Modern Phytomorphology **7**: 81–86, 2015

THE MORPHOLOGICAL CHANGES OF *PHASEOLUS VULGARIS* L. EXPOSED TO THE AQUEOUS EXTRACTS OF THE LEAVES OF *JUGLANS REGIA* L.

Katarzyna Możdżeń * & Jakub Oliwa

Abstract. To date the allelopathic interactions between plants didn't explained, whether some substances are produced by plants to counteract competition or these substances are randomly generated and they are transferred from generation to generation. For that reason an attempt was made to investigate the influence of the aqueous extracts of dried leaves of walnut (*Juglans regia* L.) at concentrations of 3, 5 and 10% on the germination, growth, weight and morphology of bean (*Phaseolus vulgaris* L. cv. *Laurina*). The results indicate that increasing concentration of extract has a stimulating effect on seeds germination in comparison to the control. Extracts at a concentration of 3% and 10 % inhibited the growth of the majority of organs and caused reduction of their fresh and dry weight. Whereas the extract at a concentration of 5% stimulated plant growth.

Key words: *Juglans regia, Phaseolus vulgaris*, biometry of plants, fresh and dry weight, germination

*Department of Plant Physiology, Institute of Biology, Pedagogical University, Podchorążych St. 2, 30-084 Cracow, Poland; * kasiamozdzen@interia.pl*

Introduction

There is a strong correlation between the substances produced by plants and environmental factors in the allelopathic interactions. In case of deficiency of mineral components, high or low temperatures or attack of pathogens the formation of allelopathic substances is observed. Rate of their synthesis depends on the time and intensity of environmental factors and genetic factors of plants (Kołodziejczyk-Nieckuła 1994). The level of their toxicity is determined by processes of transport, transformation and retention in the soil, where they are permanently or periodically absorbed by soil colloids and humic compounds (Oleszek *et al.* 2001). These substances are then absorbed by roots and transported to the other organs of plant, where they fulfill regulatory functions at the cellular and tissue levels. The main sources of these substances are compounds released from decomposing plant biomass, especially in early spring, when the soil is poorly oxygenated (Wójcik-Wojtkowiak 1998).

The practical application of allelopathy has become the subject of many studies, for example of the impacts of weeds and pests on cultivated plants and methods to control them (Widera 1994; Azizi & Fuji 2006). This aspect has become especially important from the point of view of reduction the use of pesticides in agriculture and gardening, due to environmental degradation. Substances of plant origin on the basis of which will be possible to produce non-toxic and readily biodegradable natural herbicides, have received great attention in recent years (Sobótka 1997; Gniazdowska 2004).

The leaves of walnut (*Juglans regia* L.) contain compounds from the group of flavonols and kaempferol which have an allelopathic impact on the other plants (Zhang *et al.* 2008). In this aspect, the aim of this study was to investigate the influence of aqueous extracts of the leaves of walnut on the germination of seeds, growth and morphology of bean (*Phaseolus vulgaris* L. cv. *Laurina*).

Material and methods

The plant material were seeds of bean (*Ph. vulgaris* cv. *Laurina*) from the National Seed Central (POLAN) in Cracow and the leaves of

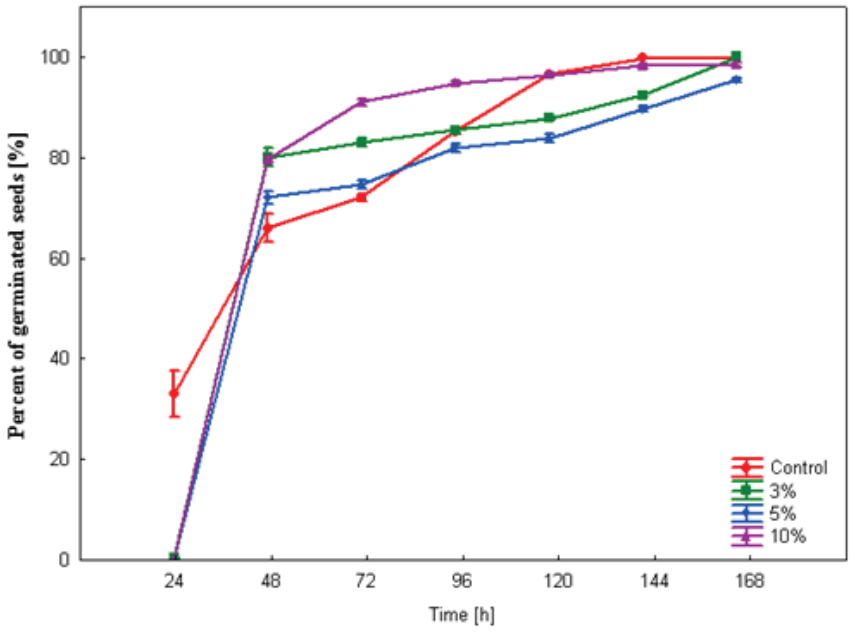

Fig. 1. The influence of the aqueous extracts of the leaves of *Juglans regia* on a percent of germinated seeds of *Phaseolus vulgaris* cv. *Laurina*.

