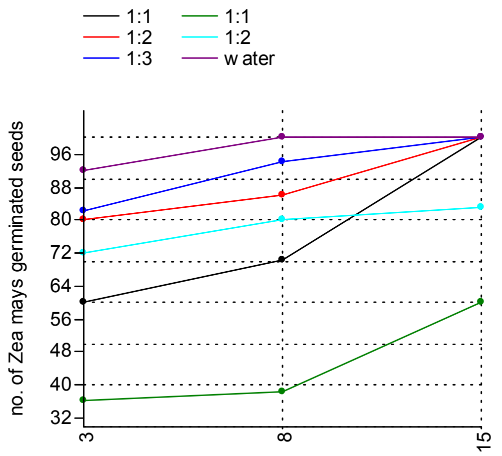

Fig. 4. No. of germinated seeds of *Zea mays*

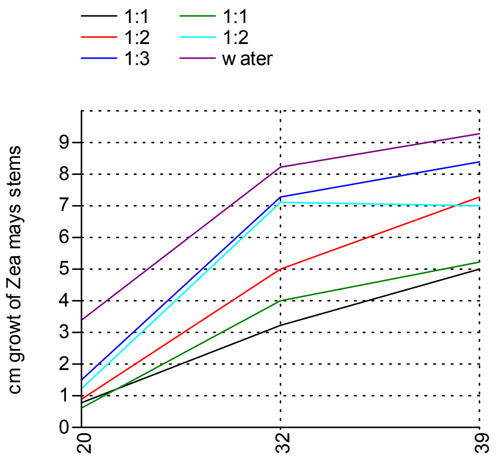

Fig. 5. *Zea mays* growth

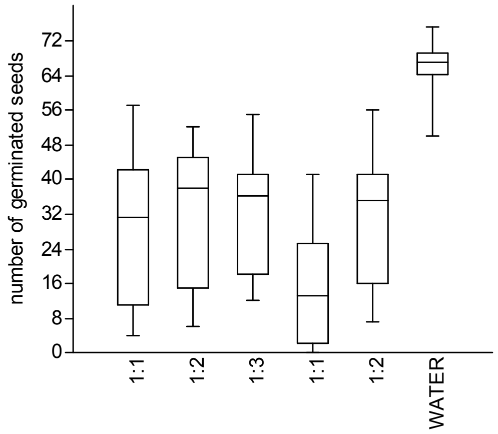

Fig. 6. Average germination in different experimental variants.

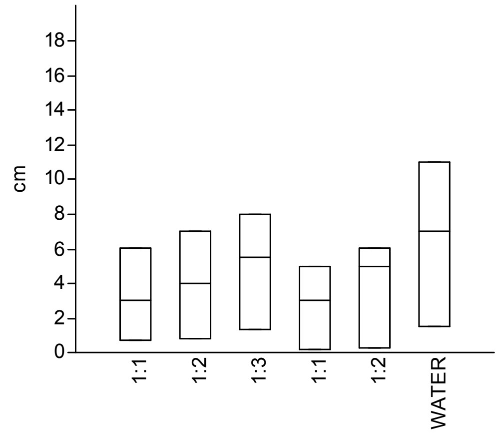

Fig. 7. Mean of seedlings growth in different experimental variants.

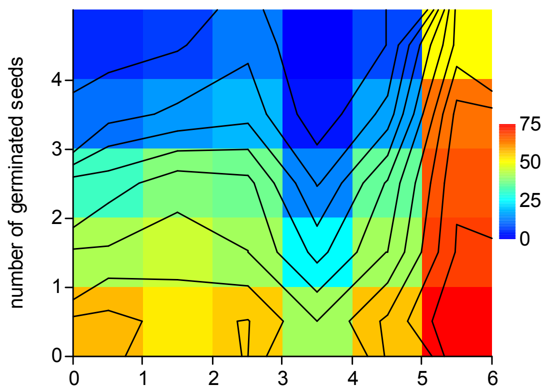

Fig. 8. Germinated Seeds Of *Lycopersicon esculentum*.

