Modern Phytomorphology **5**: 93–99, 2014

ALLELOPATHIC INFLUENCE OF AQUEOUS EXTRACTS FROM THE LEAVES OF *MORUS ALBA* L. ON SEED GERMINATION AND SEEDLING GROWTH OF *CUCUMIS SATIVUS* L. AND *SINAPSIS ALBA* L.

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Abstract. The aim of the present study was to elucidate impact of the aqueous extracts from leaves of *Morus alba* L. on germination, growth and photosynthetic activity of *Cucumis sativus* L. and *Sinapis alba* L. Plants were grown for 21 days at the temperature 25°C (day) and 18°C (night), within 12/12 hours photoperiod, light intensity 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and relative humidity 60-70% (day/night). Our experiments proved that allelopathic compounds in aqueous extracts of the leaves *M. alba* at high concentrations, reduce power and energy of germination. Biometric analysis of seedlings and adult plants grown showed that allelopathic substances have stimulating or inhibiting function depending on the stage of treatment. Moreover, they cause changes in chlorophyll contents and activity of photosystem II (PS II).

Key words: *Morus alba, Cucumis sativus, Sinapis alba*, allelopathy, morphology, leaves and seed germination

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Introduction

The term of allelopathy (from gr. *allelon* = mutually and *pathos* = suffering, feeling, sensitivity) was introduced by Molish in 1937. It means the biochemical interactions which occur between all types of plants, including microorganisms. These interactions are mediated by allelopathic substances which are secreted from the some organisms (donors) have effect on the others (acceptors) (Rice 1984; Oleszek 2001; Gniazdowska *et al.* 2004). Allelopathic compounds produced by higher plants are mostly secondary metabolites, which are synthesized by the acetic acid and shikimic acid pathways. Phenolics, cyanogenic glycosides, quinones, lactones, organic acids and volatile terpenes belong to the most active compounds (Kopcewicz & Lewak 2002).

Allelopathic substances are present in all organs of the vegetative and generative organs of plants (Rice 1984). The main source of allelopathic compounds are leaves. Quantity of allelopathic compounds in plant depends on its age. It was found that they are released in much larger quantities from young plants than from ripening or aging plants (Ahmed & Wardle 1994; Wójcik-Wojtkowiak 1998).

Allelopathic compounds are the most active early in spring, in soils with weak oxygenation. They are released into the environment by processes of evaporation, leaching from the aerial parts, by sweating of the root system or the decomposition of dead plant tissues (Wójcik-Wojtkowiak 1998; Gniazdowska *et al.* 2004). Currently, research is focused on use of the allelopathy phenomenon in protecting plants against insects, pests, nematodes and increasing their resistance to weeds (Widera 1994; Gniazdowska *et al.* 2004). Specifically, investigators are looking for the products, containing allelopathic compounds, on the basis of which it would be possible to synthesise natural biodegradable herbicides.

The aim of the study was to investigate the effect of aqueous extracts from the leaves of *Morus alba* L. on the germination, growth and photosynthetic activity of seedlings and adult plants of *Cucumis sativus* L. and *Sinapis alba* L.