walnut (*J. regia*) collected in the summer in the south-eastern Poland and then dried at room temperature.

In the first experiment, extracts at concentrations of 3, 5 and 10% were prepared. For this purpose 3, 5 and 10g of ground, dried leaves of walnut were weighed and drenched by distilled water. After 24 hours these extracts were filtered through Whatman's paper on a Buchner's funnel. Afterwards on the Petri's dishes, previously sterilized with filter paper and soaked by prepared extracts, one hundred seeds of *P. vulgaris* were placed. Thus prepared material was kept in darkness at 25°C for 7 days to determine an impact of extract on the energy and the strength of germination.

In the second experiment, changes in length, weight and water content in the organs of *P. vulgaris*, were determined which grown from seeds germinating on distilled water and which were watered with extracts during the growth. Germinated seeds were planted into pots filled with clean river sand and were placed in a greenhouse at the turn of July and August in 2014. Once a week, the plants were watered with standard medium and on the other days with aqueous extracts.

The statistical analysis was performed by parametric statistical test – ANOVA/MANOVA, using Duncan's test, at probability level $p \leq 0.05$, by means of Statistica for Windows 10.0.

Results

In the first day a greater percentage of germinated seeds were found in the control plants in comparison to the seeds on media saturated with extracts of walnut. After 48 hours a higher percentage of germinated seeds were observed on the Petri's dishes with extracts at concentration 3 and 10%, while the lowest in the control one. In the following days there was an increase in the number of germinated seeds in each of media with extracts. After 7 days the lowest percentage of germinated seeds was observed in case of seeds watered with extract at a concentration of 5%, in comparison to the other extracts and distilled water (Fig. 1).

Biometrical analysis of a root demonstrated significant growth inhibition of this examined

organ of plants, which were watered with extract at concentration of 3% in comparison to the control sample. There was no change in the length of the root in all other cases. The control plants have the longest hypocotyl of all other plants. The shortest hypocotyl have plants, which were watered with 3 and 10% extracts in comparison to 5% extract and control sample. In case of epicotyl a significant stimulation of its length was found in the specimens watered with a solution of 5% in comparison to control sample and the other concentration of solutions. The shortest epicotyl had the plants watered with extract of 3%. For extract of 10% no change in the length of epicotyl was observed in comparison to the control sample. The length of the remainder of the shoot was significantly stimulated by the extract of 5% and was inhibited by the extract of 10% in comparison to the other concentrations and control sample (Tab. 1).

The fresh weight of organs of *P. vulgaris*, which were watered with 5% extract of walnut leaves was greater than the fresh weight of plants organs in control sample. The smallest values of fresh weight of root and hypocotyl were recorded in the case of the plants watered by extract 10% while in the case of the petioles, the leaf blades and the remainder of the shoot in plants, which were watered with a solution at concentration of 3% (Tab. 2). The values of dry weight of plants in control and plants watered with extract 5% were similar. The extract of 5% caused decrease of the value of dry weight in comparison to control sample and to the other used extracts (Tab. 3).

The differences in percentage of water content in the organs of plants watered with extracts were statistically significant as compared to the control plants. In the case of the root the largest water content was found in the plants watered with extract of 10%. For the hypocotyl significant decrease of water content in plants watered with extract of walnut leaves at concentration of 5% was observed, compared to the control plants and the extract at concentration of 3%. In the epicotyl there was no difference in the values of the examined parameter. The leaf petioles had the largest water content at a concentration of 3% and the smallest at concentration of 10%. The smallest water content in the leaf blades was observed in the plants watered with extract of 10%, as compared to the other concentrations of the extracts and control sample (Tab. 4).

