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Bioadsorption of lead(II) and cadmium(II) ions onto the lyophilized cell surface of *Pseudomonas aeruginosa* in aqueous suspension

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Abstract. Biosorption of Cd(II) and Pb(II) ions from aqueous solution using lyophilized *Pseudomonas aeruginosa* (PAOI) cells was studied under various experimental conditions. The effect of pH, initial metal concentration and adsorption time on bioadsorption was investigated. The optimum pH value range for Cd(II) adsorption was found to be 4.0-7.0, and for Pb(II) it was 4.0-5.0. Pb(II) and Cd(II) bioadsorption equilibrium was analyzed by using Langmuir model. The maximum uptake capacity of Pb(II) and Cd(II) was estimated to be 164 mg g^{-1} and 132 mg g^{-1}, respectively.

Keywords: heavy metals, biosorption, *Pseudomonas aeruginosa*, pH, kinetics, isothermh

1 Introduction

Heavy metal pollution is one of the most important environmental problems today. In recent years, applying biotechnology in controlling and removing metal pollution has been paid much attention, and gradually becomes hot topic in the field of metal pollution control because of its potential application. Alternative process is biosorption, which utilizes various certain natural materials of biological origin, including bacteria, fungi, yeast and algae. These biosorbents possess metal-sequestering property and can be used to decrease the concentration of heavy metal ions out of dilute complex solutions with high efficiency and quickly, therefore it is an ideal candidate for the treatment of high volume and low concentration complex wastewaters. Therefore researches on biosorption have become an active field for the removal of metal ions or organic compounds [1, 2].

The capability of some living microorganisms to accumulate metallic elements have been observed at first from toxicology point of view. However, inactive/dead microbial biomass can also passively bind metal ions via various physicochemical mechanisms. Mechanisms responsible for biosorption, although understood to a limited extent, may be one or combination of ion exchange, complexation, coordination, adsorption, electrostatic interaction, chelation and microprecipitation [1, 3]. The uptake of heavy metals by biomass is usually classified into three categories: (1) cell surface binding, (2) intracellular accumulation and (3) extracellular accumulation [1, 4]. Being metabolism-independent, the cell surface binding can occur in either living or inactivated microorganisms, whereas the intracellular and extracellular accumulation of metals are usually energy-driven processes, and thus can take place in living cells [5].

Several investigations have reported that *Pseudomonas aeruginosa* displays efficiency for metal uptake [6, 7, 8]. Chang and Chen studied the biosorption of cooper(II), lead(II) and cadmium(II) ions on *P. aeruginosa* and the multi-metal adsorption results showed that lead and cooper significantly inhibited the adsorption of cadmium, while the effects of cadmium on the adsorption of cooper and lead were limited [9]. They also reported the combined effects of two or more metal ions on inactivated *P. aeruginosa* depend on the number of the metals competing for binding sites, metal combination, levels of metal ion concentration, order of metal addition, and residence time [9], [10]. Leung et al. selected *Pseudomonas* as biosorbent for lead, cooper and nickel, among 12 bacteria isolated from activated sludge. They reported the maximum sorption capacity 271.7 and 46.8 mg g^{-1} for lead(II) and copper(II) ions, respectively

[9, 10]. Kang et al. the Cr(III) and Cr(VI) biosorption studied onto the cell surface of *P. aeruginosa*. Batch experiments were conducted with various initial concentrations of chromium ions to obtain the sorption capacity and isotherm. It was found that the sorption isotherm of *P. aeruginosa* for Cr(III) was described well by Langmuir isotherm model, while Cr(VI) appeared to fit Freundlich model. The results of FT-IR analysis suggested that the chromium binding sites on the bacterial cell surface were most likely carboxyl and amine groups. The bacterial surface of *P. aeruginosa* seemed to engage in reductive and adsorptive reactions with respect to Cr(VI) biosorption [11].

In this study the biosorption characteristics of lyophilized *Pseudomonas aeruginosa* bacterial cells for cadmium(II) and lead(II) ions in aqueous suspension are being presented. The effect of pH, contact time and initial heavy-metal concentration on biosorption were investigated for both heavy-metal ions. Bioadsorption isotherms were determined for cadmium(II) and lead(II) ions in batch systems in the initial concentration range of 5-250 mg l^{-1}.

2 Materials and Methods

Bacterial strain and cultivation

The microorganism used in this study was *Pseudomonas aeruginosa* (PAO1). The strain was cultivated in Mueller-Hinton broth (Difco) using shaken flasks. They were incubated at 37 °C and the liquid cultures were agitated at 220 rpm. The reproduction curve was performed by using OD_{600} values by spectrophotometry (Fig. 1., Spectronic, Genesys 5, Milton Roy Company, USA).

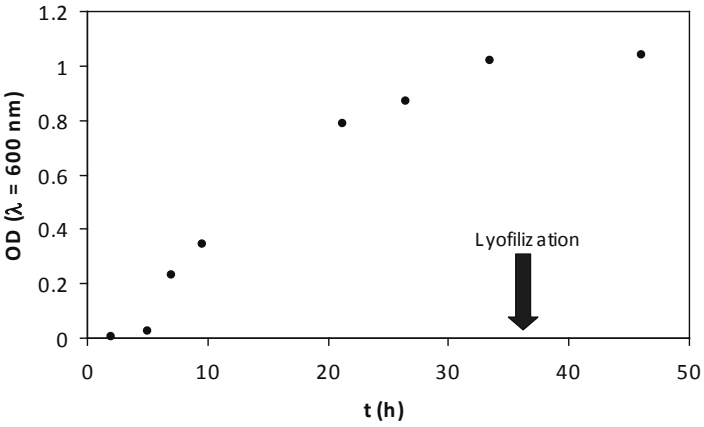


Figure 1: Time-course profiles of growth by *Pseudomonas aeruginosa* PAO1 cells.

In Fig. 1 the optical densities of bacterial cell suspension were presented against incubation time. The minimum inhibitory concentration (MIC) was determined with half dilution method by using 96 mmol l^{-1} initial heavy-metal concentration. The concentrations of heavy-metals were 96.0, 48.0, 24.0, 12.0, 6.0, 3.0 and 1.5 mmol l^{-1}. The MIC values were determined after solid plate cultivation and the cell number was compared with the control culture.

Preparation of biosorbents

Cells were harvested by centrifugation (10000 rpm, 30 min) at early-stationary phase of growth, after 38 h incubation. Cells were twice rinsed with physiological salt solution, then freeze-dried (lyophilization) after centrifugation.

Preparation of heavy-metal solution

The heavy-metal test solutions containing Pb(II) and Cd(II) ions were prepared from PbNO3 (Fluka, Germany) and $CdNO_3 \cdot H_2O$ (Fluka, Germany) in the concentration range of 5-500 mg l^{-1}. The pH values of the adsorption systems were adjusted by using 0.1 M NaOH and 0.1 M HCl solutions.

Analysis of heavy-metals

The concentration of heavy-metals in solutions was measured by Atomic Absorption Spectrometer (Perkin – Elmer 2380) at 217 nm for Pb(II) and 228 nm for Cd(II). Before measurement the heavy-metal solutions were diluted with deionized water to ensure that the heavy-metal concentration in the sample was linearly dependent on the absorbance detected. The calibration of cadmium(II) and Pb(II) was made with standard cadmium and lead solution (Scharlau) in the concentration range of 0-2.5 mg l^{-1} for Cd(II and 0-10 mg l^{-1} for Pb(II).

Study of pH effect on biosorption

The effect of pH on Cd(II) and Pb(II) adsorption was investigated by *Pseudomonas aeruginosa* biomass in aqueous suspension. To determine the optimum pH range of bioadsorption, adsorption measurements with 25 and 50 mg l^{-1} solutions were performed for both Cd(II) and Pb(II) ions in the pH range of 3.0-8.0. The suspension concentration was 1 g l^{-1}. The initial pH of the suspension was 5.6 and the expected pH was regulated with 0.1 M NaOH and 0.1 M HCl solutions, then the adsorption systems were agitated at 250 rpm. After 24 hours samples were taken from the adsorption systems and were spin-dried

at 10000 rpm till 10 minutes and diluted for analysis by atomic absorption spectrophotometry.

Kinetics study of biosorption

In the Cd(II) and Pb(II) biosorption kinetics study by *Pseudomonas aeruginosa* the concentration of Cd(II) and Pb(II) ions were 50 mg l^{-1} at suspension concentration of 1 g l^{-1}. Samples were taken from the solutions at desired intervals and the metal concentrations of the supernatants were measured after centrifugation. The supernatants were spin-dried at 5500 rpm till 10 minutes and diluted for analysis by atomic absorption spectrophotometry.

Determination of bioadsorption isotherms

The biomasses (1g l^{-1}) were suspended in heavy-metal solutions in the glass containers, which were gently agitated at room temperature. For the determination of adsorption isotherms for Pb(II) and Cd(II) solutions in the concentration range of 5-250 mg l^{-1} were used. After 24 hours incubation, samples were taken from the suspensions, and the biomass was separated from the heavy-metal solution at 10 000 rpm for 5 min, and then the heavy-metal content of the supernatant was measured by AAS. The metal uptake was calculated as follows:

$$q = \frac{(c_0 - c_e) \cdot V}{m} \tag{1}$$

q is the adsorbed amount of heavy-metals (mg g^{-1}),
c_0 is the initial heavy-metal concentration (mg l^{-1}),
c_e is the heavy-.metal concentration in the adsorption equilibrium (mg l^{-1}),
V is the volume of the solution (l), and
m is the mass of biosorbent (g).
All experiments were performed in duplicates.

3 Results and discussions

Determination of minimum inhibitory concentration for cadmium(II) and lead(II) ions

Free cells of *P. aeruginosa* were susceptible to the heavy-metals tested. The measured value of MICs for cadmium(II) was 20 mmol l^{-1} and for lead(II) was 12 mmol l^{-1} , respectively. These values were close to previously reported for *P. aeruginosa* (15 mmol l^{-1} for Pb(II)) [12]. The growth medium having

complex rich composition (Mueller-Hinton broth) was used, which resulted in a high level of complexation between the metal cations and components of the growth medium. The residual concentrations of supernatant in the heavy-metal stock solutions were determined after centrifugation. Due to precipitation the MIC value was found to be 6 mmol l^{-1} for cadmium(II) and 1.8 mmol l^{-1} for lead(II).

pH effect on cadmium(II) and lead(II) bioadsorption

It has been shown that the affinity of cationic species towards the functional groups present in the cellular surface is strongly dependent on the pH [13]. Fig. 2a and 2b summarize the results of the adsorption of Cd(II) and Pb(II) ions by *Pseudomonas aeruginosa* PAO1 bacterial cells as a function of pH at initial concentrations of 25 and 50 mg l^{-1}.

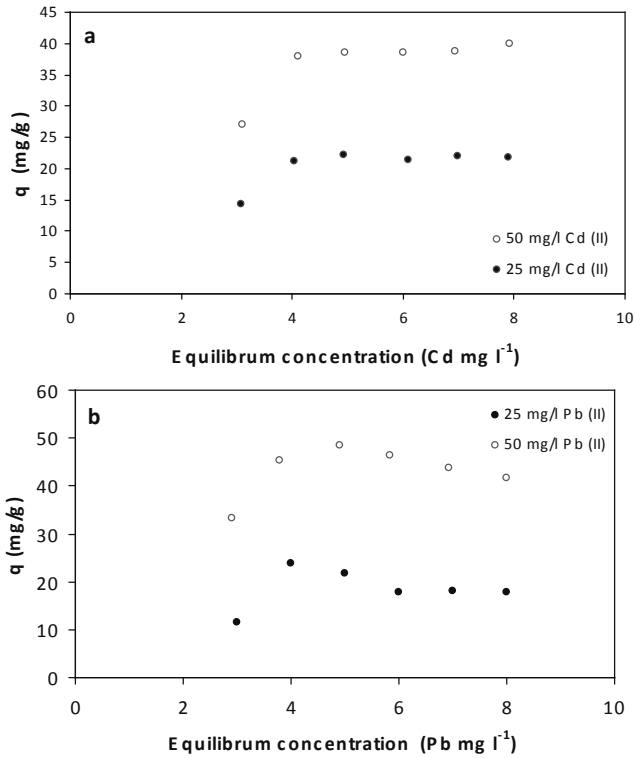
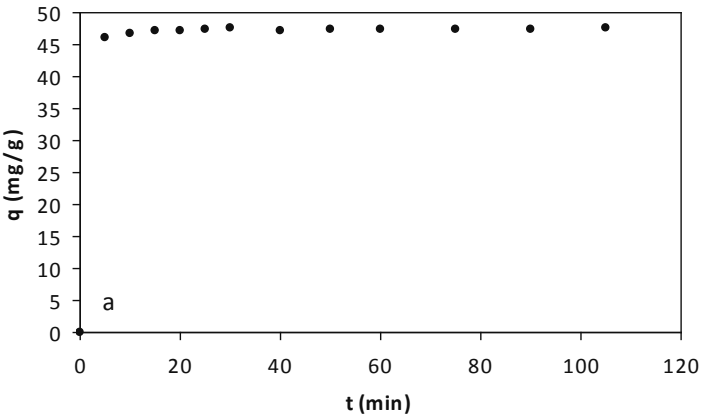


Figure 2: Effect of pH on (a) Cd(II) and (b) Pb(II) biosorption by *Pseudomonas aeruginosa* PAO1 bacterial biomass. Initial concentrations: 25 and 50 mg l^{-1}, contact time: 24 h, biomass concentration: 1 g l^{-1}, temperature: 22.5°C.

