

SHORT COMMUNICATION

Effects of walnut phenolics on germination of dandelion seeds

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ABSTRACT

The effects of walnut phenolics were determined on the seeds germination of dandelion. The walnut methanolic extract contained eight phenolic compounds: catechin, chlorogenic acid, *p*- and *o*-coumaric acids, ferulic acid, tannic acid, caffeic acid and syringic acid. The catechin was the major phenolic compound in all the organs of walnut. The application of walnut extracts in the soil, reduced the seeds germination of dandelion (*Taraxacum officinale* Web). While comparing the effects of extract on other monocotyledonous and dicotyledonous plants, the germination of wheat and bluebottle seeds was slightly inhibited, but that of corn poppy and red deadnettle seeds was drastically inhibited than dandelion seeds. Moreover some identified phenolics present in walnut tissues, also reduced the germination of dandelion seeds and the catechin was most strongest inhibitor. Possible role of walnut phenolics as allelopathic agents against dandelion is discussed.

Keywords: Allelochemicals, bluebottle, *Centaurea cyanus*, corn poppy, dandelion, *Lamium purpureum*, *Papaver rhoeas*, red deadnettle, seed germination, *Taraxacum officinale*, *Triticum aestivum*, walnut phenolics, winter wheat

INTRODUCTION

Dandelion is common weed in Poland in agricultural fields, grass lands, orchards, parks and home gardens. As there is a strong regulation of herbicides use, hence, the environmental friendly allelopathic methods of weed control are needed (3,19). The allelopathic action of walnut (*Juglans regia* L.) to higher plants is well documented (6,11), but still little is known about its mechanism and possible uses to control weeds. The juglone is major walnut naphthoquinone and is allelopathic to various plants (23,24). The other bioactive substances (phenolics, diarylheptanoids, α -tetralone derivatives and/or terpenoids) present in walnut had been less studied (5,10). However, they participate in allelopathic interactions with other higher plants during the growing season. Recently the antibacterial and antioxidant potential of walnut has been proved (2,12,15,16). The environmental significance of walnut allelochemicals considers the effects of (i). root exudates, (ii). fallen walnut leaves and/or (iii). broken husks on the germination of surrounding plants. Thus it seems important to determine the content, chemical composition and allelopathic potential of walnut allelochemicals.

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This study aimed to determine (i). the phenolic composition of tissues of walnut roots, fallen leaves and broken husks, (ii). effects of methanolic extracts of walnut leaves on seeds germination of dandelion (*Taraxacum officinale* Web), wheat (*Triticum aestivum* L.), bluebottle (*Centaurea cyanus* L.), corn poppy (*Papaver rhoeas* L.) and red deadnettle (*Lamium purpureum* L.) and (iii). the effects of (+)-catechin, caffeic and chlorogenic acids on the seeds germination of dandelion.

MATERIAL AND METHODS

The fallen leaves, broken husks and roots of walnut were collected from the home gardens in Siedlce, Poland, during the second half of October, 2008. The collected plant material was placed in dry ice and immediately freeze dried. The lyophilized tissues were then powdered, placed in dark glass bottles and kept at 0-4°C until the analysis.

Extraction and determination of walnut phenolics

1g of the freeze dried walnut leaves, husks or roots were shaken with 100 ml of 80% methanol for 1 h and the slurry was filtered through Whatman 4 filter paper and then centrifuged at 5 000 rpm for 15 min. The supernatant was evaporated on a rotary evaporator until the water phase and then extracted three times with equal volumes of ethyl acetate. The ethyl acetate fractions were combined and evaporated at 30°C on a rotary evaporator until dryness. Then the dry residue was dissolved in 2 ml of 80% methanol and filtered through Supelco 0.45µm filter system. The phenolic extracts thus obtained were separated using the HPLC Varian System equipped with a Microsorb C18 column and UV detector operated at 300 nm and fluorescence detector. The separation was run in isocratic conditions and elution of studied compounds was performed with methanol-distilled water mixture (25:75; v/v) to which 1% acetic acid was added. The walnut phenolics were identified and quantified based on the retention time and as per International standards. All analyses were done in three replicates and content of identified compounds was expressed as an average molar concentration per gram of freeze dried material.

Effect of walnut extracts on germination of tested plant seeds

Influence of walnut on germination of dandelion seeds was examined using the crude extract obtained from the leaf tissues of walnut. Twenty five dandelion seeds previously soaked for 12 h in tested crude extract were placed in Petri dishes (15 cm dia) filled with the Whatman 4 filter paper and then were treated with 15 ml of 4% methanol walnut extract (the crude methanol extract was evaporated to the water phase and made-up with methanol to 4% final solution). During the bioassays, the control seeds were prepared as well and then were treated with 4% methanol solution in the same way. Similar seeds germination tests were also done with winter wheat cv. Tonacja, bluebottle corn poppy and red deadnettle. All assays were done in four independent replicates (four Petri dishes contained 25 seeds each). The germinated seeds were placed in environmental cabinet (23°C and 16h light/8h dark) for 8 days and the numbers of dandelion seedlings were monitored daily.

