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RESEARCH ARTICLE

Allelopathic activity and chemical composition of rapeseed extracts on Jungle rice

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Abstract

Jungle rice is one of the most serious grass weeds of maize in Egypt. Laboratory experiments were conducted to evaluate the allelopathic effects of extracts of rapeseed on the germination and seedling growth of jungle rice (*Echiochloa colona*). The inhibitory effect of rapeseed extracts on plant growth, using jungle rice as a bioassay material was investigated to test different variants of biological activity of rapeseed aerial parts, five extracts; in three solvents methanol, chloroform and water extraction respectively.Analysis of chemical composition of methanolic extract of rapeseed aerial parts using HPLC. chloroformic extract induced slight inhibition of the growth, whereas methanolic extracts .

Introduction

The phenomenon of allelopathy, where a plant species chemically interferes with the germination, growth or development of other plant species has been known for over 2000 years. Statements as early as 300 years BC points to the phenomenon that many crop plants, including chick pea (*Cicerarietinum*) and barley (*Hordeumvulgare*), inhibit the growth of weeds and crop plants other than barley (Rice, 1984). Allelopathic interactions between plants have been studied in both managed and natural ecosystems. In agricultural systems allelopathy can be part of the interactions between cropped plants and between crops and weeds. Therefore this phenomenon affect the economical outcome of the plant production. Both crop and weed species with allelopathic activity are known (e.g. Inderjit and Dakshini 1998, Inderjit and Foy 1999, Putnam and Weston 1985, Weston 1996). It has been shown that allelopathy can be used for pest, weeds, insects, nematodes and pathogens management. Allelopathy is one of the potential areas of research resulted in control of weed growth and development. Allelopathic crops, when used as cover crops, mulch, smother crops, green manures, or grown in rotational sequences, are helpful in reducing noxious weeds and plant pathogens, that leading to improve the quality of soil and crop yield.

Another research area within allelopathy is the search and development of new herbicides on the way of isolation, purification and identification of active compounds from allelopathic plants (Duke 1998, Macias *et al.*, 1997, Macias *et al*., 1998a, 1998b). These compounds are often referred as natural herbicides. For example the phenolic acids beta-phenyl-lactic acid (PLA) and betahydroxybutyric acid (BHA) had been identified as allelochemicals in water extracts of rye residues (Narwal 1996). The two hydroxamic acids DIBOA (2,4-dihydroxy-1,4(2H)- benzoxazin-3-one) and BOA (2(3H)-benzoxazolinone) had also been isolated from root and shoot tissue of rye and were found to be toxic to many weed species (Barnes and Putnam 1986, 1987). Rye root exudates containing hydroxamates also inhibited the development of a wild oat, *Avena fatua*, in a seed germination test (Pérez &Ormemeño-Núñez 1991). The two alkaloids, gramine (N,N-dimethyl-3-amino-methylindole) and hordenine (N,N dimethyltyramine) have been confirmed to play an important role in the phytotoxic ability of barley (Lovett and Hoult1995, Overland 1966).

From the agronomic point of view, the research in allelopathy provides perspectives of a reduced reliance only on traditional herbicides – chemical substances, bearing in mind that weed control can be also effectively obtained due to release of allelochemicals from the crop. Moreover also in cropping systems, where chemical herbicides are not used, for example in organic farming, crop cultivars with enhanced allelopathic activity could be the part of the weed management strategy. This strategy, however, needs to be supported by the fundamental knowledge about allelochemicals involved in allelopathy, which have to be isolated in an amount adequate for identification and for further characterisation in bioassays.

Rapeseed is used frequently as a prior crop to small grains in conventional or organic agriculture, based on careful soil preparation, thus the influence of the residues of this crop may influence the growth and development of next one in sequence. Reduced growth of crops and weeds is often reported following addition of *Brassica* residues to soil or following *Brassica* spp. (Golami *et al,* 1996; Khatib *et al,* 1997). Similar results were obtained by Younesabadi (2005), who reported that *A.retroflexus* and *A.theopherasti* germination was reduced through rapeseed treatment but cotton germination had no change. Independent on such information, our knowledge about the allelochemical potency of rapeseed still remains fragmentary, therefore the aim of this project was to evaluate the influence of the extracts from aerial parts of *Brassica napus* (canola) var saur4, on the germination and growth of model plant *Echinochloa colona* . The preliminary study on chemical composition of these extracts were also performed using two chromatographic methods: the thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC).