In both cases, metal uptake by the biomass increases with increasing pH till it reaches a maximum after which the metal uptake decreases. The bacterial cell wall contains negatively charged functional groups such as carboxyl, phosphate, imidazole and amino groups. They are primarily responsible for the anionic character and metal binding capacity of the cell wall by Gram-negative bacteria [14]. Increasing pH increases the negative charge on the cell surface, which favors the adsorption of the heavy-metal cations. In addition, metal ions undergo hydrolysis as the pH inceases, so strong acidic pH range (pH $<$ 3) is not appropriate for adsorption. High alkaline pH (pH $>$ 8) results metal precipitation. So the effect of pH was determined in the pH range of 3.0-8.0. Optimum pH values were found to be at 4.0-7.0 for cadmium(II) and 4.0-5.0 for lead(II) biosorption. Other investigators like Chang et al. reported that the maximum pH by inactivated and resting cells of *P. aeruginosa* PU21 was 5.5 for lead(II) and 6.0 for cadmium(II) [5, 15]. For *P. pudita* it was 6.0 [16].

Time-course of biosorption

The time-course profiles for the adsorption of Pb(II) and Cd(II) ions by freeze-dried bacterial cells are shown in Fig. 3a-b. and 4a-b. Fig. 3a and 4a represent the adsorbed amounts of Cd(II) and Pb(II) by the biomass, respectively, in the function of contact time. In Fig. 3b and 4b the adsorption efficiencies by the biomass for Cd(II) and for Pb(II) are presented against contact time. The metal concentration decreased rapidly during the first 10 minutes and remined nearly constant after 20 minutes of adsorption, suggesting that the biosorption was very fast and reached saturation within 20 minutes.



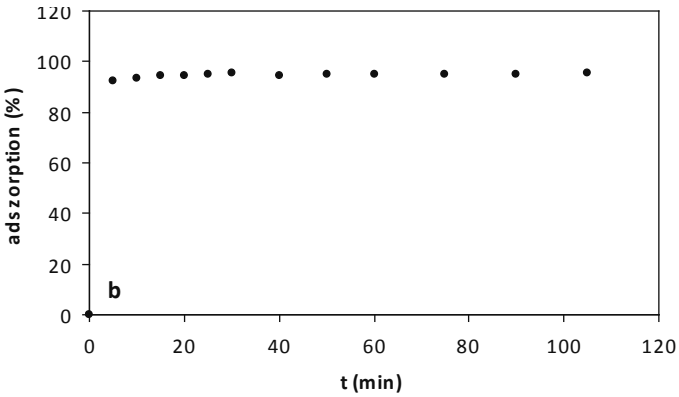


Figure 3: Biosorption of Cd(II) by lyophilized cells of *Pseudomonas aeruginosa* PAO1 (pH = 5.6) as a function of time. Biomass concentration: 1 g l^{-1}, initial concentration 50 mg l^{-1}, temperature: 22.5°C.

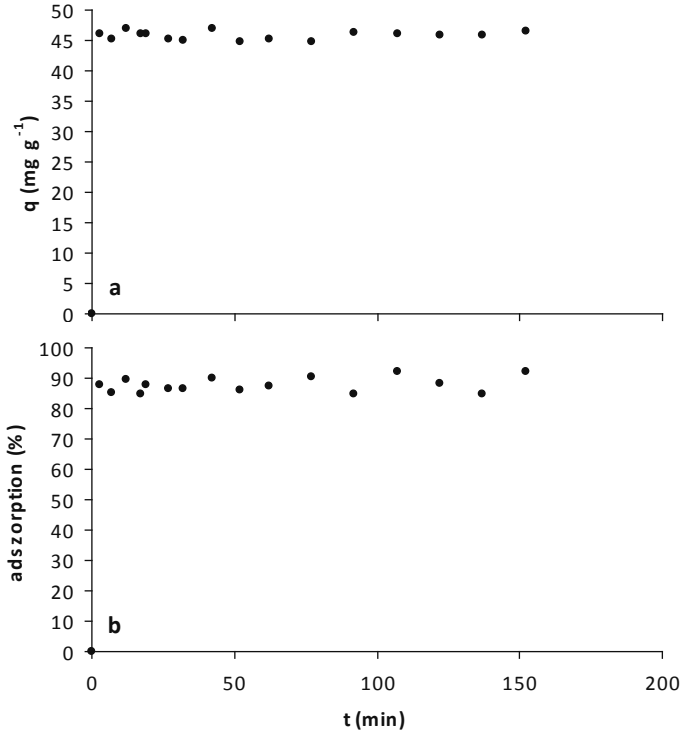


Figure 4: Biosorption of Pb(II) by lyophilized cells of *Pseudomonas aeruginosa* PAO1 (pH = 5.6) as a function of time. Biomass concentration: 1 g l^{-1}, initial concentration 50 mg l^{-1}, temperature: 22.5°C.

This finding is in agreement with earlier studies [5,15]. The cells can accumulate metal ions by their surface and intracellular binding sites. The metal adsorption capacity of lyophilized cells for Cd(II) was 47.6 mg g^{-1} (95 %) and for Pb(II) it was 46.6 mg g^{-1} (92 %), which values do not show significant difference at initial 50 mg l^{-1} metal concentration. Further examination of bioadsorption equilibrium is needed.

Bioadsorption isotherms

Cadmium(II) and lead(II) sorption performance on lyophilized bacterial cells of *P. aeruginosa* PAO1 was achieved by the biosorption equilibrium measurements at initial concentration of 5-250 mg l^{-1} for both metals at pH 5.6. Biomass concentration was 1 g l^{-1}. The equilibrium bioadsorption isotherms determined for both heavy-metals using batch technique shows that metal uptake by bacterial biomass was a chemically equilibrated and saturable mechanism (Fig. 5.) Thus, there was an increase in metal uptake as long as binding sites were free. Preferential adsorption mechanism can be observed for Pb(II) adsorption in comparison with Cd(II) adsorption process. Experimental data were applied to adsorption model given by Langmuir, where its mathematical formulas can be expressed as:

$$q_e = \frac{q_{max} b c_e}{1 + b c_e} \tag{2}$$

and its linear form is represented by the following equation:

$$\frac{c_e}{q} = \frac{1}{q_{max} \cdot b} + \frac{c_e}{q_{\min}} \tag{3}$$

where b is the adsorption equilibrium constant including the affinity of binding sites (l mg^{-1}), ce and qe are unadsorbed metal ions in solution and adsorbed metal ions on the biosorbent at equilibrium, respectively, qmax is the maximum amount of metal ion per unit weight of adsorbent to form a complex monolayer on the surface (mg g^{-1}) [1, 2, 5, 13, 15, 17].

The calculated qmax Langmuir parameter gave a correlation with the experimental value. The calculated qmax value obtained for Pb(II) was 163.9 mg g^{-1}, it was higher than that for Cd(II): 131.6 mg g^{-1}. The experimental qmax value for Pb(II) was 155 mg g^{-1}, while it was 117 mg g^{-1} for Cd(II). The values of equilibrium constant b for Pb(II) and Cd(II) were calculated to be 0.508 l mg^{-1} and 0.054 l mg^{-1}, respectively, which indicates that, lyophilized cells of *P. aeruginosa* possesses a higher adsorption affinity for Pb(II) ions as

compared to that for Cd(II) ions. Such correlation led us to conclude that the energy of adsorption is more favorable for lead (II) ions that for Cd(II) ions.

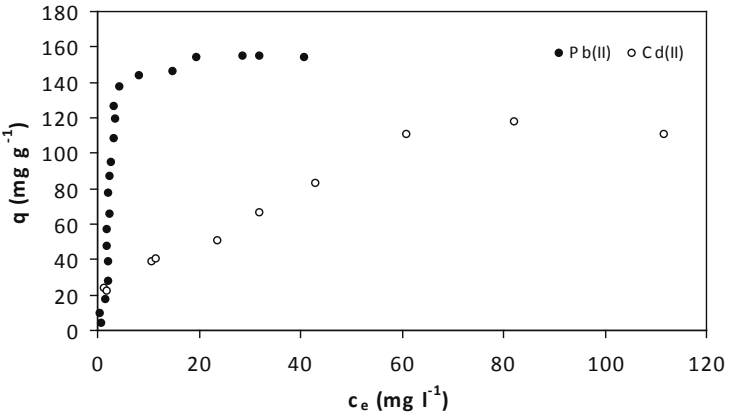


Figure 5: Bioadsorption isotherms of freeze-dried *Pseudomonas aeruginosa* PAO1 bacterial cells.for Cd(II) and Pb(II) ions in the initial heavy-metal concentration of 5-250 mg l^{-1} . Biomass concentration: 1 g l^{-1}, pH = 5.6, temperature: 22.5°C.

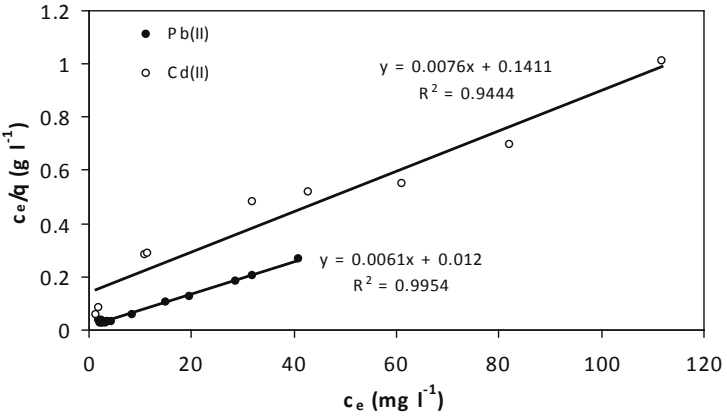


Figure 6: Bioadsorption isotherms of freeze-dried *Pseudomonas aeruginosa* PAO1 bacterial cells.for Cd(II) and Pb(II) ions in the initial heavy-metal concentration of 5-250 mg l^{-1} . Biomass concentration: 1 g l^{-1}, pH = 5.6, temperature: 22.5°C.

4 Conclusion

Lyophilized bacterial cells of *Pseudomonas aeruginosa* PAO1 was able to adsorb cadmium(II) and lead(II) ions with considerably high capacities. As the pH increased, the metal adsorption capacity increased significantly. However, with the restriction of forming insoluble metal hydroxides at high pH values, the optimal operating pH in this study was 4.0-7.0 for biosorption of Cd(II) and 4.0-5.0 for biosorption of Pb(II), respectively. At low initial concentrations of Cd(II) and Pb(II) ions there was no significant difference in adsorption capacities. The biosorption was very fast and reached saturation within 20 minutes. Adsorption data were well described by the Langmuir model. The maximum uptake capacity of Pb(II) and Cd(II) ions was estimated to be 164 mg g^{-1} and 132 mg g^{-1}, respectively. Biomass of *Pseudomonas aeruginosa* PAO1 appears to have the possibility to be an effective adsorbent for the removal of heavy-metals from polluted wastewaters.

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Comparative studies on the cultivation and phylogenetics of King Oyster Mushroom (*Pleurotus eryngii* (DC.: Fr.) Quél.) strains

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Abstract. The king oyster mushroom (*Pleurotus eryngii*) is still not a well known species amongst producers, despite its excellent taste and relatively easy cultivation technologies. Nevertheless, European growers show growing interest towards it. We collected king oyster mushroom strains from various habitats to get a better view about their vegetative growth, molecular based taxonomic relationship and cultivation parameters. In the *in vitro* experiments we investigated the growth of vegetative mycelia at various temperatures and pH. All strains were cultivated on lignocellulose substrate for 60 days under adequate climatic conditions and yield quantity, flushes, number and average weight of fruiting bodies

were determined. Biological Efficiency (BE%) and Productivity (P%) were calculated for each strains. We investigated taxonomic relationships among *P. eryngii* isolates by means of RAPD-PCR method. Twenty-five random primers were tested and six of them supplied us with sufficient data for generation of a neighbour-joining tree.

Results of the cultivation experiments showed that environmental conditions resulted in a very different growth rate of the various P. eryngii strains on different temperature and pH. Calculated for 100 kg substrate the highest yield was produced by the Ple-4V strain (41.5 kg), whereas the lowest yield was found at the PEL isolate (9 kg). The average yield of the investigated strains was 27.53 kg. Average weight per fruiting body was 19.95 g. We found the highest BE% at the Ple-4V and the lowest BE% at the PEL strains, 156.18% and 28.52%, respectively. These results showed that cultivation properties of the isolates are very different. The neighbour-joining tree revealed the taxonomic relations amongst the Hungarian, Malaysian, Italian and Dutch isolates. No correlation was found between the taxonomical position and growth rate of the investigated isolates. The average yield showed that the *P. eryngii* might be a suitable alternative of the popular tree oyster mushroom (*P. ostreatus*). Our results can be useful for subsequent cross breeding experiments and the dataset might support further investigations.