Effect of selected phenolics on seeds germination

The effects of four identified phenolics (*p*-coumaric, caffeic and chlorogenic acids and catechin) were also studied on the seeds germination of dandelion. The tested compounds were dissolved in 4% methanol and 15 ml solutions were added per Petri dish, prepared as described previously. All the tested phenolics were applied at 3 concentrations (0,01 mM, 0,1 mM and 1 mM) and the seeds germination was recorded daily. The data was expressed as seeds germinated (%) after 8 days over the control (100% germination). All the bioassays were run in four independent replications for each treatment.

RESULTS AND DISCUSSION

Field observations revealed the absence of dandelion plants in the vicinity of walnut trees. The extracts from walnut fallen leaves, broken husks and roots contained 8 phenolics (-catechin, chlorogenic acid, *p*- and *o*-coumaric acids, ferulic acid, tannic acid, caffeic acid and syringic acid), however, the catechin was major compound (Table 1). These phenolic acids were present in very less amounts and their concentrations varied with the plant organs. The fallen leaves were slightly rich in *o*-coumaric acid and chlorogenic acids, the broken husks were rich in caffeic and syringic acids and roots had higher concentration of syringic and chlorogenic acids. Interestingly, the *o*-coumaric acid was absent in roots (Table 1).

Table 1. The Contents ($\mu\text{M/g}$ of freeze dry weight) of identified phenolic compounds in walnut plant parts.

Phenolic compound	Leaves	Husks	Roots
Catechin	44.3 \pm 0.6 a	25.0 \pm 0.3 a	69.3 \pm 0.5 a
Chlorogenic acid	2.0 \pm 0.2 c	1.1 \pm 0.1 e	2.7 \pm 0.1 c
<i>p</i> -Coumaric acid	1.2 \pm 0.08 d	1.2 \pm 0.06 e	1.3 \pm 0.05 d
<i>o</i> -Coumaric acid	3.7 \pm 0.2 b	1.8 \pm 0.1 d	Not detected
Ferulic acid	1.0 \pm 0.02 de	1.0 \pm 0.01 e	0.6 \pm 0.02 e
Tannic acid	0.5 \pm 0.01 e	1.0 \pm 0.03 e	1.6 \pm 0.05 d
Caffeic acid	1.0 \pm 0.01 de	6.0 \pm 0.1 b	1.7 \pm 0.02 d
Syringic acid	1.5 \pm 0.01 cd	4.0 \pm 0.2 c	5.0 \pm 0.1 b

Values in columns not followed by the same letters are significantly different at 0.01 level (Duncan's test)

The walnut leaf extracts inhibited the seeds germination of dandelion by 55% over the non-treated control (Fig. 1). The walnut phenolics caused least inhibition (14.6%) in seeds germination of winter wheat cv. Tonacja, 42.1% inhibition in bluebottle seeds and 89.1% in seeds of corn poppy, while germination of red deadnettle seeds was completely inhibited (Fig. 1).

The tested phenolics also reduced the seeds germination of dandelion, especially at higher concentrations and catechin was the strongest inhibitor (Fig. 2). The phenylpropanoids at 1mM concentration completely inhibited the germination of dandelion seeds, while its 0.01mM concentration, showed slight stimulation (117%). The chlorogenic acid reduced the germination at all studied concentrations and the germination

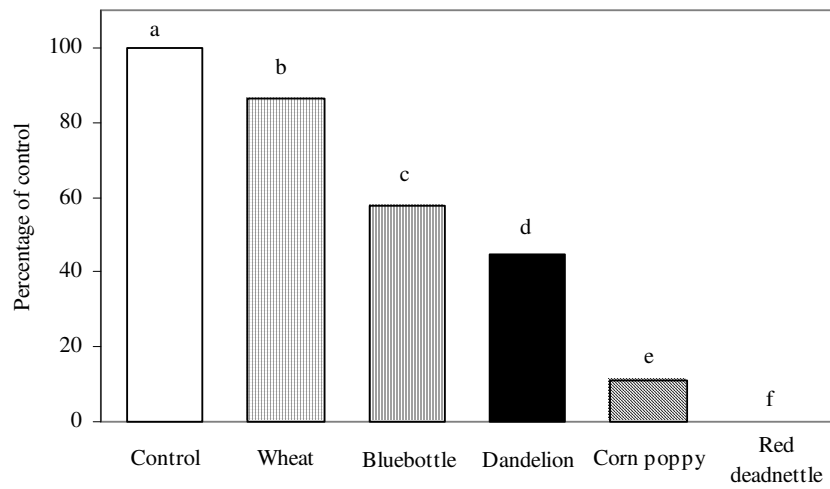


Figure 1. Effects of walnut leaves on seed germination (%) of test plants 8 days after sowing. Values not followed by the same letters are significantly different at 0.01 level (Duncan's test)