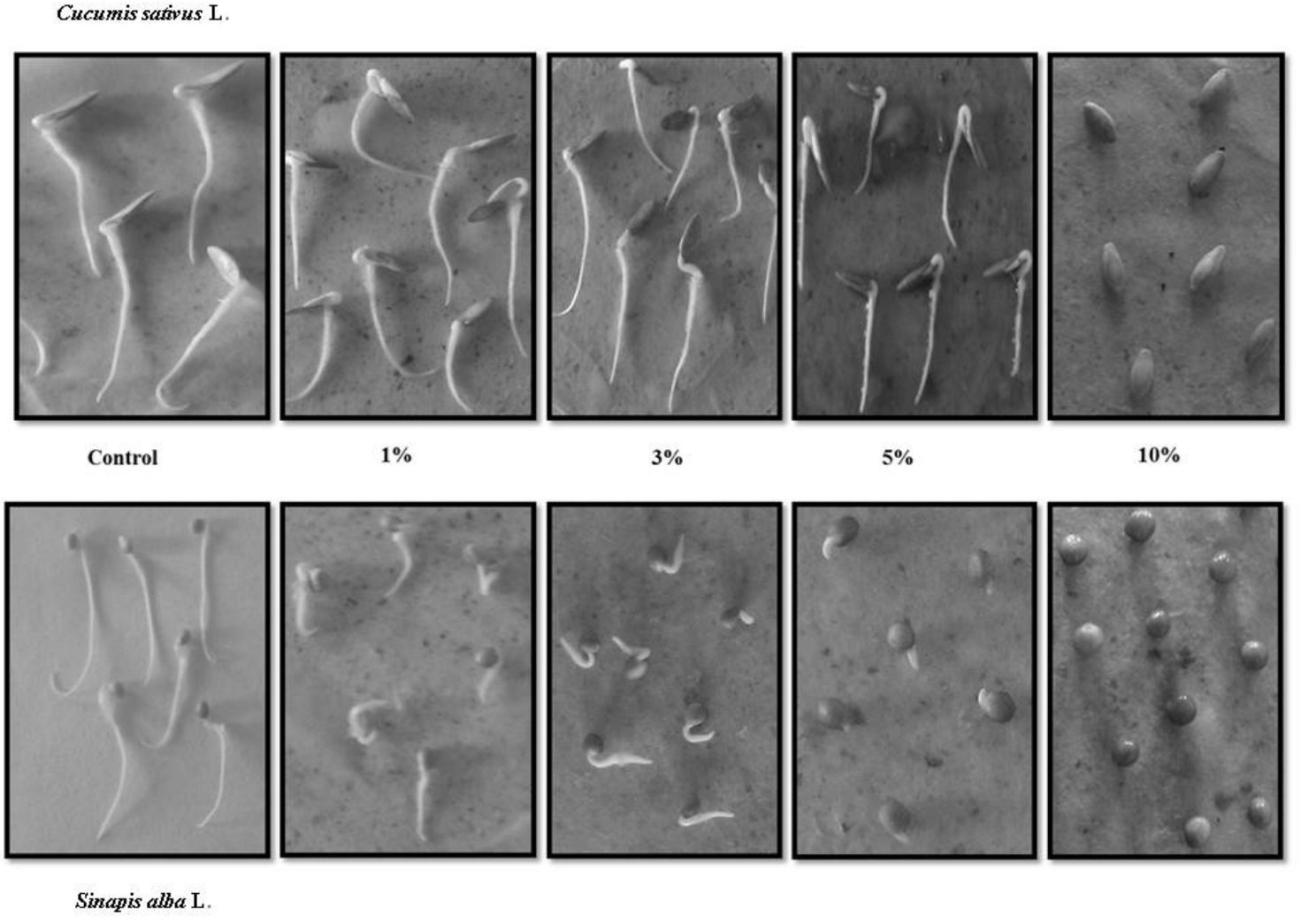

Fig. 1. The germination of seeds of *Cucumis sativus* and *Sinapis alba* treated with aqueous extracts (1, 3, 5, 10%) from the leaves of *Morus alba*.

Material and methods

The main object of the experiment was to determine the influence of allelopathic substances contained in the aqueous extracts from the leaves of *Morus alba*, on the seeds of *Cucumis sativus* cv. *'Andrus F1'* and *Sinapis alba* cv. *'Barge'*.

In the first stage of the experiment, we determined the effect of extracts from the leaves of *M. alba* on the strength and energy of germination and growth of seedlings of *C. sativus* and *S. alba*. Before tretment, aqueous extracts from leaves were prepared, concentrations 1, 3, 5 and 10%. Specifically, finely grounded leaves were put into distilled water and incubated for 24h at 25°C. After that the solutions were filtered and stored in the fridge.

On the second stage of the experiment, 5 randomly selected germinated seeds of *C. sativus* and *S. alba* both from control and treated by extracts groups were planted into the soil.

On the third stage of the experiment, the influence of aqueous extracts of the leaves on the growth of plants from seeds germinated on distilled water was checked. For this purpose, the plants were watered by aqueous extracts of *M. alba* leaves. All seedlings were also watered with distilled water and a standard nutrient mixture 'Steiner'. Plants were grown at 25°C (day) and 18°C (night), within 12/12 h photoperiod, at light intensity 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 60-70% relative humidity (day/night).