Keywords: vegetative mycelium, RAPD-PCR, neighbour-joining tree, biological efficiency

1 Introduction

Edible mushrooms are low energy foods with high nutritional value. Though their role in the healthy, reform and dietetic nourishment is significant, mushroom consumption in Hungary is still not remarkable. Amongst the traditionally cultivated and potentially new mushroom species, the king oyster mushroom (*Pleurotus eryngii*) bears extraordinary opportunities for cultivation and consumption.

Pleurotus species belong to the *Pleurotaceae* family, a member of the *Basidiomycota* phylum. There are white rot saprobe and facultative parasitic species in the genus that degrade polymers of lignin and cellulose, as well. *Pleurotus eryngii* varieties can be found in pastures, meadows, gardens and seldom in grassy forest clearings and hilly areas. This species is mainly associated with members of the *Apiaceae* (*Umbelliferae*) plant family in its natural habitats [1, 2, 3, 4].

Taxonomic relationships of the host-specialized *P. eryngii* species complex have not obviously defined yet. According to the latest phylogenetic research,

the following taxons are suggested: *P. eryngii* var. *eryngii*, *P. eryngii* var. *ferulae*, *P. eryngii* var. *elaeoselini*, *P. eryngii* var. *nebrodensis*, P. *hadamardii*, *P. fossulatus* [5, 6] and the newly described variety *P. eryngii* var. *tingitanus* [7, 8]. The taxonomy debates could be ended by comprehensive molecular biology, speciation and co-evolutionary researches.

Intensive production of the species started in Hungary in the 1950's, on mixture of composted hay and sawdust [9, 10, 11, 12]. In the 1960s Véssey tried to produce the mushroom on sterile substrate [13]. Cailleux & Diop used sterile medium consisting of wheat straw and oat for the production [14]. There are much more raw materials that can be used for cultivation, than those mentioned in the literature. Most of these materials originate from agriculture and forestry, such as straw of different grains, sawdust, cotton-straw, soybean straw, corn stalk, *Cyperus alternifolius* stalk, corn flour, cooked grain seeds, malt, bamboo powder, cornmeal, ricebran etc.[15, 16, 17, 18, 19, 20]. The substrate can be produced either by wet heat treatment and dry heat treatment (xerotherm method) or by sterile technology [8, 21]. A critical point in the preparation of the substrate is the adjustment of nitrogen content, since both the too low or too high level of nitrogen results a decrease in the amount of mushroom [22]. The danger of supplementation is the emergence of various moulds (e.g. *Trichoderma* spp., *Penicillium* spp. etc.) on the blocks and later on the fruiting bodies.

Generally, cultivation is carried out on blocks, in bottles or in plastic bags. Casing material can be applied that might be the same as which used for the cultivation of white button mushroom (*Agaricus bisporus*). Fruiting bodies appear on both of the non-cased and cased substrate, as well. Győrfi & Hajdú [20] found significant yield increase compared to the non-cased cultures, when casing soil mixtures were used in different thickness. Another possible cultivation method is when the spawn run blocks are sunken 25-30 cm deep into the soil and then covered by 3 cm of casing soil.

Mushroom strains vary in their ability to convert substrate materials into mushrooms as measured by a simple formula known as the "Biological Efficiency (BE) Formula". BE = weight of fresh mushroom fruiting bodies / weight of dry substrate × 100 [12, 23]. Sonnenberg et al. [22] published that BE is often very low (10-15%) in Germany and the Netherlands, but it could be increased 20-25% in cultivation experiments. Kirbag & Akyüz [12] reported 48-85% BE when agricultural waste was used as substrate. Rodriguez Estrada et al. [24] varied supplementation and casing and reached 179%. Productivity (P) is used sometimes by producers as an indicator of yield quantity calculated for wet substrate weight. Productivity (P) = (fresh mushroom weight / fresh

substrate weight) × 100 [25].

The aim of this study was to determine the yield of wild *P. eryngii* isolates and their taxonomical relations on the basis of RAPD-PCR fingerprints.

2 Materials and methods

Isolation of strains: isolates were collected from various grassy areas in Hungary. Inoculation onto malt agar plates was made by the use of plectenchyma originated from the broken pileus, and then the plates were incubated on 25°C. After incubation samples were taken from the regular sectors of the vegetative mycelia and inoculated three more times onto new agar plates. Strains were maintained on slant agar in test tubes and replicas were stored in liquid nitrogen.

Three strains (PE-SZM, PEL and PES) originated from the Strain Research Laboratory of the Hungarian Mushroom Growers' Association in vegetative mycelial form. Originally, the PE-SZM originated from Malaysia, whereas the PEL and PES were collected in Italy and in the Netherlands, respectively.

Investigation of the effect of temperature: 10 days old vegetative mycelial mats of the 15 strains were inoculated into the centre of malt agar plates, one strain on each plate. Mycelial mats were collected by 0.8 cm diameter cork borer. Incubations were performed in the following temperatures: 5°C, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C. Incubations on each temperature were repeated three times with each strain, the control strains were grown on 25°C. In addition to the collected strains in this experiment the Korona 357 (K 357) *P. ostreatus* industrial hybrid strain, originated from the crossing of the popular Gyurkó HK 35 hybrid, was investigated, as well.

Investigation of the effect of pH: pH of the malt agar plates was set between 4 and 9 in 0.5 steps by 1 M HCl and 1 M NaOH. Inoculations were made the same way as in the temperature experiments in three replicates and the plates were incubated on 24°C. The pH of the control malt agar plates was set to 6, and the K 357 industrial hybrid was used as a control strain.

Spawn making: rye based spawn was produced from each isolates. 5.5 l rye was cooked then thinly covered with gypsum. 200 g rye was filled into a 500 ml Erlenmeyer flask and sterilized on 121°C for 2 hours. After cooling, the rye was inoculated by 1.5-2 cm diameter agar discs covered with 7 days old mycelia and then incubated on 25°C for 10 days.

Substrate and cultivation: 900 g wet substrate was filled into 2,000 ml polypropylene bags. Composition of substrate was the following (calculated

for air dry material): beech sawdust 65%, wheat bran 17%, beech chips 9%, gypsum 3.5%, soy supplement (Promycel 480) 5.5%. Moisture of substrate was set to 60%. The bags were closed by paper plugs, then covered by aluminium foil and autoclaved on 121°C for 2.5 hours. After cooling, the substrate was inoculated by 10 m/m% spawn.

Dry weight content was calculated after drying the substrate at 105°C until constant mass. Dry weight of initial and spent substrates was determined. In order to calculate productivity, initial wet mass and spent wet mass of the substrate were measured.

Spawn running was carried out on 25°C. Due to the 10 m/m% rate used for spawning, all the substrates were evenly colonized in 10 days from the inoculation, then the bags were cooled onto 10°C. On the 12th day the bags were opened and the substrate within was cased with peat based casing soil. It was followed by spraying with water in order to lightly moisturize the soil then the bags were covered with veil foil. From this point 12 hours of light and 12 hours of darkness by turns and 95% relative humidity was adjusted. On the 15th day air temperature was raised to 17.5°C. On the 17th day the mycelia reached the surface of the casing soil at the edges of the bag. On the 19th day the veil foil was removed, the level of CO_2 dropped below 800 ppm and initiation of fruiting was facilitated.

After the appearance of primordia the temperature was raised to 19°C (+/- 2°C) and the casing soil, the floor and walls of the growing house were sprayed with water.

Quantity of yield and number of fruiting bodies were registered and calculated for 100 kg of substrate. Average weight of fruiting bodies was determined. BE and P values and their differences were calculated for initial and spent substrates. Based on the average results of the strains, growing characteristics of the species were deduced.

Grouping of the strains on the basis of yield was made by Tukey correlation analysis tool of the SPSS 15 software.

DNA-extraction: a modified protocol of Shure [26] was used for DNA extraction. Thirty mg of freeze-dried mycelium powder was placed into a microcentrifuge tube, then 325 μl DNA isolation buffer (0.6 M NaCl, 0.1 M Tris (pH 7), 40 mM EDTA, 4% Sarcosyl, 1% SDS), 325 μl urea (60 g urea dissolved in 100 ml distilled water) and 6.5 μl 1 M $Na_2O_5S_2$ was pipetted onto it and the mix was vortexed for 1 min. The tubes were then incubated on 37°C for 45 min and vortexed for 15 s after 15 and 30 min of incubation. The proteins were precipitated by 650 μl phenol:chloroform:isoamyl alcohol 25:24:1 solution, then the samples were centrifuged at 9,500 g for 7 min. The

supernatant was pipetted into a new tube. The phenol-chloroform treatment was repeated twice, then the water phase was pipetted into a new Eppendorf tube and the DNA was precipitated with 70% (v/v) cold isopropanol. The tubes were incubated for 5 min then centrifuged at 9,500 g for 7 min. The isopropanol was removed from the tube and the pellet was washed three times with 1 ml 70% ethanol. The DNA was dried in vacuum concentrator, and then dissolved in 80 μl 1×TE buffer. The DNA solution was treated with 2 μl RNase on 37°C for 45 min, and then stored on -20°C. Quality and quantity of the DNA was measured with a NanoDrop 1000 (Thermo Fisher Scientific, USA) spectrophotometer.

RAPD-PCR: after the RAPD-PCR conditions were optimized, DNA extracts of the 15 P. eryngii isolates were tested with a P. ostreatus strain, which was chosen as outgroup. For RAPD the OpA and OpB random decamers (Operon Technologies) were used. The PCR reaction volume was 25 μl that contained 0.5 μl $MgCl_2$ (25 mM), 2.5 μl Taq buffer, 0.5 μl dNTP (10 mM), 1 μl primer (10 pM), 0.125 μl Taq polymerase (5 U μl^{-1}; Fermentas, Canada), 2 μl template DNA (200 ng μl^{-1}) and 18.375 μl distilled water. PCR amplifications were performed in a Corbett Research PCR Thermal Cycler (Corbett Life Science, Australia) using the following cycling parameters: initial denaturation at 95°C for 2 min, followed by 35 cycles of amplification comprising a denaturation step for 1 min at 95°C, annealing at 34°C for 1 min, and extension at 72°C for 1 min and a 5 min final extension step at 72°C.

Eight μl of the PCR product was electrophoresed at 110 V for 1 h on a 1% agarose gel (SeaKem LE Agarose, Lonza) stained with GelRed (Biotium, USA) in 1×Tris Borate EDTA buffer. A 100 bp BenchTop ladder (Promega, USA) was used as a molecular size marker.

Construction of phylogenetic tree: RAPD-PCR experiments were repeated three times and the resulted band patterns were scored visually. Presence or absence of bands was recorded in a binary matrix, separately for each primer. Genetic distance matrices were calculated using PHYLTOOLS software package and Nei-Li coefficient. The neighbour-joining tree was generated by the NEIGHBOR program of the PHYLIP software package [27, 28].

3 Results and discussion

Strain collection: in the period of 2006-2008 12 strains from different areas of Hungary were isolated (Figure 1) and deposited in the Laboratory of Microbiology of the Eszterházy Károly College (Eger).

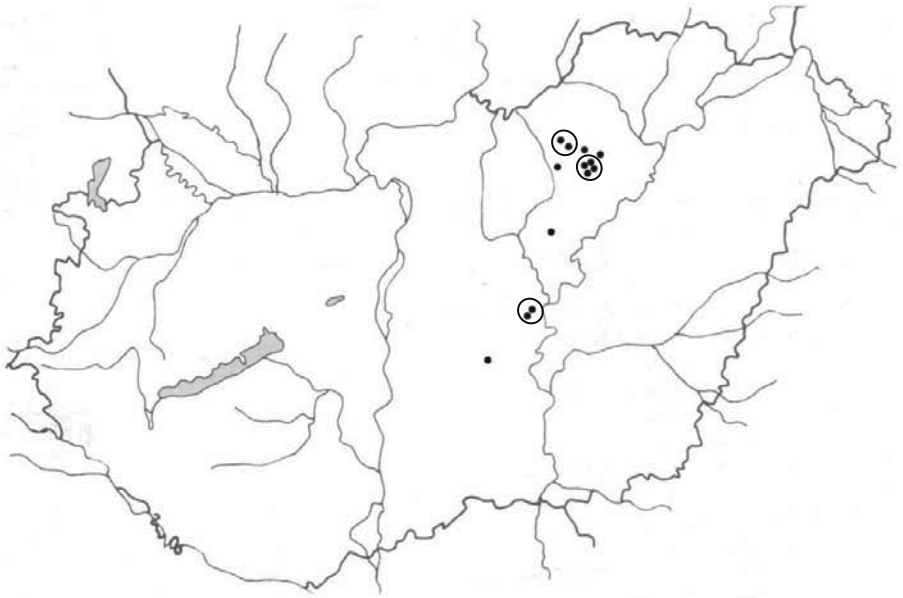

Figure 1: Location of Hungarian strains. 1: Kecskemét; 2: Tószeg (two isolates); 3: Hevesi Füves Puszta (Heves Grass Plains); 4: Demjén; 5: Novaj (four isolates); 6: Bogács; 7: Síkfőkút; 8: Eger, Pásztorvölgy (two isolates)

Growth rate of vegetative mycelia on different temperatures: mycelia of the various strains showed very similar growth rate on minimum and maximum temperatures. In contrast to this, significant differences of the growth rate were noticed on optimal temperature, which was between 25-30°C for most of the strains. In average, the isolates showed 6.6-8 mm daily growth and the PEC and PEFi strains produced the highest growth rate. Since the optimal temperature range is used for spawn run in the course of cultivation, it is worth determining the growth rate of strains before mass production, because significant differences can be found between them. Table 1 shows the time required by the strains to reach the maximum colony diameter (8 cm) on a given temperature. Grey fields show the shortest time the strains needed to reach the maximum colony diameter and the lowermost line gives a summary about the number of strains that reached the maximum diameter on the given temperatures.

pH tolerance: *Pleurotus* species have high pH tolerance, but significant differences in the optimum pH value were found among the strains. A strongly limiting value for some strains was pH 4, whereas others grew almost at the same rate as at higher pH. It was surprising that some isolates showed a rela-

tively fast rate of growth on alkaline pH (8-9). This feature may be used for protection against competitive organisms and microparasites in order to avoid the use of pesticides.