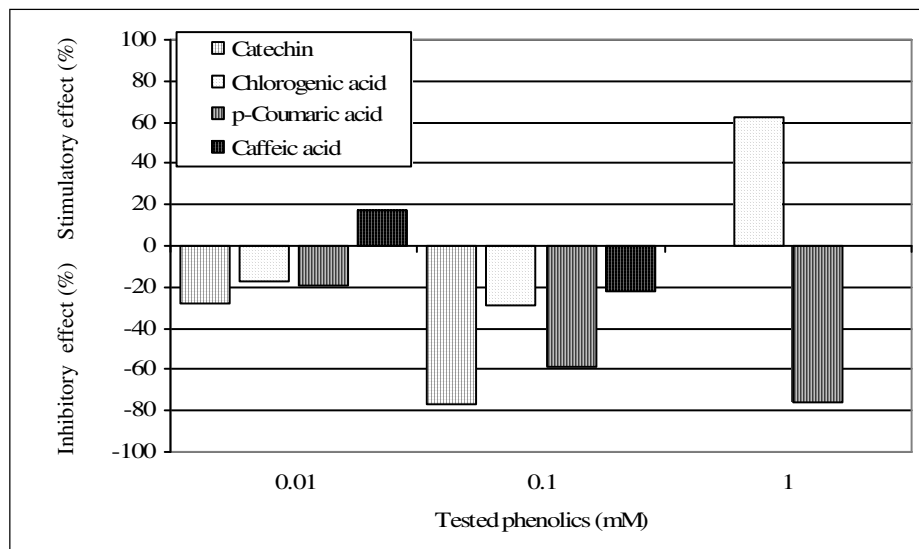


Figure 2. Inhibitory/stimulatory effects of phenolic compounds on seeds germination of dandelion at various concentrations.

was concentration dependent. The *p*-coumaric acid also decreased the seeds germination of dandelion at all studied concentrations but it was more inhibitory than chlorogenic acid (Fig. 2).

The phenolics present in walnut extracts reduced the germination not only of dandelion seeds but also of other tested plant species. The walnut extracts contained a variety of the phenolic compounds, including soluble and condensed tannin-like compounds, phenolpropanoids and phenolic acids. On the other hand, some quercetin glycosides and quinic acid derivatives were also found in tissues of walnut leaves collected in May (15). It is likely that they were also present in the studied plant material but were not detected, owing to the different extraction conditions, HPLC separation and detection methods used. Generally, the selected phenolic compounds inhibited the seeds germination of dandelion. On the other hand phenolics are also allelopathic to other plant spp. (1,13,20,21,22).

In field conditions, the phenolic compounds exerted an allelopathic effect not only towards major weeds (e.g. corn cockle and wild oat) but also against crops (4,9,18). Our results also showed slight reduction in germination of winter wheat seeds. However, the seeds germination of weeds (dandelion, bluebottle, corn poppy and red deadnettle) was drastically inhibited. The phenolic compounds released from the decomposed plant residues in the soil act as allelopathic agents (7,14,25). However, their effects are often modified by the soil microbes, which changes the chemical properties and phytotoxicity of plant allelochemicals (9,17,18). Usually the plant phenolics are active in the soil, even for over two months after the application of plant residues (17). The plant residues are usually decomposed slowly, hence bioactive phenolic compounds might be released slowly into the soil for a certain period. The process is often slowed down by low temperatures during the fall and winter. Hence, the higher concentration of phenolics in the soil might be prolonged and exerts a stronger effect on the seed germination (17). Thus, the absence of dandelion plants in neighborhood of walnut trees might be related to the exudation of phenolic compounds into the soil. On the other hand, it has been suggested that walnut allelopathic agents might be present in the soil around walnut trees (6). For example, the juglone was found present in the surface and deeper layers of soil, several meters around walnut trees and its phytotoxicity was proved.

CONCLUSIONS

The walnut phenolics might be useful allelopathic agents to control various weeds, including the dandelion. Their effects might be even stronger due to (i) the presence of juglone and other naphthoquinone derivatives in the walnut tissues (24) and (ii) possible synergistic allelopathic effects. Therefore further allelopathic studies of walnut compounds and possible application of fallen leaves and/or broken husks in ecological agriculture are required.

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