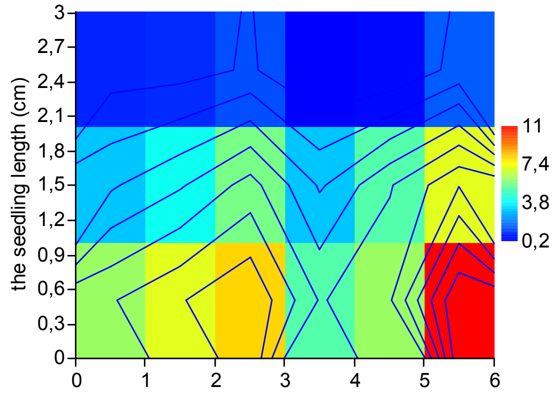

Fig. 9. The stem length of *Lycopersicon esculentum* seedling.

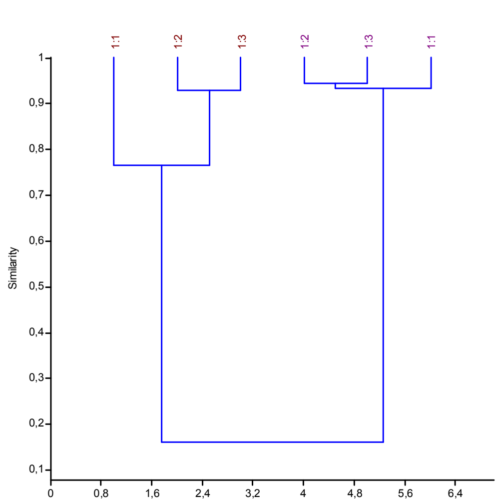

Fig. 10. Cluster analysis between seeds germination of *Lycopersicon* & *Zea* at different dilution of rhizomes extract (according Euclidian distance)

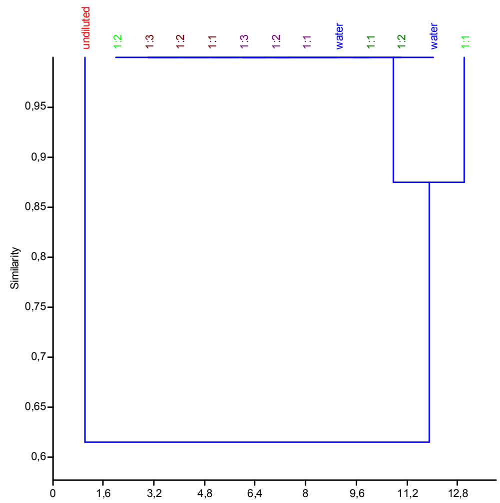

Fig. 11. Cluster analysis between seed germination and plantlet growth at different dilution of *Asarum* extracts (according Jaccard index); Light green – *Lycopersicon plantlets; dark green* – *Zea mays* plantlets; *purple* – *Lycopersicon* seeds; *brown* – *Zea mays* seeds