Table 1: Time (days) required by the different strains to reach maximum colony diameter (8 cm) on certain temperatures. C: K 357 P. ostreatus hybrid control.

Strain/Temp.	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C
PEP	41	32	24	**20**	**20**	23	-
PEC	41	32	24	10	**7**	10	-
PEF	41	32	24	12	**10**	**10**	-
PE-SZM	-	32	19	**12**	**12**	**12**	-
Ple-5V	41	32	19	**12**	**12**	**12**	-
Ple-4V	41	32	14	**10**	12	12	-
Ple-3V	41	32	19	17	12	**10**	-
Ple-2V	41	32	14	**10**	**10**	**10**	-
Ple-1V	41	32	14	**10**	**10**	**10**	-
Ple-6V	41	32	19	**12**	**12**	**12**	-
PEFi	41	32	14	12	**7**	10	-
PES	41	32	14	12	**10**	**10**	-
PEA	41	32	19	12	**10**	**10**	-
PEL	-	32	24	**12**	**12**	17	-
PEG	41	32	19	**12**	14	14	-
C (*P.o.*)	41	32	10	7	10	10	-
Mean	41.00	32.00	18.67	12.33	11.47	12.13	-
Deviation	0.000	0.000	3.994	2.717	3.137	3.603	-
Prevalence	0	0	0	9	12	9	0

Table 2 shows the connection between pH values and the time required for the strains to reach the maximum colony diameter. We found that the isolates grew faster on two pH values: one optimum was at the acidic pH 4.5, the other one was in the alkaline pH 7.5-8.5 range. Since the result was obtained from the mean growth rate of 15 strains and the strains did not show significant differences, it is more characteristic for the species than for its different strains.

Yield of various isolates: the average yield was calculated for 100 kg substrate. The highest yield was produced by the Ple-4V and Ple-5V strains, 41.5 kg and 39.5 kg, respectively. The lowest yield was found at the PEL and PEG strains, 9 kg and 11 kg, respectively. The average yield calculated for 100 kg substrate was 27.53 kg. Average weight of fruiting bodies was 19.95 g. Average yield of strains and weight of fruiting bodies is shown in Figure 2. We

performed Tukey cluster analysis and found that only two strains (PE-SZM and PES) can be grouped together, the remaining isolates show continuous transition from the lower towards the higher yield.

Table 2: Time (days) required by the different strains to reach maximum colony diameter (8 cm) depending on the pH of agar plates. C: K 357 P. ostreatus hybrid control.

Strain/pH	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**
PEA	14	14	14	13	13	13	11	**8**	11	10	11
PEC	17	11	9	10	9	**8**	**8**	**8**	**8**	9	9
PEG	14	**13**	14	14	14	17	14	14	14	14	14
PEF	17	14	14	14	13	13	12	**11**	14	**11**	**11**
PEFi	11	11	10	10	10	10	**8**	**8**	**8**	9	9
PEL	14	14	14	14	14	**13**	14	14	14	17	16
PEP	**17**	**17**	19	22	22	22	22	22	22	22	22
PES	18	17	17	17	15	14	13	13	**11**	**11**	**11**
PE-SZM	14	14	**11**	14	13	13	13	13	13	13	14
Ple-1V	14	11	10	10	10	10	10	**9**	11	10	10
Ple-2V	11	**9**	**9**	10	11	13	10	10	**9**	**9**	11
Ple-3V	14	**11**	**11**	13	13	14	**11**	**11**	**11**	**11**	**11**
Ple-4V	14	11	11	13	11	13	11	11	11	**10**	11
Ple-5V	14	14	14	13	13	13	13	16	16	**11**	17
Ple-6V	14	14	14	13	14	13	13	**11**	**11**	13	14
C (P.o.)	9	9	9	9	9	9	8	8	8	8	9
Mean	14.47	13.00	12.73	13.33	13.00	13.27	12.40	11.93	12.27	12.00	12.73
Deviation	2.031	2.299	2.915	3.109	3.047	3.173	3.397	3.693	3.535	3.525	3.515
Prevalence	1	4	3	0	0	2	3	7	6	6	3

The dataset suggests that not only the genetic background had an effect on the relatively high average yield, but other factors were also responsible. Casing inhibited desiccation of lignocellulose substrate, ensured even moisture content and water uptake for the mushroom and attenuated errors of climatization. Changing of climatic conditions (temperature, CO_2 level, RH, air flow) played an important role, as well. An additional key factor was the raise of nitrogen level, what was assured by use of nitrogen containing soy supplement (Promycel 480). Sterile cultivation technology, low substrate quantity and 60 days long cultivation (with more flushes depending on the strain) were also important factors.

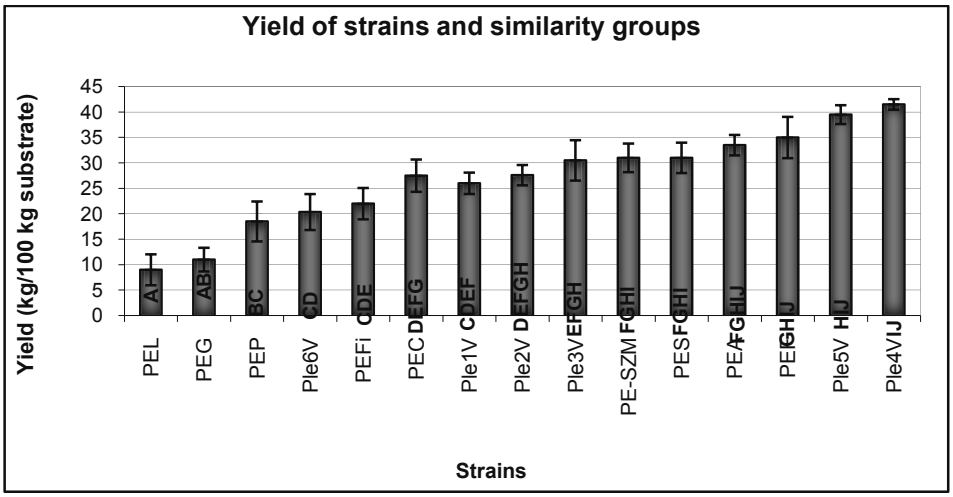

Figure 2: Yield amount of strains calculated for 100 kg substrate (kg), average weight of fruiting body (g), value of similarity and dissimilarity between strains. The more identical characters in the bars mean higher degree of similarity; while less identical character refer to less similarity.

The 12-6 days spawn running period given by Stamets [23] had been shortened to 8-0 days in our case, since we used high (10%) spawn rate. Even so, we could harvest the first flush only at the 27-28th day, which was very close to the 20-29th day described earlier [23]. However, other authors stated that they could harvest the first flush only at the 38th day [24] or in a 37-54 day period [12], depending on the composition of the substrate.

Biological efficiency and productivity: a relatively high BE value was resulted for most of the strains when the weight of the spent substrate was used in the calculations. Especially high BE value was found at the Ple-4V (156.18%) and Ple-5V (140.03%) strains. The lowest BE was found at the PEL (28.52%) and PEG (37.82%) strains. BE value of the species, calculated as average BE of the strains, was 98.41%, whereas the productivity was 44.36%. As long as the calculations were made with the initial substrates instead of the spent substrates, both the BE and P values were lower, since the weight of the substrate changed during cultivation. Average BE calculated for the initial substrate was 25.9%, the productivity was 16.83%. We think it is important to calculate these two parameters (BE and P) for both the initial and spent substrate, because in the literature it is not stated which version was used for calculations, though, it might be the reason of the serious differences of BE values published by different authors.

Table 3 summarizes our results: quantity of yield calculated for 100 kg substrate, number of fruiting bodies, mean weight of fruiting bodies, biological efficiency and productivity, for each investigated strains.

Table 3: Summary of the main results calculated for 100 kg substrate. Mean weight values were rounded to the first decimal.

Strain	Quantity of yield (kg/100 kg)	Total nr. of FBs (pcs/100 kg)	[a]Mean weight of FB (g)	BE % spent substrate	P % spent substrate	[b]BE % (D)	[c]P % (D)
PEL	9	1.050	8.6	28.52	11.11	4.8	2.11
PEG	11	1.500	7.3	37.82	14.29	8.9	3.29
PEP	18.5	550	33.6	56.31	20.56	7.6	2.06
Ple-6V	20.35	1.050	19.4	74.35	32.82	20.8	12.47
PEFi	22	1.100	20	72.29	31.65	14.4	9.65
Ple-1V	26	1.400	18.6	97.33	44.83	28.9	18.83
PEC	27.5	1.600	17.2	100.28	45.45	27.9	17.95
Ple-2V	27.6	1.400	19.7	104.38	45.62	31.7	18.02
Ple-3V	30.5	2.450	12.4	109.43	58.65	29.1	28.15
PES	31	1.500	20.7	111.2	43.06	29.6	12.06
PE-SZM	31	2.150	14.4	120	55.36	38.4	24.36
PEA	33.5	1.700	19.7	118.44	45.58	30.2	12.08
PEF	35	1.400	25	119.7	53.44	27.5	18.44
Ple-5V	39.5	1.400	28.2	140.03	65.29	36	25.79
Ple-4V	41.5	2.600	16	156.18	76.85	46.9	35.35
Mean (species)	27.53	1.488	19.95	98.41	44.36	25.9	16.83
Deviation	9.401	541.140	8.258	35.773	18.640	11.713	9.973

[a]FB: fruiting body
[b]BE % (D): BE % calculated with spent substrate – BE % calculated with initial substrate
[c]P % (D): P % calculated with spent substrate – P % calculated with initial substrate
BE% and P% values calculated with the initial substrate are not shown in the table

Molecular biology examinations: as a result of the preliminary RAPD-PCR experiments, six primers were chosen that produced differentiating bands for each strain. These decamers were the following: OPA 05, OPA 07, OPA 10, OPA 13, OPA 18 and OPB 10.

Most of the strains could be differentiated with the OPA 05 and OPA 13 decamers, but high degree of similarity was found between the Ple-1V/Ple2V and Ple3V/Ple-4V isolates, respectively. Ple-1V and Ple-2V strains were isolated from the same habitat, but the Ple-3V and Ple-4V isolates originated from different locations. Another interesting result is that the OPA 05 could not differentiate the Ple-5V and Ple-6V isolates, but OPA 13 patterns of the two isolates were not identical, though these two strains were collected from the same place in two successive years. RAPD-PCR reactions were repeated three times under previously optimized circumstances and the differentiating bands were used for calculation of distance matrices and generation of the neighbour-joining tree. Some primers resulted RAPD fingerprints that were unique for certain strains, so these primers might be suitable for characterization of these strains in the future. By cloning and sequencing of differentiating bands specific primers can be designed for a given isolate. Therefore, these primers might be valuable tools for breeders and spawn makers for origin protection and differentiation of isolates.

The neighbour-joining tree illustrates the taxonomical relations of the collected *P. eryngii* strains (Figure 3). There are two large groups on the tree: four strains were found to be slightly related to the Malaysian strain, while nine isolates were closer related to the Italian strain. It is remarkable that in one case we could isolate the same strain in both years (Ple-5V and Ple-6V in 2007 and 2008) from the same area. It is also interesting that samples from Kecskemét and Demjén show close relation, though the areas are 140 km apart from each other. Furthermore, no significant differences were found amongst the samples collected in the mountains and around Kecskemét's low landscape, which exhibit different climatic and environmental conditions.