After 21 days of cultivation, the imaging of chlorophyll fluorescence was performed, biometric analyses were conducted, content of chlorophyll *a* and *b*, biometric analysis were conducted, fresh and dry weight and water content in plants *C. sativus* and *S. alba* were determined. Chlorophyll fluorescence imaging was performed using a FluorCam – Photon Systems Instruments Company by analyzing parameters F_v/F_m, F_0, F_m. The contents of chlorophyll a and b in the leaves were determined using the method described in Barnes *et al.* (1992). Biometric measurments for *C. sativus* and *S. alba included:* roots length, hypocotyles, petioles with cotyledons, distance from the cotyledons to the first leaves with petioles, petioles of the first row, remaining part of the stalks, the rest of momentums the plant and the numbers of leaves.

Statistical analysis was performed using STATISTICA 10.0 for Windows. Results presented in this paper are average of 8-10 of the biological or technical replicates ± SD. The significance of differences between means was tested with parametric ANOVA. Comparisons of the significance of differences in average values between objects were made using Tukey's test (HSD) for heterogeneous groups – at confidence level of $p \leq 0.05$.

Results

The present study showed that extracts from leaves of *M. alba* significantly influence on the length of seedlings and selected organs of plants. The aqueous extracts of *M. alba* leaves inhibited the process of germination of seeds (Fig. 1). Seeds germinated slow and less seeds were germinated. Reduction of germination energy was more profound with the increased concentrations of allelopathic substances These observations were further confirmed by biometric analysis of seedlings *C. sativus* and *S. alba* (Tab. 1).

Growth disturbance during the embryonic stage has influence on the growth of seedlings. In the case of *S. alba* the extracts of *M. alba* slowed growth of the roots in every stage of the experiment but they stimulated their growth in the case of *C. sativus*. Significant differences were observed in the length of hypocotyl of *C. sativus* treated with extracts in phase of germination. In the phase of growth, 5% extract of *M. alba* demonstrated the inhibitory effect on the length of hypocotyl, the first leaves petioles, and petioles of the first row of *S. alba*. In addition, the extracts used in the phase of germination of *S. alba* increased the length of petioles cotyledons, and petioles of the first row. Generally, aqueous extracts of the leaves of *M. alba* showed an inhibitory effect especially on plants treated by extracts in the phase of growth (Fig. 2).

