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Effect of chamomile and common agrimony extracts on biomass of pea roots in the cadmium-present environment

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Abstract The paper is focused on the evaluation of the allelopathic effect of *Matricaria chamomilla* L. and *Agrimonia eupatoria* L. shoot extracts of two concentrations (5 g.dm^{-3}; 10 g.dm^{-3}) on fresh (FW) and dry weight (DW) of pea roots. Effects of herbal extracts were tested also in roots exposed to cadmium (5 mg.dm^{-3}). The results of the experiments point to a significant effect of the herbal extracts themselves on the growth of pea roots, with the observed changes varied depending on the source of extract as well as on their concentration. The effect of the herbal extract was different in the conditions influenced by cadmium, depending on the tested extract and its concentration. Despite to the fact that the protective effect of plant extracts on the pea roots growing in an environment contaminated with Cd^{2+} ions was not confirmed, an interaction between the effects of plant extracts and cadmium ions on the examined growth parameters was recorded.

Key words chamomile, common agrimony, allelopathy, pea, cadmium

1. INTRODUCTION

Contamination of soil with heavy metals represents a serious environmental issue with risky impact on the health of living organisms including humans. During their lives, plants are, however, often subjected to multiple types of stress concurrently – various interactions between abiotic and biotic stressors arise. Heavy metals effects on plants are studied mainly in laboratory conditions often not taking into consideration the other factors of the environment. Specific effects of plant species on germination, growth and development of other species is called allelopathy and substances with the given effect secreted by a plant are named allelochemicals. Allelochemicals are also natural herbicides, and are produced also by crop plants. Allelochemicals include mainly alkaloids, phenols, terpenoids and glycosides (for instance benzoic acid, ferulic acid, juglone and others). Most of the allelochemicals exist in plant tissues in a non-active form, and through various chemical reactions (hydrolysis, oxidation-reduction reactions, methylation, dimethylation, etc.) compounds with specific allelopathic effects are formed (Whittaker and Fenny, 1971; Cheng and Cheng, 2015). The key factor determining the phytotoxic effect of allelochemicals is their concentration in soil water. However, similar to herbicides, in the soil environment these compounds are subjected to some retention, transformation and transport processes (Weidenhamer, 1996).

Allelopathic effects on plant germination and growth are caused by various mechanisms, including the decrease of the mitotic activity of cells in roots and shoots, the inhibition of hormonal and enzyme activity, the reduction of mineral intake, inhibition of photosynthesis and respiration, as well as the permeability of cell membranes (Gniazdowska and Bogatek, 2005; Scavo et al., 2019). The effects of allelochemicals are usually nonspecific: low concentration and short-term effect mostly stimulate all processes in plants, while at higher concentration and longer-term effect the life activity of plants is suppressed or even ceased.

Cadmium (Cd) is a non-essential element whose higher doses have negative effect on growth and metabolism of plants, and which can contaminate the different links of food chain. Significant factors that affect mobility and plant availability of cadmium in the soil comprise pH value, amount and quality of organic matter, redox potential and presence of other elements or substances. Besides the above-mentioned factors, the absorption of cadmium by plants is also affected by the species and variety of the plant, fertilization and way of cultivation (Makovníková et al., 2006). In majority of plants, cadmium is primarily accumulated in their roots, which at many plants lead to growth inhibition, changes in water regime, respiration and photosynthesis (Benavides et al., 2005).

There is a very little knowledge on the allelopathic effect of plants on other plants in the conditions of concurrent influence by heavy metals as well as on the effect of allelochemicals on the accumulation of metals in plants. Studies of Wang et al. (2018) and Wei et al. (2020) showed that heavy metals (Cu, Pb) promoted the invasion of some plant species (*Solidago canadensis*, *Erigeron annuus*, *Conyza canadensis*) and allelopathic phenomena were more severe in the presence of heavy metals. Increased accumulation of Cd by tissues of potato weed (*Galinsoga parviflora*) was observed at application of shoots of *Ramunculus sieboldii*, *Clinopodium confine*, *Mazus japonicus* and *Plantago asiatica* on the soil surface (Lin et al., 2014).