Discussion

According to Krupa (1970), germination is a complicated cycle of transformations, in which following the transition from the resting phase to the vegetative development takes place. During this process storage substances are decomposed to the simple available compounds, enzymes are activated and vitamins are synthesized. Starch, proteins and lipids are decomposed to the compounds, which are energy sources and substrates for the synthesis of new substances (Lewicki 2010).

The seeds of bean are non-endospermic seeds, which storage substances are accumulated in thickened cotyledons. They consist of embryo, storage tissue and seed coat. The seeds of bean germinated epigeally by growing up the cotyledons over the soil surface, which afterwards become green and serve as photosynthetic organs (Grzesiuk & Kulka 1981). The obtained results allow concluding that the aqueous extracts of the leaves of walnut inhibit germination of bean seeds on the first day and stimulate it for the next days. The most of seeds germinated on the media with the extract of 3% and the least with the extract of 5% in comparison to the control sample (Fig. 1).

The beginning of growth is a result of the end of germination and it's also the next phase of development. From the plants examined in this investigation, the longest organs had the specimens, watered with extracts at concentration of 5%. In contrast, the specimens watered with extract of 3% were definitely shorter in comparison with the control plants. The extract of leaves of walnut at concentration of 3% inhibited the growth of root, hypocotyl and epicotyl, while stimulated the remainder of the shoot (Tab. 1).

Tab. 1. The influence of the aqueous extracts of the leaves of *Juglans regia* on the length of the above-ground and underground organs of *Phaseolus vulgaris* cv. *Laurina*; the average values of 5 repetitions, which were marked (on the same line) by different letters, are significantly different by Duncan's test at probability level $p \leq 0.05$.

Organ	Extracts concentration [%]			
	0	3	5	10
	Length [cm]			
Root	19.82[a]	18.10[b]	19.78[a]	19.89[a]
Hypocotyl	6.43[a]	5.00[c]	5.85[b]	4.81[c]
Epycotyl	2.83[b]	2.36[c]	3.32[a]	2.83[b]
The remainder of the shoot	13.35[c]	14.55[b]	15.09[a]	12.64[d]

Tab. 2. The influence of the aqueous extracts of the leaves of *Juglans regia* on the fresh weight of the above-ground and underground organs of *Phaseolus vulgaris* cv. *Laurina*; the average values of 5 repetitions, which were marked (on the same line) by different letters, are significantly different by Duncan's test at probability level $p \leq 0.05$.

Organ	Extracts concentration [%]			
	0	3	5	10
	Fresh weight [g]			
Root	5.11[c]	5.62[b]	6.08[a]	4.44[d]
Hypocotyl	0,51[a]	0,30[c]	0,40[b]	0,25[d]
Epycotyl	0,16[a]	0,12[b]	0,16[a]	0,06[c]
Petioles	2.54[b]	1.87[d]	3.19[a]	1.94[c]
Leaf blades	0.16[a]	0.06[d]	0.09[b]	0.08[c]
The remainder of the shoot	2.09[b]	1.62[d]	2.58[a]	1.69[c]

Independently from concentration, the extracts used in this examination caused changes in the values of fresh and dry weight of *P. vulgaris* compared to the control plants. There was decrease of the fresh weight of plants at all concentration levels of prepared extracts in comparison to the control sample, except the fresh weight of the root, epicotyl and remainder of the shoot, when the plants was watered with extract of 5% (Tab. 2). The plants, watered with extracts of 3 and 10%, had slightly larger values of dry weight of root and smaller values of the other organs. Whereas the plants, which were watered with extract of 5%, had significantly larger values of dry weight of examined organs except of hypocotyl (Tab. 3). The largest changes in the water content in the examined organs of plants were found in plants watered with extract of 10%. In case of the other levels of concentrations, the values of this examined parameter were similar or slightly deviated from control values (Tab. 4).

According to WÓJCIK-WOJTKOWIAK *et al.* (1998) the reason of inhibiting the growth and weight reduction of the examined plant organs may be substances, which are present as neutral for other plants in above-grounds and only in the green parts of walnut. These substances are activated after a longer residence time in the soil, even to two meters deep (HARBORN 1997). The allelopathic substances of walnut cause disturbances in the functioning of cell membranes and as a result reducing the absorption of mineral substances. In addition, they have an inhibitory effect on the production of chlorophyll, photosynthetic activity of PSII and thus the deactivation of the photosynthesis process and the reduction of weight production (GNIAZDOWSKA *et al.* 2004).