Whole project was carried out at the Faculty of Chemistry, Opole University in the frame of international agreement between Polish and Egypt governments in the area of scientific activity and exchange.

MATERIALS AND METHODS

Experiments were carried out during September 2013 in the Department of Analytical and Ecological Chemistry, Faculty of Chemistry, Opole University, Poland.

Plant material

Brassica napus (canola) var saur4 was obtained from Egypt. Jungle rice seeds were collected from maize fields in Egypt. The plants were randomly taken from field in the boom stage. Then the seeds were isolated, dried and powdered. Powdered seeds were the basic material for description of alleloptahic activity.

Extractions

To test different variants of biological activity of rapeseed aerial parts, five extracts; in three solvents and two ways of extraction, were prepared as follows: five samples, each of the 15 grams weight of aerial parts of rapeseed plant Canola (*Brassica napus*) were treated in various extraction procedures. Three samples were located in Soxhlet apparatuses and extracted for four hours with methanol, chloroform and water extraction respectively. Two samples of plants were located in glass flasks and extracted with methanol and chloroform extraction in ultrasonic bath of temperature 25 C. When the process of extraction had been finished, all extracts were evaporated to dryness and diluted with 50 ml of distilled, sterile water to prepare the stock solutions.

Tests on allelopathic activity of extracts towards *E. colona* as the model plant

In order to perform this test, the influence of each from among five various extracts was tested in separated set. Each set consisted of three batches (repetitions) of twenty five (25) seeds of jungle rice, which were placed on sterile filter paper in previously sterilized 9 cm Petri dishes. Petri dishes were incubated in the dark, in conditions of stable 25 C temperature.

The influence of extracts on germination of the seeds of E. colona

The study on the impact of obtained extracts on germination of jungle rice seeds started simultaneously with the incubation of seeds. Each extract in the form of stock solution was diluted ten and hundred times, and the volumes of 10 ml each of such dilutions were uniformly applied on the surface of wet filter paper located on the bottom of sterile Petri dish, then the seeds were placed with the attention. Control was prepared on the same way, of course in this case sterile, distilled water has been used instead the diluted extract. Each experiment was arranged in triplicate. After 48 hours the number of germinated seeds was recorded in each dish, and the average number in each set was calculated.

The influence of extracts on growth of plants of E. colona

After germination of the seeds, when the seedlings possessed at least three millimeter long shoots and roots, Petri dishes were transported into the growth chamber, where the photoperiod was as follows: light 14 h and dark 10 h and the controlled temperature of 27^{o}C. Immediately after 10 ml volumes of the described dilutions of extracts were uniformly applied to dishes, with attention to avoid the direct application on the plants. Each experiment (dilution of the extract) was carried in three repetitions, same as the control with sterile distilled water (10 ml) instead the extract dilution. After one week of incubation, at least 15 randomly chosen plants from each repetition were harvested and weighted. The average fresh masses were then calculated.

Analysis of chemical composition of methanolic extract of rapeseed aerial parts using HPLC