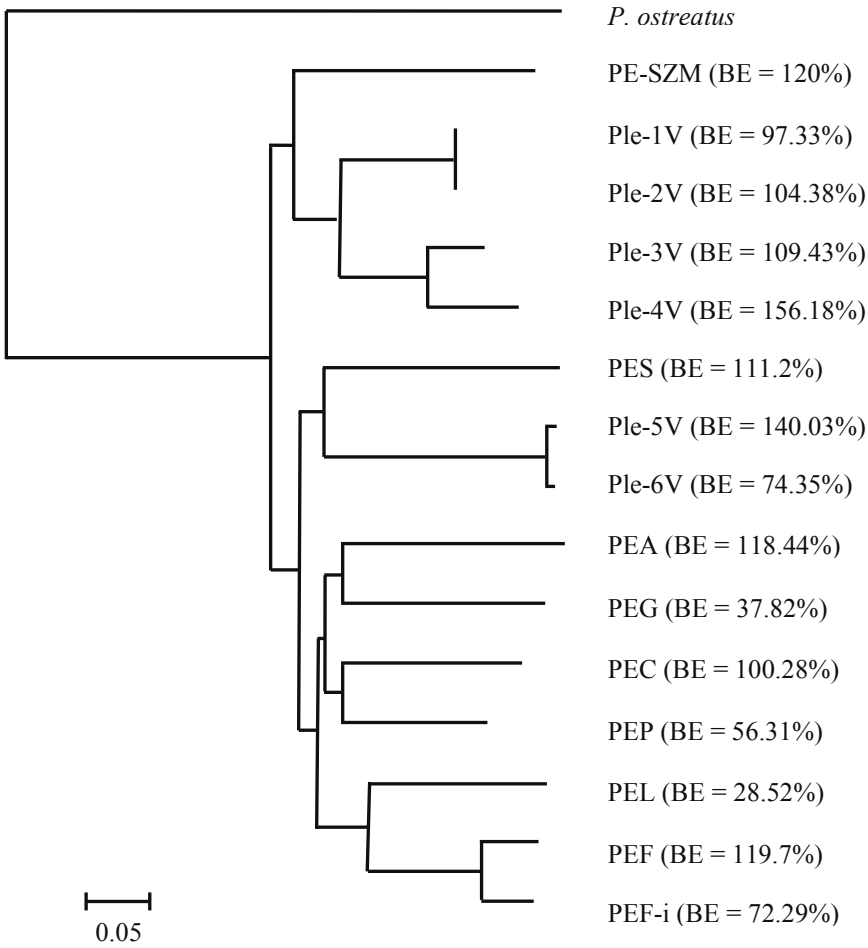

Figure 3: Dendrogram based on the RAPD analyses, created by Neighbor-Joining program [27]. The scale bar represents genetic distance. Biological efficiency (BE) is shown in brackets. Origin of strains: PE-SZM: Malaysia; Ple-1V: Hungary (Novaj 2007); Ple-2V: Hungary (Novaj 2007); Ple-3V: Hungary (Bogács); Ple-4V: Hungary (Hevesi Füves P.); Ple-5V: Hungary (Novaj 2007); Ple-6V: Hungary (Novaj 2007); PEA: Hungary (Tószeg); PEG: Hungary (Tószeg); PEC: Hungary (Pásztor-völgy); PEP: Hungary (Pásztor-völgy); PEL: Italy; PES: the Netherlands; PEF: Hungary (Kecskemét); PEF-i: Hungary (Demjén).

4 Summary

In the present research 13 wild *P. eryngii* strains of Hungarian origin were used in order to determine in vitro the growth rate of the vegetative mycelia of the species and the different strains, under various environmental conditions. Growth rate of various *P. eryngii* strains was very diverse, according to the well defined values of the environmental factors. On the basis of the average growth rate of the strains, we could conclude what are the optimum ecological values of the species, though these conclusions did not always coincide with the optimum values of the strains.

RAPD-PCR analysis was applied to examine the genetic diversity of the *P. eryngii* strains. Twenty-five primers were tested and six were chosen for further analysis and calculation of binary matrices on the basis of RAPD fingerprints. A neighbour-joining tree was generated from the matrices by the NEIGHBOR program of the PHYLIP software package. The tree reflects taxonomic relations amongst the Hungarian isolates and shows that some of them are related to a strain of Malaysian origin, whereas others to an Italian strain.

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Soil degradation processes and extreme hydrological situations, as environmental problems in the Carpathian Basin

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Abstract. Rational and sustainable use of soils – the most important, conditionally renewable, natural resources in the Carpathian Basin – are priority tasks of biomass production and environment protection. Natural conditions in the Carpathian Basin are generally favourable for rainfed biomass production. These, however, show extremely high, irregular, hardly predictable spatial and temporal variability, often extremes, and sensitively react to various natural or human-induced stresses. The main constraints are: soil degradation processes; extreme moisture regime; unfavourable changes in biogeochemical cycles of elements. Soil processes can be controlled (to a certain extent) and their unfavourable consequences can be prevented or at least moderated.

Keywords: soil multifunctionality; soil fertility; soil resilience; soil moisture control; waterlogging hazard; drought sensitivity

1 Introduction

Soils are the most important – conditionally renewable – natural resources in the Carpathian Basin. Consequently, their rational and sustainable use, protection and conservation, maintaining their desirable multifunctionality, are priority tasks of biomass production and environment protection and are key elements of sustainable development.

2 Natural conditions

The natural conditions of the Carpathian Basin (particularly the lowlands and plains) are generally favourable for rainfed biomass production [1, 9]. These conditions, however, show high and irregular, consequently hardly predictable spatial and temporal variability, often extremes and sensitively react to various natural or human-induced stresses. The generally favourable agro-ecological potential is mainly limited by three soil factors:

(1) Soil degradation processes [2, 8].
(2) Extreme moisture regime: simultaneous hazard of flood, waterlogging, over-moistening and drought sensitivity [12, 13].
(3) Unfavourable changes in the biogeochemical cycles of elements, especially of plant nutrients and environmental pollutants.

The comprehensive assessment and efficient control of these phenomena are the primary tasks of multipurpose biomass production, environment protection and sustainable rural development.

3 Limiting factors of soil multifunctionality and soil degradation processes

The main limiting factors of soil multifunctionality/fertility/productivity in Hungary are shown in Figure 1 [6].

The most important soil degradation processes in the Carpathian Basin (similarly to Europe) are summarized in Figure 2.

In spite of the large and increasing extension of degraded lands in all continents, it can be stated that soil degradation is not an unavoidable consequence of intensive (but rational!) agricultural production and social development! Because of soil resilience most of these soil degradation processes can be prevented, eliminated or at least moderated. But it needs permanent control and widely adopted soil (and water) conservation technologies, as indispensable elements of sustainable site-specific precision soil management.

In the last years the revolutionary development of in situ and laboratory analytics, remote sensing, informatics, computer technology, GIS/GPS applications, etc. have given opportunity for the control of soil degradation processes on the basis of an up-to-date comprehensive environment/soil database [7]. This was the aim of the GLASOD (Global Assessment of Soil Degradation) and PHARE-MERA international projects; and this is in the focus

of the European Soil Conservation Strategy [11]. In Hungary – based on all available soil information – the "environmental sensitivity/susceptibility/ vulnerability of soils to various soil degradation processes were comprehensively analysed, giving a good scientific basis for the development of the Hungarian Soil Conservation Strategy [3].

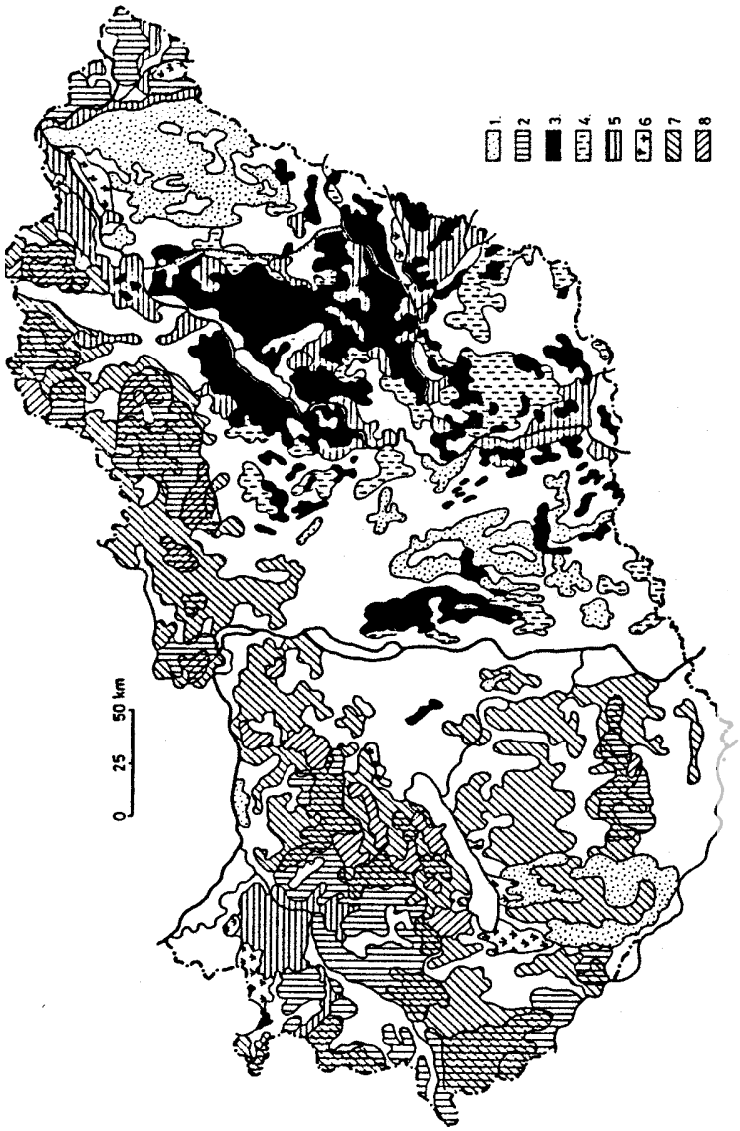

Figure 1: Map of the limiting factors of soil fertility in Hungary.
1. Extremely coarse texture (8% of the total area of Hungary). 2. Acidity (12.8%). 3. Salinity and/or sodicity (8.1%). 4. Salinity and/or sodicity in the deeper layers (2.6%). 5. Extremely heavy texture (6.8%). 6. Peat formation (1.7%). 7. Erosion (15.6%). 8. Shallow depth (2.3%).

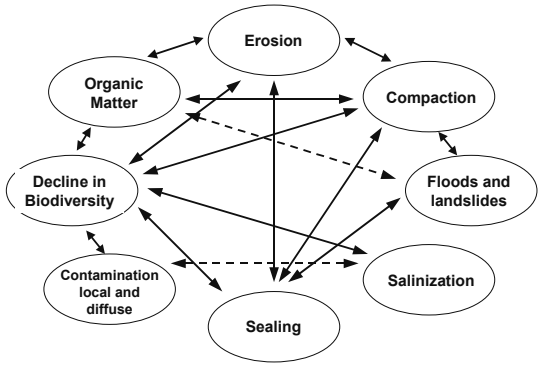

Figure 2: The main soil degradation processes in the Carpathian Basin

4 Extreme moisture regime

It can be predicted with high probability that in future water will be the determining (hopefully not limiting) factor of food security and environmental safety in the Carpathian Basin [4, 5]. Consequently, the risk reduction of extreme hydrological events and soil moisture regimes, and the increase of water use efficiency will be the key issues of soil and water management, biomass production.

Limited water resources

The water resources are limited and an increasing water demand must be satisfied from these limited resources [5].

The average 500-600 mm annual precipitation in the Pannonian Plains shows extremely high territorial and temporal (Figure 3) variability – even at micro-scale. Under such conditions a considerable part of the precipitation is lost by surface runoff, downward filtration and evaporation. Precipitation will not be more in the future. On the contrary, it might be less and its spatial and time variability is expected to increase. It results in increasing risk (frequency, intensity) of extreme weather events (high intensity rains, droughts etc.) with their hydrological (flood, waterlogging, over-moistening), ecological (droughts, crop damages, yield reduction) and environmental (erosion-sedimentation, infrastructure damages, landscape destruction) consequences.

The available quantity of surface waters (rivers) will not increase, particularly not in the critical low-water periods.

A considerable part of the subsurface waters (especially in the poorly drained lowlands) is of poor quality (high salinity, alkalinity, sodicity), threatening with harmful salinisation/sodification processes. The over-exploitation of groundwater may result in serious environmental deterioration: "desertification symptoms" appear.

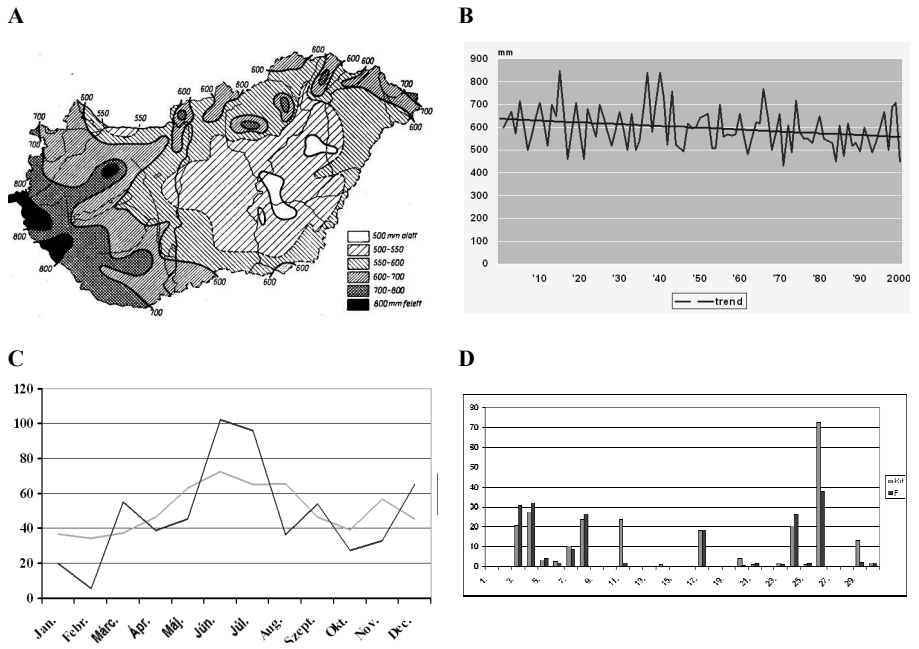

Figure 3: Territorial and time distribution of atmospheric precipitation in Hungary. A. Geographical distribution of the 100-year average annual precipitation. B. Average annual precipitation in Hungary in the 20^{th} century. C. Monthly distribution of the long-term average and 2008 annual precipitation. D. Daily distribution of monthly precipitation (May 2008) at two nearby meteorological stations

(Hydro)Physical properties of soils

There are two additional reasons of extreme soil moisture regime:

- the heterogeneous microrelief of the "flat" lowland;
- the highly variable, sometimes mosaic-like soil cover and the unfavourable physical and hydrophysical properties of some soils [10, 13].