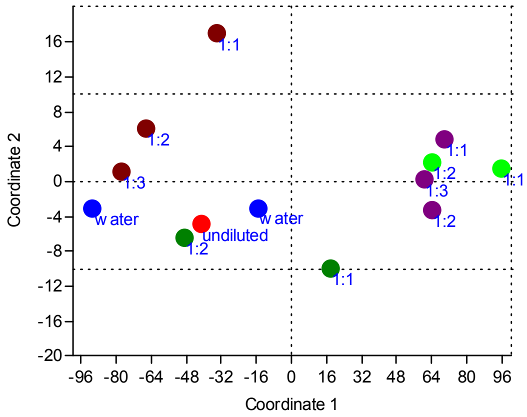

Fig.12. PCO Scatter diagram

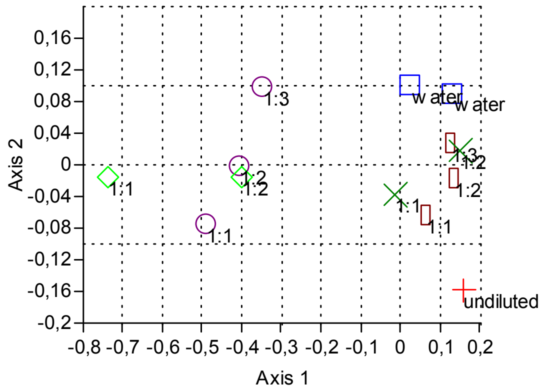

Fig. 13. Correspondence analysis diagram

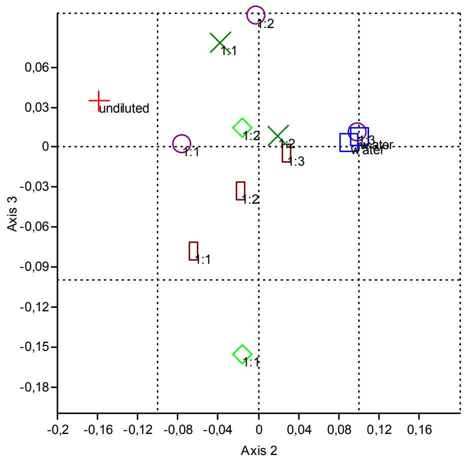

Fig. 14. The analysis of a number of key factors.

those corresponding to samples that belong to the same species and not those corresponding to samples tested at the same dilution. The PCO, the correspondence analysis and the Detrending correspondence analysis (Fig. 12, 13), reveal all the considerable differences among the samples tested. Each of these tests suggests a high degree of dispersion in terms of the response of the samples considered. The samples make up groups depending on the species and on the degree of dilution. The analysis of a number of key factors reveals (Fig.14) that the gap is small between the sample tested with *Zea mays* seeds, undiluted *Asarum* seedlings extract and the witness samples for germination. This proves that immature seedlings hold in little quantity compounds with allelopathic properties. On the other hand, the concentration of active compounds grows as the leaves grow up. This supports the hypothesis that leaves concentrate the highest proportion of active compounds in charge with allelopathic activities. Different plant tissues such as leaves and rhizomes, can release different amounts of allelochemicals into the surrounding environment [4, 18]. Study on the water melon showed that the various plant parts had not significant differences in the bioassay test [7]. Nevertheless significant differences could be observed among the parts of the *Botrichloa* [18, 17] and also *Asarum europaeum,* affecting seedling growth of plants tests during the experiment.

Allelopathic potential of the test plant could be used in biological control of weeds. In this respect, the allelopatical relationship should be tested both with the crop, in this case with tomato and corn, and weed species. Effect on weeds will be the subject of further study.

The range of species tested, in terms of allelopathy is very high. Most aim to identifying the antagonistic mechanisms between the crop and associated weeds. It was revealed that inhibition of *Artemisia* species exerts on wheat [12]. Studies on the other species of invasive or non-invasive plants, like *Ludwigia* [5], *Chenopodium* [9], have demonstrated that the inhibitory effect can be modulated by soil characteristics and other biotic and abiotic factors. Other study reveals that if the toxicants are produced continuously in the environment without control the affected species may become extinct [6]. Therefore, the effect of allelopathy of *Asarum*, demonstrated that highly active, will be further tested in various environmental conditions and in different concentrations and periods.

To conclude, the *Asarum europaeum* species rely on bio-chemical mechanisms to survive and to compete by means of biologically – active compounds with allelopathic effect. These compounds accumulate prevalently in leaves and to a smaller extent in rhizomes. The allelopathic effect takes the form of a delaying mechanism, for monocotyledonous plants and the form of inhibitory effect for dicotyledonous plants. Inhibition occurs in terms of germination and in terms of the growth process as well. These results are promising to meet the challenge, which is the identification of bio-selective herbicide [21]

The species that are „receptive" to allelopathic compounds develop various resistance mechanisms, conferring to plants different sensitivities. It is demonstrated by relevant experiments that monocotyledonous plants are more resilient against the inhibitory effect on germination and on the growth process.

We think that gaining more insight on the phenomena of bio-chemical inhibition and also on the ways to counteract inhibitory activities will allow for a better understanding of ecology – specific competition and survival mechanisms within phytocoenoses.

Revealing details about the biology of plants with potential to be applied in agriculture and forests management is a key outcome of this research exercise.

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