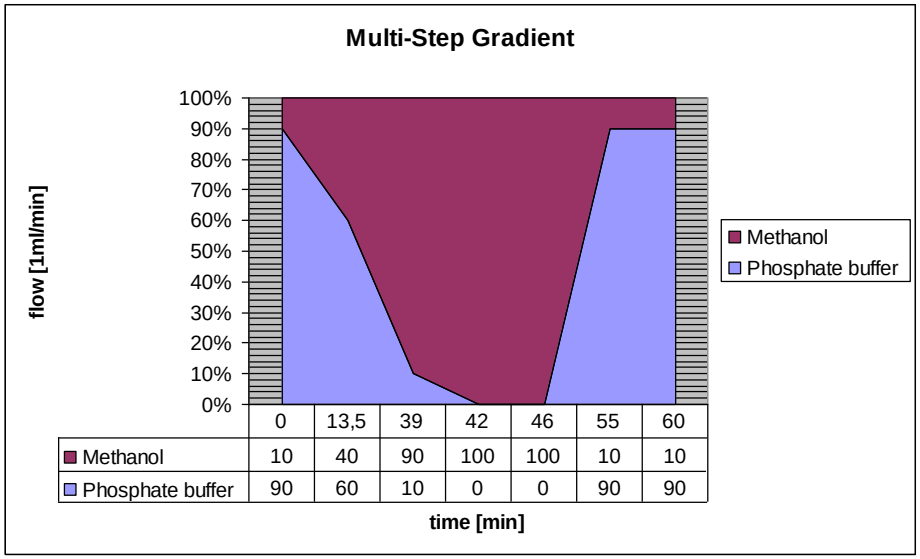
High-performance liquid chromatography specification:DionexUltiMate 3000 with Photodiode Array Detector was used as chromatograph

*Column:*Gemini 5u C18 110A; 250x4.60mm 5 µm

Mobile phase: A-Phosphate buffer 10mM: 2.6807g of $Na_2HPO_4*7H_2O$ was dissolved in 1 dm^3 of MQ water, pH was range to 2.5, using 85% H_3PO_4. *B- Methanol:*Chromasolv ® gradient grade for HPLC ≥99.%, bought in SIGMA-ALDRICH.

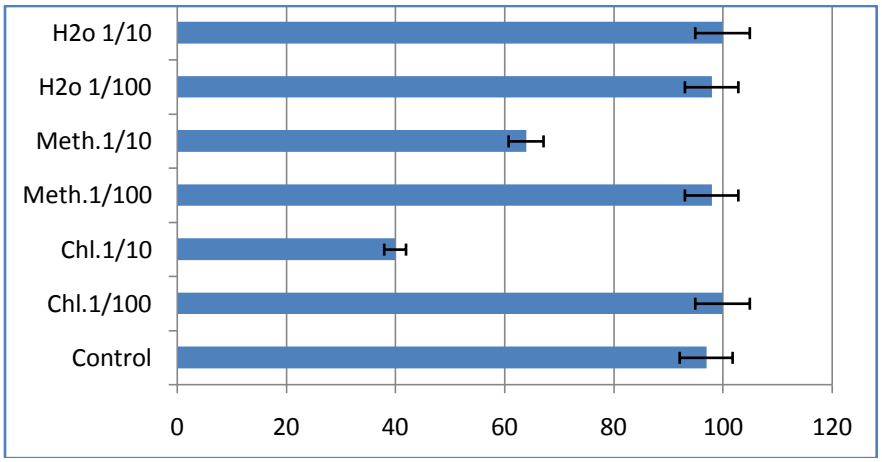
Separation of compounds was held in gradient: Flow= 1 ml/min

RESULTS AND DISCUSSION



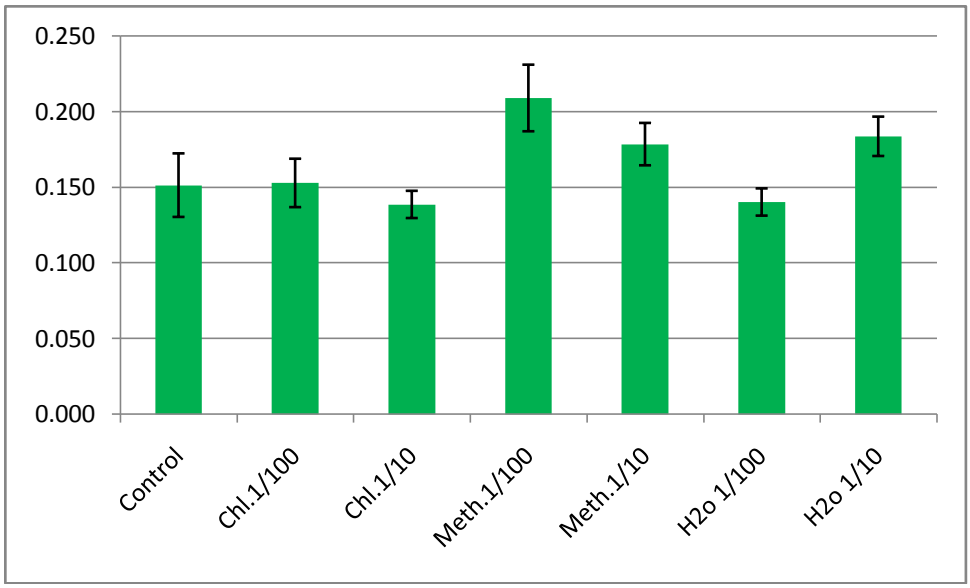
The results obtained during the study on the influence of extracts on germination of jungle rice plants (Fig. 1) indicate that only ten times diluted stock solutions of mixture of substances extracted in organic solvents; chloroform and methanol, significantly inhibited the germination of tested plants.