According to our comprehensive assessment 43% of Hungarian soils can be characterized by unfavourable, 26% by moderately (un)favourable and 31% by favourable moisture regime, as illustrated by Figure 4, indicating the main reasons of various moisture conditions.

In the last years a comprehensive soil survey-analysis-categorization-mapping-monitoring system was developed in Hungary for the exact characterization of hydrophysical properties, modelling and forecast of water and solute regimes of soils. The system was used efficiently in the planning, implementation, operation, maintenance and control of land use and agricultural water management activities [10, 14].

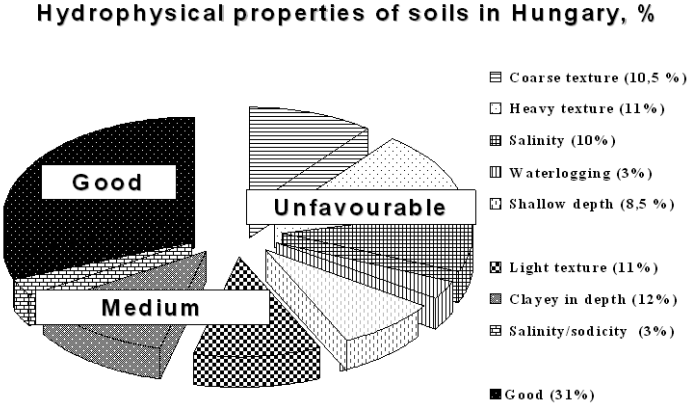

Figure 4: Moisture regimes of soils in Hungary and their reasons

The schematic map of these – quantitatively characterized – soil hydrophysical categories is presented in Figure 5.

5 Soil as water reservoir – extreme hydrological events

Under the given natural conditions it is an important fact that soil is the largest potential natural water reservoir (water storage capacity) in the Carpathian Basin. The 0-100 cm soil layer may store about half of the average annual precipitation and about 50% of it is "available moisture content". In many cases, however, this huge potential water storage capacity is not utilized because of:

- limited infiltration due to water saturated pore volume: frozen topsoil; nearly impermeable soil surface or near surface soil horizon;
- poor water retention.

As a consequence of these limitations the risk/hazard of extreme hydrological/soil moisture events (as flood, waterlogging, over-moistening vs. droughts) are characteristic features in the Carpathian Basin and occur with increasing frequency and intensity, often in the same year on the same area.

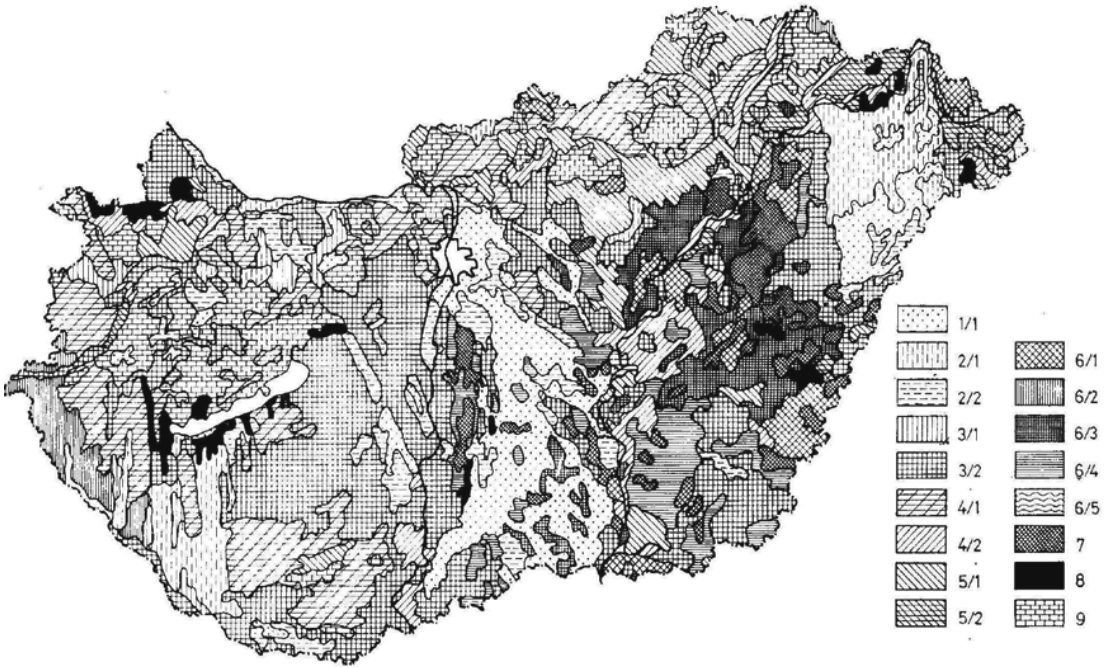

Figure 5: Hydrophysical characteristics of soils in Hungary

1.Soils with very high IR, P and K; low FC; very poor WR. 2. Soils with high IR, P and K; medium PC; and poor WR. 3. Soils with good IR, P and K; good FC; and good WR. 4. Soils with moderate IR, P and K; high FC; and good WR. 5. Soils with moderate IR, poor P and K; high PC and high WR. 6. Soils with unfavourable water management: very low IR and K. 7. Soils with extremely unfavourable water management due to high salinity/sodicity: extremely low AMR, IR and K. 8. Soils with good IR, P and K; and very high FC (organic soils). 9. Soils with extreme moisture regime due to shallow depth. *The main profile variants:* (1) texture becomes lighter with depth (soils formed on relatively light-textured parent material): 2/1, 3/1. (2) uniform texture within the profile: 1/1, 2/2, 3/2, 4/2, 5/2. (3) relative clay accumulation in the horizon B: 4/1, 5/1. Profile variants of category 6: 6/1: highly compacted, heavy-textured soils with poor structure; 6/2: pseudogleys; 6/3. deep meadow solonetzes and solonetzic meadow soils; 6/4: soils with salinity/sodicity in the deeper horizons; 6/5: peaty meadow soils

6 Unfavourable changes in the biogeochemical cycles of elements

Soil moisture regime has distinguished significance in soil productivity. It determines the water supply of plants, influences the air- and heat regimes, biological activity and plant nutrient status of soil. In most of the cases it determines the agro-ecological potential; the biomass production of various natural and agro-ecosystems; environmental sensitivity and the hazard of soil and water pollution. Extreme hydrological situations and soil moisture regime result in unfavourable changes in the biogeochemical cycles of elements (plant nutrients, pollutants) [13, 14].

7 Control of extreme moisture regime

The control of extreme field water cycle requires a special "two-way" ("double faced") soil moisture control in the Carpathian lowlands. Because the "active" water management actions, such as irrigation and drainage are faced with serious economical and environmental limitations [12] all efforts have to be taken

- to reduce evaporation, surface runoff and filtration losses;
- to increase the available moisture content of the soil: to help infiltration; increase the water storage capacity; reduce the immobile moisture content;
- to improve the vertical and horizontal drainage condition of the soil profile or the given area (prevention of over-saturation and waterlogging).

Most of these "moisture management actions" are – at the same time – efficient environment control measures [12, 13].

8 Conclusion

Sustainable and rational land use, soil and water management, including the risk reduction of extreme hydrological events and soil moisture regime requires continuous actions. This permanent control may prevent, eliminate or at least reduce undesirable soil processes and their harmful economical/ecological/ environmental/social consequences; and may satisfy the conditions for the "quality maintenance" of this "conditionally renewable" natural resource.

Control – with priorities of preventive actions – can be efficient only on the basis of comprehensive database, risk assessment, impact analysis and prognosis.

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Insecticidal potential of *Capparis decidua* on biochemical and enzymatic parameters of *Tribolium castaneum* (Herbst)

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Abstract. An insecticidal effect of solvent extract of *Caparis decidua* was exercised. Six extract, acetone, chloroform, petroleum ether, methanol, hexane and water has shown very low LD_{50} values i. e. 1.5 μg/gm, 1.2 μg/gm, 1.57 μg/gm, 0.3 μg/gm and 2.0 μg/gm. These solvent extracts have exerted toxic effects on biochemical and enzymatic parameters of *Tribolium castaneum* extracts of *C. decidua* has shown very potent activity against *T. castaneum* when insects were treated with 40% and 80% of 24-hLD50. Hexane extract has displayed a significant (p ¡ 0.05) inhibition in the level of glycogen (34.12%), protein (44.19%), DNA (41.46%), RNA (33.33%) and amino acid (30.63%). It also inhibit the activity of acid phosphatase (55.88%), alkaline phosphatase (72.90%), glutamate pyruvate transaminase (69.45%) and glutamate oxaloacetate transaminase (77.02%), lactic dehydrogenase (85.50%) and acetylcholinesterase (69.85) at a very low concentrations after 16 h treatment.

Keywords: *Tribolium castanem*, *Capparis decidua* natural pesticides, protein, amino acid glycogen, ACP, ALP

1 Introduction

Beetles (Coleoptera) and moths (Lepidoptera) are stored grain insect pest (Lepidoptera) which cause heavy loses and damage to food grains. Of these beetles are for more diversified and are highly destructive stored grain insects in comparison to moths. Both grubs and adult insects attack the stored food material while among the moth, only the caterpillars are harmful life stage that causes the damage. Besides, there are certain insect pests which do not bread in stored grains but their presence in the stores is harmful because they generate filth, noxious smell and debris. These insects are cockroaches, ants, crickets, silverfiches, pscolids, and *T. castaneum*. Few mites also cause infestation in grain flour and other stored products. Few major stored grain pests are *Sitophilus oryzae* Linn. (Rice weevil), *Trogaderma grariarium* (Kuapre beetle), *Rhizopertha dominca* (Fabr), *Tribolium castaneum* (Herbst) (Rust red flour beetle), *Sitotraga cerealella* [1]. Grain and flour moth, Bruchus chinensis (Pulse beetle). Among all the stored grain insects Tribolium is a dangerous stored grain pest that damages food grains, occurs in storehouses and godowns and has a worldwide distribution [2]. It eat the entire content of the grain and leave the hollow shell of grain behind [3]. Therefore, control of stored grain insects is highly essential. For this purpose framers and ware house owners have used synthetic chemicals mainly fumigants to control surging population of stored grain insects. But with the time due to repetitive use of synthetic pesticides, insects become resistant and are resurging enormously. Besides this, synthetic chemicals were proved highly toxic to non-tagret organisms, entered in the food chain and put adverse impact on the environment [4]. Hence, their use should be restricted to minimum. Thus, insect pests have developed resistance to many commercially available synthetic pesticides [5, 6] hence, new safe alternatives are being searched in form of bio-organic pesticides [7].

However, present plant species '*Capparis decidua*' selected for investigation possess very high insecticidal activity and belong to family Capparidaceae and is an indigenous medicinal plant, commonly known as 'Kureel' in Hindi. It is a densely branching shrub with scanty, small, caduceus leaves. Barks, leaves and roots of *C. decidua* have been claimed to relieve variety of ailments such as toothache, cough, asthma, intermittent fever and rheumatism [8]. The powdered fruit of *C. decidua* is used in anti-diabetic formulations [9]. From the above plant species both solvent and aqueous extracts prepared and tested. In the present study, insecticidal effects of *C. decidua* and its mixtures were observed on biochemical and enzymatic parameters of *Tribolium castaneum*.

2 Materials and methods

Insect culture

Adult insects of *Tribolium castaneum* (Herbst) were collected from the food grain store houses available in local market in Gorakhpur. The beetles were reared on healthy, clean and un-infested wheat seeds in glass jars and capped with muslin cloth for ventilation. Culture was maintained in laboratory under controlled temperature (28 ± 2°C), relative humidity (75 ± 5% RH) and a photoperiod of 12: 12 (L:D) h in B.O.D. Insects were reared in glass jars on gram seeds and each time early age beetles were used for the experiments.

Collection of plant material

Stems of *Capparis decidua* were collected from different places of western part of India especially from state of Rajasthan. Specimens were identified by applying standard taxonomic key specially by observing inflorescence and family formula with the help of a taxonomic expert. Fresh plant material was used to prepare extracts. Plant material was dried, chopped, grounded and milled to make powder in domestic grinder.