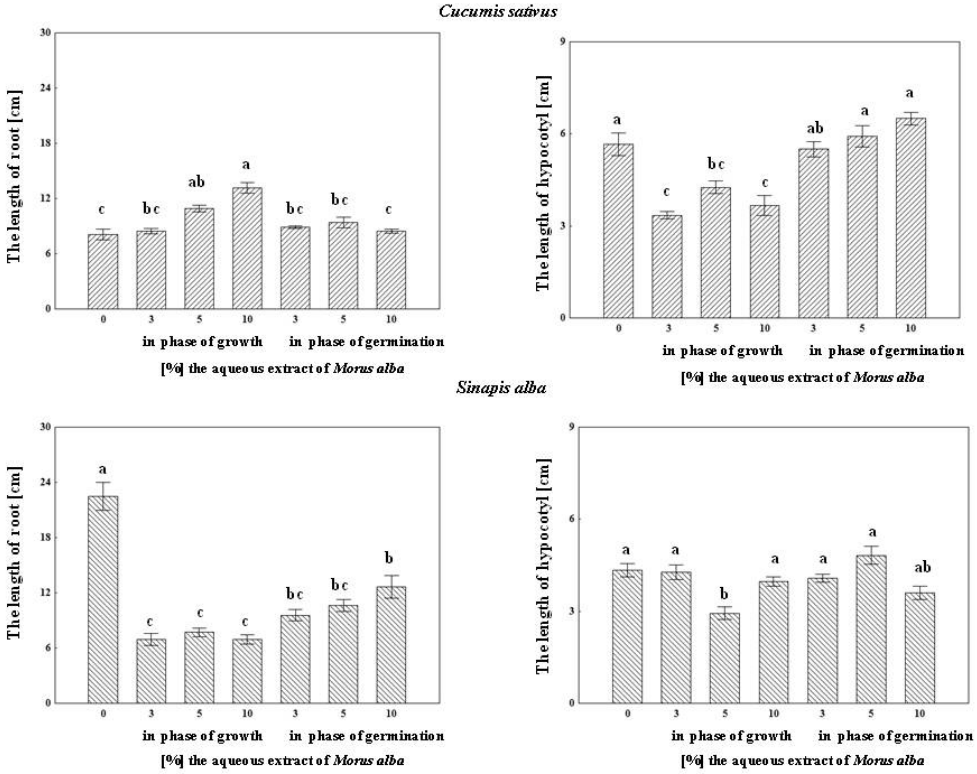

Fig. 2. The length of roots and hypocotyles in *Cucumis sativus* and *Sinapis alba* treated by (%) aqueous extracts from the leaves of *Morus alba* in the phase of germination or growth. The mean values of 8-10 replicates ± SD are shown. Values with different letters are significantly different at $p \leq 0.05$ by Tukey's test for inhomogeneous groups (HSD).

Moreover, the presence of allelopathic substances contributed to changes in the water content in the different organs of plants. The higher concentrated extracts of *M. alba* by which plants were watered caused a decrease in fresh and dry weight compairing to control plants.

The aqueous extracts from the leaves of *M. alba* had impacted also on the chlorophyll fluorescence. The differences in the maximal fluorescence (F_m), on the edge and in the upper part of leaf blade were observed in the case of *S. alba* leaves. The value F_0 was decreased in leaves treated with the aqueous extracts during the germination, but was increased in the plants watered by allelopathic extracts in the phase of growth. Differences in values of F_v/F_m were observed in leaves treated with extracts in the highest concentrations. For *C. sativus* the higest differences between the studied parameters were observed in control and plants treated by 10% extracts during germination (Fig. 3 A, B).

Generally, allelopathic compounds led to an increase in the concentration of chlorophyll in leaves of *S. alba* and *C. sativus* whatever stage of treatment was (Fig. 4).

Discussion

The differences both in time and dynamics of germination of seeds were observed in the experiment (Fig. 1). Seeds of *S. alba* started germination after 24 h and *C. sativus* – after 48 h, which may be associated with their structure, genetics or age. With increasing concentration of the allelopathic substances in the aqueous extracts from leaves of *M. alba* the reduction of energy of germination and the length of seedlings were observed. This effect was most evident when we applied an extract of 10% concentration, when the germination