Figure 1: The average percentage germination of *E. colona* seeds in dependence on the influence of rapeseed extract in appropriate solvent

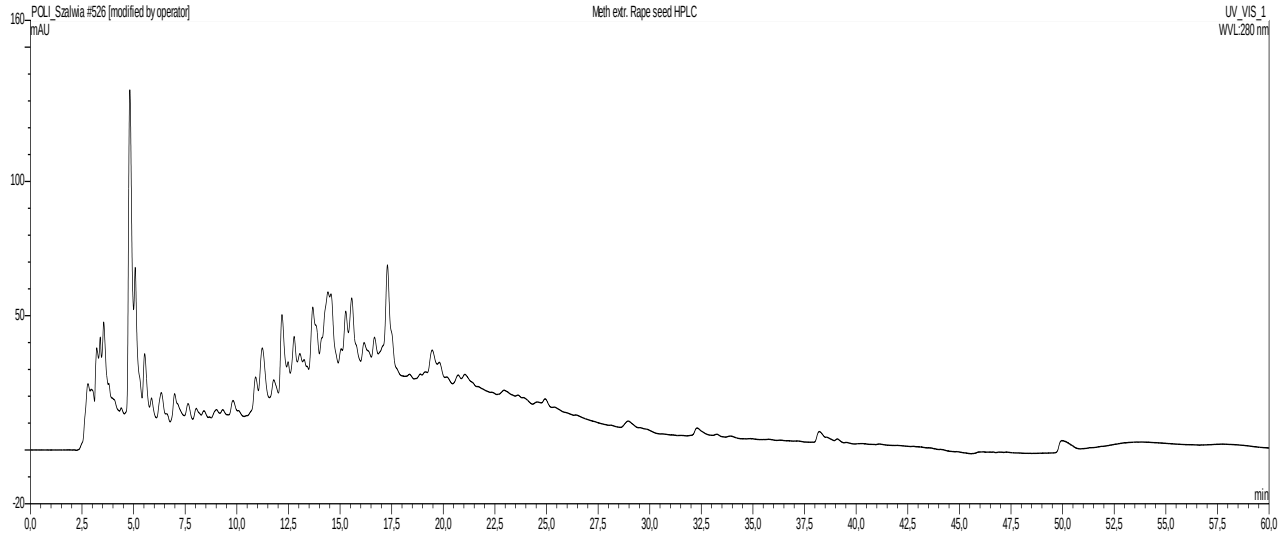


Regarding the results of the study on the influence of the rapeseed extracts on the growth of model plants, it can be stated that ten times diluted chloroformic extract induced slight inhibition of the growth, whereas methanolic extracts as well as more concentrated water extract significantly stimulated the fresh mass of tested plants (Fig.2).

Figure 2: The average fresh weight of *E. colona* seeds in dependence on the influence of rapeseed extract in appropriate solvent



Considering, however the basic statistical analysis it seems to be clear that only more diluted rapeseed extract in methanol significantly influenced the growth of tested plant by its stimulation.
Preliminary results of HPLC analysis of this extract proved its complex chemical composition, as the mixture of many substances, as it can be seen on the chromatogram attached below:



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