Preparation of extracts

Stem of *C. decidua* was collected and chopped in to small pieces, dried and pulverized to make fine powder in an electric grinder. The powdered stem (200 gm) was then extracted with various solvent according to their polarity. Extracts were allowed to evaporate in a speed vac to get residue. It was dried and weighed and re-dissolved in known volume of different solvents. Dissolved residues were stored in cold at 4°C temperature for experimental purpose.

Toxicity bio-assays

Adults of *Tribolium castaneum* were exposed with various increasing concentrations of each plant extracts separately. For this purpose, separate filter paper strips (1 cm^2) were coated with different concentrations of plant extracts were placed in the glass culture tubes and open ends were plugged with cotton balls. The coated filter paper strips were air-dried before application. Only solvent treated filter papers were strips used to set control. Ten adult insects were released culture in glass culture tubes (10 cm Height × 4 cm diameter). For each extract, five different concentrations were used and for

each concentration six replicates were set. Mortality in Tribolium castaneum was recorded after 24 hr in presence and absence of various plants extracts separately. LD_{50} values were determined by Probit method [10]. LD50 values were calculated in μg/gm body weight of the insect.

Determination of glycogen

Glycogen contents were measured according to method of Dubois et al. [11]. For this purpose 500 mg of *T. castaneum* were homogenized in 2 ml of 5% Tri-chloro acetic acid with the help of glass-glass homogenizer and centrifuged. Optical density of the reactant was read at 530 nm. Glycogen contents in unknown (supernatant) were calculated by using standard curve drawn with known amount of glucose. The blank was set by taking 0.50 ml of 5% TCA and 6 ml of concentrate H_2SO_4. The amount of glycogen was expressed in gm/100gm of body weight of *T. castaneum.* Three treatments were performed at three trials. Data obtained was statistically analyzed by using ANOVA method.

Determination of total free amino acid

Level of free amino acids was determined following Spies [12]. A total 500 mg of *T. castaneum* were homogeninzed in 2 ml of 95% ethyl alcohol. Homogenate was centrifuged at 15,000 $\times$ g for 20 minutes and supernatant was separated. For estimation of total free amino acids 0.1 ml of supernatant was taken and to it 0.1 ml of distilled water and 2.0 ml Ninhydrin reagent were mixed. The reaction mixture was kept in boiling water for 15 minutes. A total of 2 ml of 5.0 % ethyl alcohol was added to the above boiled mixture. A violet color was developed in the reaction mixture which was measured at 575 nm. For calculating the total free amino acid content standard curve was prepared by using known amount of glycine and was expressed in gm/100gm body weight of *T. castaneum.* Three replicates were used and data is statistically analyzed by ANOVA method.

Determination of nucleic acids

Level of nucleic acids in the whole body extracts of *T. castaneum* was estimated according to method of Scheidner [13]. For this purpose a total 500 mg of *T. castaneum* were fed with 40% and 80% of LD50 of different solvent extracts of C. decidua separately. Insects were scarified and homogenized in 5%TCA with glass-glass homogenizer at 15,000 $\times$ g for 25 minutes.

DNA estimation

For DNA estimation, 0.2 ml of supernatant was taken and it was diluted by adding 3.8 ml of distilled water. Then 4.0 ml of diphenylamine reagent (1 gm of diphenylamine, 100 glacial acetic acid and 2.5 ml of conc. H_2SO_4) were added to it. The mixtures were kept in boiling water bath for 10 minutes. A blue color was developed in the solution which is measured at 595 nm (O.D.).

RNA estimation

For RNA estimation 0.2 ml of supernatant was taken and it was diluted by adding 4.8 ml of distilled water. Now 2 ml of orcinol reagent (1 gm orcinol, 100 ml conc. HCl and 0.5 gm ferric acid) was added to it. The solution was kept in boiling water bath for 10 minutes, a green color was developed, which was measured at 660 nm. In both cases three replicates were set and data obtained was statistically analyzed by ANOVA method.

Determination of total protein

Total proteins of *T. castaneum* were estimated according to Lowry et al. [14]. For this purpose 500 mg of *T. castaneum* were treated with 40% and 80% of LD_{50} of different solvent extracts of C. decidua. These treated *T. castaneum* were homogenized in 4.0 ml of 10% TCA with the help of glass-glass homogenizer. The obtained homogenate was centrifuged at 15,000 × g for 15 minutes. Each experiment was performed three times. Standard curve was prepared by using 10 μg, 20 μg, 40 μg, 80 μg and 100μg of Bovine serum albumen. Data obtained was statistically analyzed by ANOVA method.

In vivo Determination of enzymatic parameters

To observe the effect on enzymatic parameters 500 mg of adult termite workers were provided sub-lethal doses (40% and 80% of LD_{50}) of different solvent extract of *C. deciduas* was provided. Insects were sacrificed at the 4 h interval up to 16 h for measurement of various enzyme levels. Insects were homogenized in phosphate saline buffer (pH 6.9) in a glass-glass homogenizer and centrifuged at 4°C for 25 minutes at 15,000 × g. Supernatant was isolated in a glass tube and used as enzyme source.

Determination of acid and alkaline phosphatase

Level of alkaline phophatase level was determined according to the method of Bergmeyer [15]. For this purpose 500 mg of *T. castaneum* were homogenized in 1 ml of PBS buffer at 4°C and centrifuged at 15,000 × g for 15 min. A 0.2 ml of supernatant was taken in a test tube and 1.0 ml of acid buffer substrate solution was added. Contents were mixed thoroughly and incubated for 30 minutes at 37°C. Now 4.0 ml of 0.10N NaOH solution was added to the incubation mixture. Similarly, for determination of ALP, 0.10 ml of supernatant was taken in a test tube and 1.0 ml of alkaline buffer substrate was mixed with it. The mixture was mixed thoroughly and incubated for 30 minutes at 37°C. Now 5.0 ml of 0.02 N NaOH was added to the incubation mixture. The reaction was stopped by adding excess of NaOH. The p-nitrophenol formed as result of hydrolysis of p-nitrophenyl phosphate gave a yellow colour with NaOH. Optical density was measured at 420 nm. Standard curve was drawn with the help of different concentrations of p-nitrophenol. Enzyme activity was expressed as μ moles of p-nitrophenol formed /30min/mg protein.

Determination of lactic dehydrogenase

Activity of lactic dehydrogenase was measured according to the method of Annon [16]. For this purpose, 100 mg of insects were homogenized in 1.0 ml of 0.1 M phosphate buffer (pH 7.5) in ice bath and centrifuged at 10000 × g for 30 minutes in cold centrifuge at 4°C. Supernatant was used as enzyme source. For determination of enzyme activity 0.05 ml of enzyme source was added to 0.50 ml of pyruvate substrate. Now the contents were incubated at 37°C for 45 minutes. Now 0.50 ml of 2,4- dinitrophenyl hydrazine solution was added and the contents were mixture and kept at the room temperature. After 20 minutes, 5.0 ml of 0.4 N NaOH was mixed and left for 30 minutes at room temperature. The optical density was measured at 540 nm and it was converted to LDH unit by drawing a standard curve. Enzyme activity has been expressed as μ moles of pyruvate reduced/45min/mg protein.

Determination of glutamate pyruvate transaminase and glutamic–oxaloacetic transaminase

GPT and GOT activity was measured according to the method of Reitman and Frankel [17]. A total of 500 mg T. castaneum were homogenized in 2 ml ice cold PBS buffer and centrifuged at 15,000 × g for 15 min at 4°C. For determining the activity of GPT, 0.10 ml of enzyme source was taken and

0.50 ml of GPT substrate. Similarly, for determination of GOT, 0.10 ml of enzyme source was taken and 0.50 ml of GOT substrate was added to it. Now 0.50 ml of 2, 4-dinitrophenyl hydrazine solution was added and contents were left stand for 15 minutes at room temperature. Then 5.0 ml of 0.4 N NaOH was added and mixed well and allowed to stand at room temperature for 20 minutes. The optical density was read at 505 nm after setting the blank. Standard curve was prepared by using oxaloacetic acid as working standard. The enzyme activity was expressed in units of glutamate pyruvate transaminase or glutamate oxaloacetate transaminase activity/ hr/mg protein

Determination of acetylcholinesterase

Acetylcholinesterase activity was determined according to the method of Ellman et al. [18]. For this purpose 500 mg treated *T. castaneum* were homogenized 50 mM phosphate buffer (pH 8) in ice bath and centrifuged at 1000 × g for 30 minutes in cold centrifuge at 4°C. To the supernatant 0.10 ml (5 × 10-4 M) of freshly prepared acetylcholinethioiodide solution, 0.05 ml of DTNB reagent (chromogenic agent) and 1.45 ml of PBS (pH 6.9) were added. The changes in optical density were monitored at 412 nm regularly for three minutes at 25°C. Enzyme activity has been expressed as moles 'SH' hydrolysed per minute per mg protein.

Statistical analysis

The LD_{50} for each extract was determined by using Probit analysis. Mean, standard deviation, standard error and Student t-test were applied [19].

3 Results

Toxicity determination

The solvent extracts of *C. deciduas* have shown potent toxicity against the insect *T. castaneum* as have shown very low LD50 i.e. 1.5 μg/gm, 1.2μg/gm, 1.2 μg/gm, 1.57 μg/gm, 0.3 μg/gm and 2.0 μg/gm of body weight of *T. castaneum* for acetone, chloroform, petroleum ether, methanol, hexane and water extracts respectively (Table 1).

Table 1: LD_{50} of different extracts of *C. deciduas* against *T. castaneum* (Herbst)

Solvent extract	LD_{50} (μg/gm)	UCL	LCL	Slope function
Acetone	1.5	2.724	0.825	1.98
Chloroform	1.2	2.198	0.655	2.00
Petroleum ether	1.2	2.313	0.622	2.12
Methanol	1.57	2.902	0.849	2.02
Hexane	0.3	0.562	0.160	2.05
Water	2.0	6.296	2.541	1.68

Determination of bio-molecules

Treatment of *T. castaneum* with sub-lethal concentration of *C. decidua* acetone, chloroform, petroleum ether, methanol hexane and water extracts have significantly depleted the glycogen content up to 36.66%, 52.09%, 47.76%, 58.08%, 34.12% and 60.84% after 16 hr. (Table2-7). Same extracts have also retard the protein synthesis and cut down it's level up to 57.44%, 46.36%, 47.57%, 46.45%, 44.19 and 54.85%. Similarly, the solvent extracts significantly inhibited the DNA content up to 58.15%, 46.96%, 42.93%, 50.08% 41.46, 54.85% and the RNA was inhabited up to 26.20% 46.82%, 61.27%, 33.33%, 47.39% and 33.91% In a similar way the amino acid content was also found to be reduced up to 52.05%, 41.76%, 28.08%, 53.87%, 30.63% and 46% (Table 2-7).

Determination of enzymes

Significant alteration in the activity of certain metabolic enzymes of *T. castaneum* was found with respect to treatment with sub-lethal concentration of different solvent extracts of *C. decidua*. Hexane extract has shown higher inhibitory activity against the enzymes and significantly reduced the body content of ACP (55.88%), ALP (72.90%), GPT (69.45%), GOT (77.02%), LDH (86.74%), and AChE (69.85%). Contrary to this, aqueous extract have shown lower activity against the enzymes and have shown lesser inhibition i. e. 81.54%, 87.37%, 94.36%, 92.74%, 96.69% and 91.58% in ACP, ALP, GPT, GOT, LDH and AChE contents (Table 8-13). Meanwhile, acetone chloroform petroleum ether and methanol have shown moderate activity against these enzymes, data presented in tables 8-13.

Table 2: Effect of 40% and 80% of LD_{50} of *Capparis decidua* acetone fraction on glycogen, protein, DNA, RNA and amino acid of *Tribolium castaneum* (Herbst).

Parameters	0 (Control)	Time (in h)							
		4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	2.01±0.04 (100)	1.413±0.055 (70.29)	1.203±0.026 (59.85)	1.340±0.032 (66.66)	1.157±0.014 (57.56)	1.230±0.023 (61.19)	0.957±0.015 (47.61)	0.900±0.017 (44.77)	0.737±0.023 (36.66)
Protein	9.187±0.070 (100)	9.097±0.038 (99.02)	8.057±0.096 (87.70)	8.210±0.021 (89.36)	7.750±0.046 (84.36)	7.787±0.052 (84.76)	6.037±0.135 (65.71)	5.717±0.059 (62.23)	5.277±0.106 (57.44)
D.N.A.	0.545±0.001 (100)	0.517±0.0038 (94.84)	0.442±0.0014 (81.08)	0.472±0.0056 (86.59	0.418±0.0032 (76.68)	0.210±0.005 (75.95)	0.366±0.0014 (67.14)	0.339±0.0018 (62.19)	0.317±0.0063 (58.15)
R.N.A.	0.519±0.007 (100)	0.354±0.0011 (68.21)	0.326±0.0038 (62.81)	0.315±0.0056 (60.69)	0.262±0.0050 (50.48)	0.210±0.005 (40.46)	0.193±0.0029 (37.19)	0.174±0.0061 (33.53)	0.136±0.0041 (26.20)
Amino acid	0.826±0.005 (100)	0.778±0.0022 (94.18)	0.723±0.0061 (87.53)	0.611±0.002 (73.97)	0.520±0.0072 (62.95)	0.556±0.002 (67.31)	0.488±0.0076 (59.08)	0.478±0.0027 (57.87)	0.430±0.005 (52.05)

Values are mean ±SE of three replicates

Table 3: Effect of 40% and 80% of LD_{50} of *Capparis decidua* chloroform fraction on glycogen, protein, DNA, RNA and amino acid of *Tribolium castaneum* (Herbst).