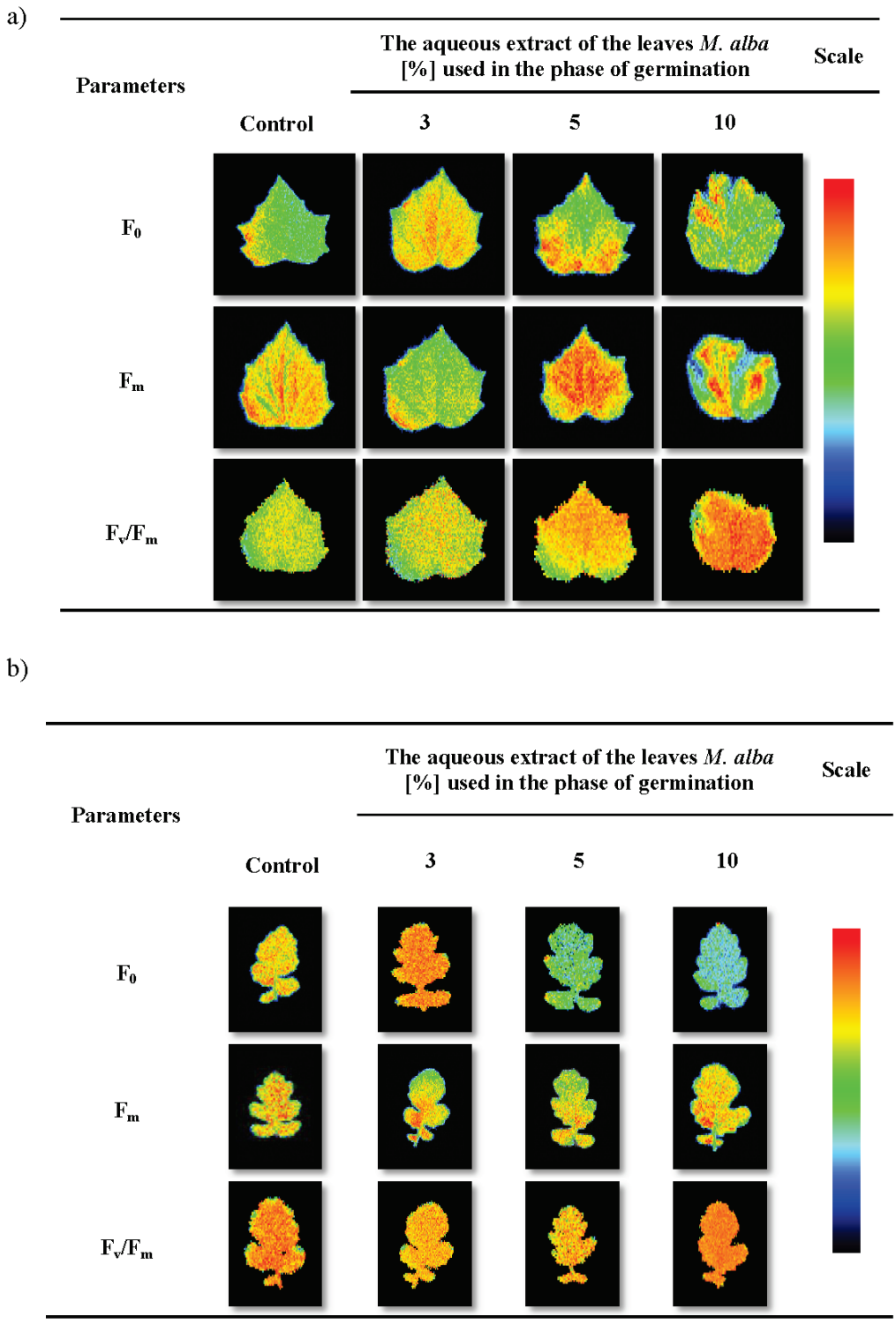

Fig. 3. Imaging of chlorophyll *a* fluorescence in leaves of studied plants based on the parameters F_0, F_m, F_v/F_m treated by aqueous extracts from the leaves of *Morus alba* in the phase of germination: **A** –*Cucumis sativus*; **B** –*Sinapis alba*.