Extracts of medicinal plants are studied mainly in connection with their effects on human health, while little is known on their effects on other plants. The aim of the work was to verify the allelopathic effect of the extract of chamomile (*Matricaria chamomilla* L.) and common agrimony (*Agrimonia eupatoria* L.) on the content of root biomass of pea. The effects of the herbal extract were simultaneously tested also in plants exposed to cadmium.

2. MATERIALS AND METHODS

Seeds of pea (*Pisum sativum* L. cv. Gloriosa) were surface-sterilized for 5 minutes with sodium hypochlorite prior to germination. Water extract from dry matter of shoots of chamomile (*Matricaria chamomilla* L.) and common agrimony (*Agrimonia eupatoria* L.) were prepared as follows: 1 g of dry matter was flooded with 100 ml of distilled water (10 $g.dm^{-3}$) and was left to extract for 24 hours at 25 °C (Javaid et al., 2006). The extracts were filtered through a Whatman No. 1 filter paper. These extracts were further diluted to obtain solutions of concentrations 5 $g.dm^{-3}$. The sterilized seeds (22 seeds) were placed onto Petri dishes lined with double layer of filter paper and flooded with the individual extract of chamomile, common agrimony and cadmium solution (5 $mg.dm^{-3}$) according to the scheme presented below (Tab. 1).

Table 1 The scheme of the experiments

Variant of the experiment	Control samples	Samples with the extracts of medicinal plant and cadmium	
Extract of chamomile	Distilled water	Extract of chamomile (5 $g.dm^{-3}$)	Extract of chamomile (10 $g.dm^{-3}$)
Extract of chamomile and ions of Cd^{2+}	Extract of Cd^{2+} (5 $mg.dm^{-3}$)	Extract of chamomile (5 $g.dm^{-3}$) + Cd^{2+} (5 $mg.dm^{-3}$)	Extract of chamomile (10 $g.dm^{-3}$) + Cd^{2+} (5 $mg.dm^{-3}$)
Extract of common agrimony	Distilled water	Extract of common agrimony (5 $g.dm^{-3}$)	Extract of common agrimony (10 $g.dm^{-3}$)
Extract of common agrimony and ions of Cd^{2+}	Extract of Cd^{2+} (5 $mg.dm^{-3}$)	Extract of common agrimony (5 $g.dm^{-3}$) + Cd^{2+} (5 $mg.dm^{-3}$)	Extract of common agrimony (10 $g.dm^{-3}$) + Cd^{2+} (5 $mg.dm^{-3}$)

Cadmium was applied in the form of solution of $Cd(NO_3).4H_2O$. The volume of the distilled water and extracts applied in each variant of the experiment was 20 ml.

The seeds of pea were subsequently left to germinate in an incubator for 4 days (96 hours) at the temperature of 25 °C. After the 4 days, the fresh weight (FW) and the dry weight (DW) of the roots were determined. For each treatment, three replicates were carried out in a completely randomized design. Data for the examined parameters were statistically processed with software MS Excel 2010 and XLSTAT 2003. The differences between the data sets were determined through Student's t-test and Kruskal-Wallis' non-parametric test. The effects of herbal extract and heavy metal on the examined parameter were evaluated by two-way analysis of variance – ANOVA.

3. RESULTS AND DISCUSSION

Effects of medicinal plants (chamomile, common agrimony) extracts on fresh and dry weight of roots of pea at early stages of ontogenesis (4th day of germination) were evaluated. Two concentrations of extracts (5 and 10 $g.dm^{-3}$) were applied. The effect of the herbal extracts on the examined growth parameter was evaluated also along with concurrent influence of cadmium (5 $g.dm^{-3}$). The changes were evaluated compared to the control (water).