Tab. 3. The influence of the aqueous extracts of the leaves of *Juglans regia* on the dry weight of the above-ground and underground organs of *Phaseolus vulgaris* cv. *Laurina*; the average values of 5 repetitions, which were marked (on the same line) by different letters, are significantly different by Duncan's test at probability level $p \leq 0.05$.

Organ	Extracts concentration [%]			
	0	3	5	10
	Dry weight [g]			
Root	0.57^{b}	0.58^{b}	0.70^{a}	0.42^{c}
Hypocotyl	0.17^{a}	0.11^{b}	0.09^{bc}	0.07^{c}
Epycotyl	0.05^{a}	0.04^{b}	0.05^{a}	0.03^{c}
Petioles	0.32^{b}	0.27^{c}	0.40^{a}	0.22^{d}
Leaf blades	0.04^{a}	0.01^{a}	0.05^{a}	0.01^{a}
The remainder of the shoot	0.51^{a}	0.16^{b}	0.59^{a}	0.18^{b}

Tab. 4. The influence of the aqueous extracts of the leaves of *Juglans regia* on the water content in the above-ground and underground organs of *Phaseolus vulgaris* cv. *Laurina*; the average values of 5 repetitions, which were marked (on the same line) by different letters, are significantly different by Duncan's test at probability level $p \leq 0.05$.

Organ	Extracts concentration [%]			
	0	3	5	10
	Water content [%]			
Root	88.93^{c}	89.72^{b}	88.46^{d}	90.62^{a}
Hypocotyl	32.92^{a}	36.28^{a}	23.84^{b}	27.91^{ab}
Epycotyl	33.19^{a}	30.97^{a}	32.49^{a}	31.24^{a}
Petioles	12.43^{b}	14.35^{a}	12.42^{b}	11.40^{c}
Leaf blades	23.55^{a}	17.61^{c}	20.77^{b}	11.23^{d}
The remainder of the shoot	24.17^{a}	9.81^{b}	22.96^{a}	10.59^{b}

The allelopathic active compounds belong to various chemical groups, what is the main reason for the problems in studying the mechanisms of allelopathic interactions. Moreover many plants secrete several active compounds, which can act comprehensively causing various changes in the morphology of plants.

Conclusion

The obtained results allow concluding that depending on the concentration of the extract and at which stage it's used, the allelopathic substances contained in aqueous extracts of the leaves *J. regia* have either stimulating or inhibiting effect on the germination and growth of *P. vulgaris*.

References

Azizi M., Fuji Y. 2006. Allelopathic effect of some medicinal plant substances on seed germination of *Amaranthus retroflexus* and *Portulaca oleraceae*. *Acta Hort.* **699**: 61–67.

Gniazdowska A., Oracz K., Bogatek R. 2004. Allelopatia-nowe interpretacje oddziaływań między roślinami. *Kosmos – Problemy Nauk Biologicznych* **52 (2)**: 207–217.

Grzesiuk S., Kulka K. 1981. Fizjologia i biochemia nasion. PWR i L.

Harborn J.B. 1997. Ekologia biochemiczna: 275–291. Wyd. PWN, Warszawa.

Kołodziejczyk-Nieckuła E. 1994. Allelopatia. *Wiedza i Życie* **10**: 28–32.

Krupa J. 1970. Rola światła w procesie kiełkowania nasion i zarodników. *Rocznik Naukowo Dydaktyczny WSP w Krakowie –Prace botaniczne II* **39**: 5–17.

Lewicki P. 2010. Kiełki nasion jako źródło cennych składników odżywczych. *ŻYWNOŚĆ. Nauka. Technologia. Jakość* **6 (73)**: 18–33.

OLESZEK W., GŁOWNIAK K., LESZCZYŃSKI B. 2001. Biochemiczne oddziaływania środowiska. Akademia Medyczna, Lublin.

SOBÓTKA W. 1997. Alleloherbicydy – wczoraj i dziś. *Postępy w ochronie roślin* **37 (1)**: 50–57.

WIDERA M. 1994. Możliwości wykorzystania zjawiska allelopatii w ochronie roślin. *Ochrona roślin* **38 (9)**: 15–16.

WÓJCIK-WOJTKOWIAK D., POLITYCKA B., WEYMAN-KACZMARKOWA W. 1998. Allelopatia. Wyd. AR, Poznań.

ZHANG H., GAO J.M., LIU W.T., TANG J.C., ZHANG X.C., JIN Z.G., XU Y.P., SHAO M.A. 2008. Allelopathic substances from walnut (*Juglans regia* L.) leaves. *Allelopathy J.* **21 (2)**: 425–431.