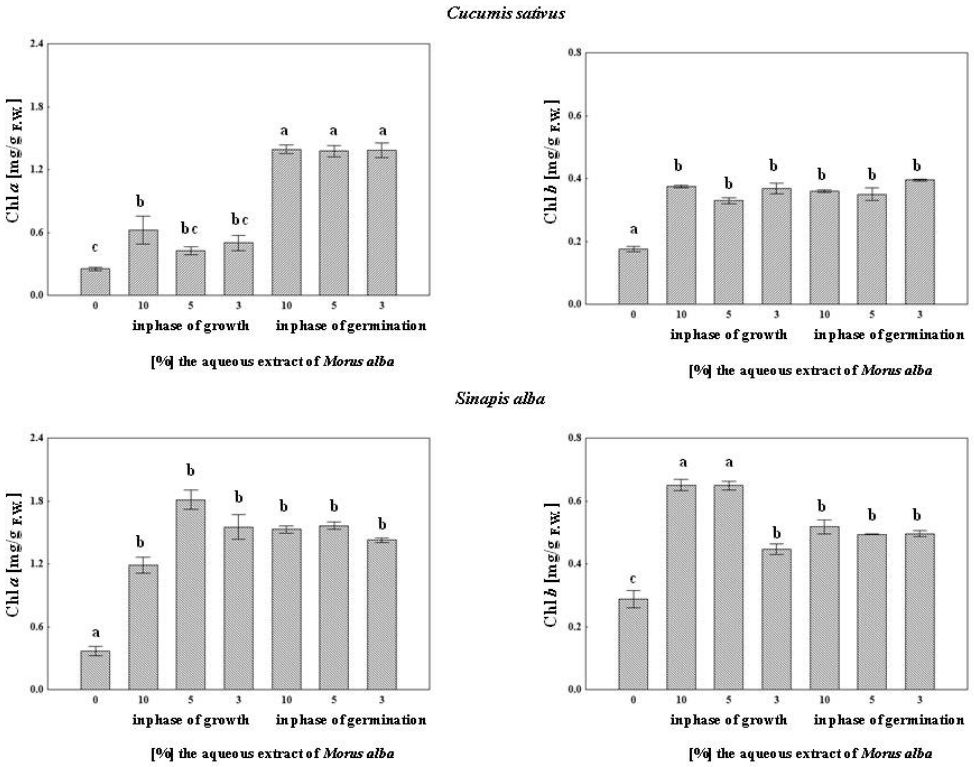

Fig. 4. The average content of chlorophyll *a* and *b* in leaves of *Cucumis sativus* and *Sinapis alba* treated with aqueous extracts of *Morus alba* in the phase of germination or growth. The mean values of 5-6 replicates ± SD are shown. Values with different letters are significantly different at $p \leq 0.05$ by Tukey's test for inhomogeneous groups (HSD).

was completely inhibited (Tab. 1).

The growth is a process extremely sensitive to the presence of allelopathic substances. Aqueous extracts from the leaves of *M. alba* in these experiments showed allelopathic influence on the growth of seedlings of *C. sativus* and *S. alba*. It was better seen on the plants watered by extracts, there allelopathic compounds inhibited the development of seedlings in comparison to the plants in control. Growth inhibition both of roots and the aerial parts of the plants (Fig. 2), decrease of fresh and dry weight, changes in fluorescence parameters and chlorophyll content, are indicators of the presence of allelopathic substances (Figs. 3, 4). Influence of allelopathic compounds on cell membranes was considered as the initial action, because their damage affects all metabolic and physiological processes. These compounds modify the operation and the permeability of membranes, impair the metabolism of proteins and lipids, leading to disturbances in the structure of cell membranes (JANICKA-RUSSAK *et al.* 2004).

Accordingly to RICE (1984), allelopathic substances have inhibitory eefect on division and elongation of cells through their distortion, deformation of the nucleus and a strong vacuolization. They reduce the water potential in the cells of the leaves, affect the degree of stomatal opening, consequently the intensity of transpiration and reduce the size of active part on the root surface, changing the appearance of root hairs. Furthermore, allelopathic compounds regulate functioning of plants, using IAA oxidase. They block the process of oxidation in PSII, causing a shortage of energy resulting from the photosynthesis. They cause interference in the active transport of molecules, synthesis of pigments and other,

Table 1. The length of seedling of *Cucumis sativus* and *Sinapis alba* treated with aqueous extracts (1, 3, 5, 10%) of *Morus alba* leaves. The mean values of 8-10 replicates ± SD are shown. Values with different letters are significantly different at $p \leq 0.05$ by Tukey's test for inhomogeneous groups (HSD).

Species of plovers	Aqueous extracts from the leaves *Morus alba* (%)				
	0	1	3	5	10
Cucumis sativus	$4,0^{a}$	$4,2^{a}$	$4,1^{a}$	$3,04^{b}$	0^{e}
Sinapis alba	$3,9^{a}$	$1,41^{c}$	$0,68^{d}$	$0,18^{de}$	0^{e}

metabolites within the plant. Short-term effects of allelopathic substances can slow uptake of phosphates, nitrates and sulphates. Uptake speed of ions and their concentration in the tissues has a significant impact on the supply of minerals in the plants. Theirs optimal level is necessary for the proper growth and development (Wójcik-Wojtkowiak 1998). Reduction in the synthesis of nutrients, intake of water and the production of energy required to maintain active transport, are manifested in the reduction of fresh and dry weight.

Conclusions

The obtained results allow concluding stimulatory and inhibitory effects of allelopathic substances of *M. alba* on the germination, growth and photosynthetic activity of *C. sativus* and *S. alba* plants, depending on concentration and stage of development on which the aqueous extracts were applied.

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