The weaker extract of common agrimony caused decrease in FW of pea roots by 21 %. In contrast, the applied concentration of cadmium showed stimulatory effect on root growth (increase in FW by 21 %). Considering the observed insignificant changes in growth of roots of faba bean caused by a wide concentration range of $Cd(NO_3).4H_2O$ (Piršelová and Ondrušková, 2021), we assume that the observed stimulatory effect is most likely a manifestation of so called hormesis (stimulation by low dose of metal) (Calabrese and Mattson, 2011; Piršelová et al., 2018).

Almost the same level of growth inhibition was caused by the stronger extract of common agrimony, and extracts of common agrimony with cadmium (Fig. 1). The weaker extract of chamomile had, on the contrary, a stimulatory effect (increase in FW by 13 %). Stimulatory effect (8 and 10 %) was observed also in variants where Cd solution and extracts of chamomile were applied simultaneously (Fig. 1).

With regard to the stimulatory effect of the weaker chamomile extract on FW, we assumed it to have a protective effect in the cadmium contaminated environment. The cadmium solution alone at the applied concentration, however, did not have a toxic effect on the germinating plants (a stimulation of growth was actually observed); thus the protective effect of the herbal extract was not shown, and, on the contrary, growth inhibition appeared. The results of the two-way analysis of variance (ANOVA) pointed to the fact that the observed inhibition rate was affected by the applied cadmium solution and the interaction between the effects of cadmium and chamomile extract ($p < 0.05$). The effect of the chamomile extract alone on the changes of FW of roots was not proven (Tab. 2). In the case of common agrimony, Cd alone did not have any effect on the biomass of pea roots (Tab. 3). To similar conclusion came, in their study, Yadav and Singh (2013) who observed lower germination of wheat seeds as well as lower content of photosynthetic pigments due to the effect of benzoic acid, but not due to the effect of Cd (7 $mg.dm^{-3}$). An interaction between the effects of Cd and benzoic acid was also observed.

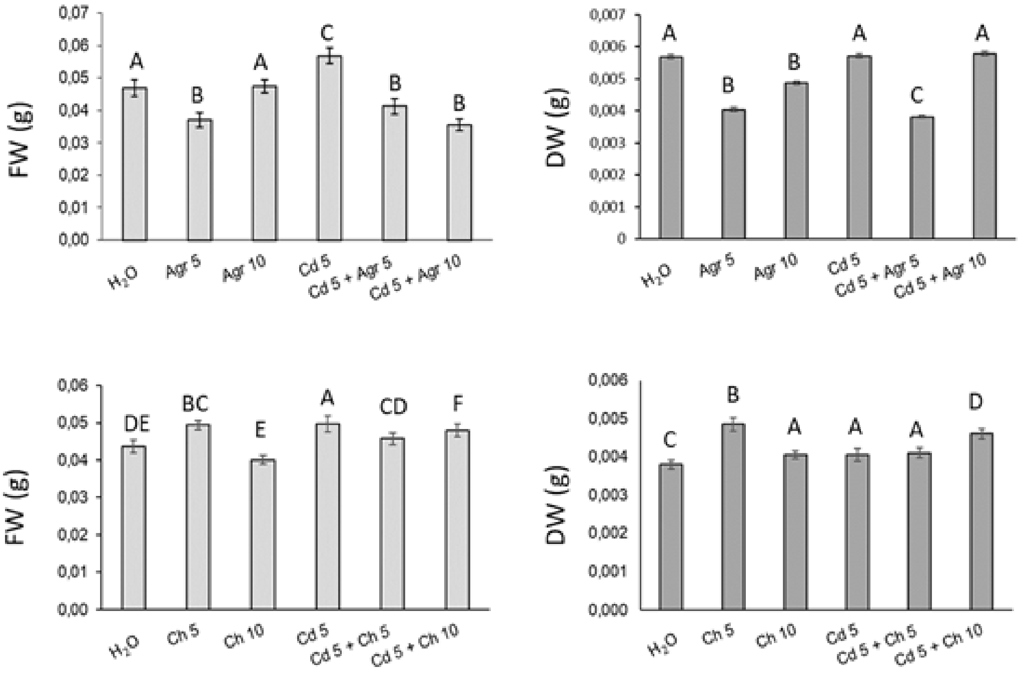

Figure 1. Effect of extracts of common agrimony (Agr), chamomile (Ch), cadmium (Cd) and their combination on fresh (FW) and dry weight (DW) of pea roots. The values represent the arithmetic mean ± standard error. Different letters indicate significant differences between samples at $p < 0.05$. The applied dose of herbal extract is expressed in $g.dm^{-3}$ solution and Cd in $mg.dm^{-3}$.

Table 2 Two-way ANOVA results of the effect of chamomile (Ch) extract, cadmium (Cd) and their interaction on the fresh weight of pea roots.

Source of variation	*Mean square*	*Degrees of freedom*	*F ratio*	*P value*
Ch extract	0.000548	2	2.696709	0.0696
Cd	0.000796	1	7.8314819	P < 0.05 *
Interaction	0.00157	2	7.7222871	P < 0.05 *

** Significance at p < 0.05*

In the case of application of common agrimony, two-way analysis of variance (ANOVA) confirmed again that inhibition rate in the case of variant with cadmium is a result of interaction between the effects of cadmium and common agrimony extract. The herbal extract alone also affects the above-mentioned changes (Tab. 3).

Dry matter content of common agrimony roots decreased (by 29 % and 14 %) due to the effect of weaker and stronger extracts of common agrimony (Fig. 1). The tested dose of cadmium did not affect the dry matter content, however the simultaneous application of cadmium and weaker extract of common agrimony resulted in decrease in dry matter content by 33 %. In contrast to the FW, simultaneous application of cadmium and stronger extract of common agrimony did not result in statistically significant changes in dry matter content (Fig. 1). Nevertheless, in contrast to FW, the stronger extract of chamomile caused a statistically significant increase in DW content by 7 % (Fig. 1).

Table 3 Two-way ANOVA results of the effect of common agrimony extract (Agr), cadmium (Cd) and their interaction on the fresh weight of pea roots.

Source of variation	*Mean square*	*Degrees of freedom*	*F ratio*	*P value*
Agr extract	0.005371	2	15.9759	P < 0.05 *
Cd	6.68E-05	1	0.397651	0.52910
Interaction	0.003932	2	11.69726	P < 0.05 *

** Significance at p < 0.05*

Effects of plants' water extracts on germination and growth parameters of plants have been examined by several authors, with most of them observing their inhibitory effect. Alvarez-Inglesias et al. (2014) examined for example phytotoxic potential of water extract of faba bean (*Vicia faba* L.) seeds on weeds: *Amaranthus retroflexus*, *Echinochloa crus-galli* and *Digitaria sanguinalis*. An inhibitory effect of rape (*Brassica napus*) water extract on the growth of *Phalus minor* (Retz.), *Convolvulus avensis* (L.) and *Sorghum halepense* (L.) was described also by Aliki et al. (2014). The inhibitory effects varied depending on the concentration of the applied extract.

4. CONCLUSIONS

Results of the experiments pointed out a significant effect of the herbal extracts alone on the growth of pea roots, with the given changes being dependent on the source of herbal extract as well as on the concentration of the extract. An interesting finding is the fact that, while the herbal extract alone had, in some variants of the experiment, an inhibitory effect on the roots' biomass content, the simultaneous application of the extract with the cadmium lead, on the contrary, to growth stimulation. Despite to the fact that the protective effect of plant extracts on the pea roots growing in an environment contaminated with Cd^{2+} ions was not confirmed, an interaction between the effects of plant extracts and cadmium ions on the examined growth parameters was recorded, which suggests the need to exploring the plants tolerance to heavy metals in broader contexts.

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