Parameters	0 (Control)	Time (in h)							
		4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	2.01±0.04 (100)	1.867±0.029 (92.88)	1.677±0.026 (83.43)	1.603±0.033 (79.75)	1.487±0.030 (73.98)	1.517±0.020 (75.47)	1.190±0.017 (59.20)	1.157±0.026 (57.56)	1.047±0.070 (52.09)
Protein	9.187±0.070 (100)	8.303±0.122 (90.38)	7.420±0.108 (80.77)	7.510±0.112 (81.75)	6.140±0.076 (66.83)	6.757±0.098 (73.55)	5.437±0.064 (59.18)	5.530±0.097 (60.19)	4.260±0.095 (46.36)
D.N.A.	0.545±0.001 (100)	0.479±0.0029 (87.870	0.430±0.0033 (78.88)	0.441±0.0048 (80.90)	0.381±0.0044 (69.89)	0.385±0.0015 (70.63)	0.364±0.0026 (66.77)	0.303±0.0018 (55.58)	0.256±0.0037 (46.96)
R.N.A.	0.519±0.007 (100)	0.439±0.0035 (84.59)	0.399±0.383 (76.88)	0.419±0.0037 (80.73)	0.383±0.0033 (73.80)	0.367±0.0067 (70.71)	0.339±0.004 (65.32)	0.311±0.0021 (59.92)	0.243±0.0035 (46.82)
Amino acid	0.826±0.005 (100)	0.682±0.0012 (82.56)	0.460±0.0030 (55.68)	0.538±0.0011 (65.13)	0.427±0.002 (51.69)	0.475±0.0017 (57.50)	0.375±0.0015 (45.40)	0.387±0.0025 (46.85)	0.345±0.0006 (41.76)

Values are mean ±SE of three replicates

Table 4: Effect of 40% and 80% of LD_{50} of *Capparis decidua* petroleum ether fraction on glycogen, protein, DNA, RNA and amino acid of *Tribolium castaneum* (Herbst)

Parameters	0 (Control)	Time (in h)							
		4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	2.01±0.04 (100)	1.710±0.017 (85.07)	1.463±0.035 (72.78)	1.463±0.023 (72.78)	1.263±0.018 (62.83)	1.317±0.024 (65.52)	1.160±0.023 (57.71)	1.190±0.023 (59.20)	0.960±0.015 (47.76)
Protein	9.187±0.070 (100)	8.453±0.070 (92.01)	7.503±0.084 (81.67)	7.247±0.110 (78.88)	6.290±0.078 (68.47)	6.747±0.062 (73.44)	5.327±0.11 (57.98)	5.447±0.090 (59.29)	4.370±0.0095 (47.57)
D.N.A.	0.545±0.001 (100)	0.368±0.0035 (67.51)	0.340±0.0023 (62.37)	0.335±0.0024 (61.45)	0.293±0.0024 (53.75)	0.325±0.0043 (59.62)	0.256±0.0014 (46.96)	0.268±0.0012 (49.16)	0.234±0.0023 (42.93)
R.N.A.	0.519±0.007 (100)	0.514±0.0075 (99.04)	0.449±0.0044 (86.51)	0.468±0.0026 (90.17)	0.403±0.0047 (77.65)	0.429±0.0029 (82.66)	0.359±0.004 (69.17)	0.362±0.0072 (69.75)	0.318±0.0043 (61.27)
Amino acid	0.826±0.005 (100)	0.558±0.0042 (67.55)	0.501±0.0027 (60.65)	0.420±0.0034 (50.84)	0.315±0.0044 (38.13)	0.357±0.0024 (43.22)	0.283±0.0036 (34.26)	0.263±0.0018 (31.84)	0.232±0.0046 (28.08)

Values are mean ±SE of three replicates

Table 5: Effect of 40% and 80% of LD_{50} of *Capparis decidua* methanol fraction on glycogen, protein, DNA, RNA and amino acid of *Tribolium castaneum* (Herbst).

Parameters	0 (Control)	Time (in h)							
		4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	2.01±0.04 (100)	1.673±0.0176 (83.23)	1.423±0.032 (70.79)	1.504±0.042 (74.82)	1.347±0.035 (67.01)	1.297±0.014 (64.52)	1.217±0.032	1.183±0.018 (58.85)	1.167±0.041 (58.08)
Protein	9.187±0.070 (100)	7.840±0.081 (85.34)	7.030±0.089 (76.52)	6.823±0.090 (74.27)	6.277±0.066 (68.32)	5.760±0.046 (62.70)	5.183±0.081 (56.42)	4.080±0.092 (44.41)	4.267±0.093 (46.45)
D.N.A.	0.545±0.001 (100)	0.461±0.0018 (84.57)	0.431±0.001 (79.08)	0.413±0.0021 (75.77)	0.363±0.0029 (66.60)	0.333±0.0018 (61.09)	0.319±0.0024 (58.52)	0.307±0.0035 (56.32)	0.273±0.0018 (50.08)
R.N.A.	0.519±0.007 (100)	0.352±0.0023 (67.82)	0.313±0.0052 (60.30)	0.322±0.0033 (62.04)	0.259±0.004 (49.90)	0.244±0.0021 (47.01)	0.228±0.0043 (43.93)	0.210±0.0038 (40.46)	0.173 ±0.0032 (33.33)
Amino acid	0.826±0.005 (100)	0.695±0.0026 (84.140	0.675±0.0017 (81.71)	0.603±0.0048 (73.00)	0.559±0.0024 (67.67)	0.546±0.0012 (66.10)	0.505±0.001 (61.13)	0.583±0.0014 (58.47)	0.445±0.0026 (53.87)

Values are mean ±SE of three replicates

Table 6: Effect of 40% and 80% of LD_{50} of *Capparis decidua* hexane fraction on glycogen, protein, DNA, RNA and amino acid of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	2.01±0.04 (100)	1.323±0.026 (65.82)	1.213±0.058 (60.35)	1.237±0.026 (61.54)	0.970±0.0029 (48.26)	1.147±0.064 (57.06)	0.767±0.014 (38.16)	1.140±0.032 (56.71)	0.700±0.032 (34.12)
Protein	9.187±0.070 (100)	7.537±0.081 (65.82)	7.347±0.052 (79.97)	7.163±0.067 (77.97)	6.803±0.066 (74.05)	6.900±0.053 (75.11)	5.250±0.032 (57.15)	4.630±0.061 (50.40)	4.060±0.070 (44.19)
D.N.A.	0.545±0.001 (100)	0.362±0.0023 (66.41)	0.313±0.0029 8	0.307±0.0014 (56.32)	0.273±0.0013 (50.08)	0.275±0.0018 (50.45)	0.254±0.0026 (46.60)	0.251±0.007 (46.04)	0.226±0.0073 (41.46)
R.N.A.	0.519±0.007 (100)	0.469±0.0049 (90.36)	0.396±0.0022 (76.30)	0.430±0.003 (82.85)	0.349±0.004 (67.24)	0.384±0.006 (73.98)	0.313±0.0018 (60.30)	0.356±0.0046 (68.59)	0.246±0.0038 (47.39)
Amino acid	0.826±0.005 (100)	0.807±0.004 (97.69)	0.667±0.0024 (80.75)	0.722±0.0012 (87.40)	0.469±0.0024 (56.78)	0.496±0.002 (60.04)	0.346±0.0011 (41.89)	0.292±0.0023 (35.35)	0.253±0.0029 (30.63)

Values are mean ±SE of three replicates

Table 7: Effect of 40% and 80% of LD_{50} of *Capparis decidua* aqueous fraction on glycogen, protein, DNA, RNA and amino acid of *Tribolium castaneum* (Herbst).

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen	2.01±0.04 (100)	1.843±0.026 (91.69)	1.740±0.023 (86.56)	1.670±0.0173 (83.08)	1.583±0.024 (78.75)	1.390±0.063 (69.15)	1.417±0.026 (70.49)	1.313±0.030 (65.32)	1.223±0.050 (60.84)
Protein	9.187±0.070 (100)	7.060±0.099 (76.85)	6.857±0.071 (74.64)	6.257±0.078 (69.19)	5.683±0.046 (61.86)	5.170±0.043 (56.27)	4.383±0.037 (47.71)	4.643±0.075 (50.54)	3.817±0.078 (54.85)
D.N.A.	0.545±0.001 (100)	0.517±0.004 (94.84)	0.482±0.0063 (88.79)	0.457±0.0029 (83.84)	0.413±0.0047 (75.76)	0.393±0.0014 (72.09)	0.341±0.0021 (62.56)	0.319±0.0029 (58.52)	0.299±0.0049 (54.85)
R.N.A.	0.519±0.007 (100)	0.425±0.0055 (81.88)	0.378±0.0037 (72.83)	0.369±0.0052 (71.09)	0.307±0.0009 (59.15)	0.309±0.0035 (59.53)	0.244±0.0032 (47.01)	0.245±0.0047 (47.20)	0.176±0.0046 (33.91)
Amino acid	0.826±0.005 (100)	0.609±0.0012 (83.53)	0.613±0.0067 (74.21)	0.585±0.0015 (70.82)	0.459±0.0024 (55.57)	0.466±0.0045 (56.41)	0.437±0.0032 (52.90)	0.413±0.0068 (50.00)	0.380±0.0026 (46.00)

Values are mean ±SE of three replicates

Table 8: Effect of 40% and 80% of LD_{50} of *Capparis decidua* acetone fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.346±0.003 (100)	2.220±0.05 (94.62)	2.124±0.022 (90.53)	2.018±0.0024 (86.01)	1.921±0.057 (81.88)	1.918±0.013 (81.75)	1.917±0.0233 (81.71)	1.915±0.0083 (81.62)	1.913±0.012 (81.54)
ALP	1.853±0.047 (100)	1.692±0.015 (91.31)	1.586±0.021 (85.59)	1.517±0.0057 (81.86)	1.502±.0.0081 (80.05)	1.481±0.011 (79.11)	1.466±0.041 (79.11)	1.424±0.003 (76.84)	1.419±0.0013 (76.57)
GPT	4.289±0.0046 (100)	4.045±0.017 (94.31)	4.039±0.0083 (94.17)	4.031±0.0018 (93.98)	4.027±0.0012 (93.89)	4.005±0.012 (93.37)	3.912±0.003 (91.21)	3.909±0.002 (91.14)	3.886±0.0013 (90.60)
GOT	3.117±0.0012 (100)	2.949±0.022 (94.61)	2.892±0.045 (92.78)	2.849±0.004 (91.40)	2.839±0.0024 (91.08)	2.821±0.031 (90.50)	2.816±0.01 (90.34)	2.807±0.012 (90.05)	2.801±0.022 (89.86)
LDH	8.316±0.0022 (100)	8.297±0.019 (99.77)	8.287±0.012 (99.65)	8.281±0.0087 (99.57)	8.259±0.015 (99.31)	8.251±0.024 (99.21)	8.241±0.05 (99.09)	8.131±0.0017 (97.77)	8.001±0.011 (96.21)
AChE	0.962±0.0009 (100)	0.918±0.012 (95.42)	0.911±0.0011 (94.69)	0.909±0.031 (94.49)	0.891±0.035 (92.61)	0.879±0.0014 (91.37)	0.852±0.007 (88.56)	0.846±0.0013 (87.94)	0.831±0.003 (86.38)

Values are mean ±SE of three replicates

Table 9: Effect of 40% and 80% of LD_{50} of *Capparis decidua* chloroform fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.346±0.003 (100)	2.198±0.0017 (93.69)	2.104±0.016 (89.68)	2.008±0.0019 (85.59)	1.891±0.018 (80.60)	1.788±0.003 (76.21)	1.751±0.0013 (74.63)	1.735±0.001 (73.95)	1.723±0.021 (73.44)
ALP	1.853±0.047 (100)	1.611±0.0053 (86.94)	1.566±0.016 (84.51)	1.502±0.0012 (81.05)	1.479±.008 (79.81)	1.471±0.0031 (79.38)	1.455±0.006 (78.52)	1.413±0.05 (76.25)	1.411±0.0023 (76.14)
GPT	4.289±0.0046 (100)	3.895±0.007 (90.81)	3.777±0.008 (88.06)	3.661±0.0018 (85.35)	3.437±0.002 (80.13)	3.405±0.012 (79.38)	3.212±0.023 (74.88)	3.189±0.013 (74.35)	3.127±0.0043 (72.90)
GOT	3.117±0.0012 (100)	2.909±0.02 (93.32)	2.812±0.015 (90.21)	2.809±0.0019 (90.01)	2.793±0.002 (89.60)	2.721±0.013 (87.29)	2.696±0.011 (86.49)	2.687±0.0021 (86.20)	2.485±0.022 (79..72)
LDH	8.316±0.0022 (100)	8.167±0.019 (98.20)	8.127±0.021 (97.72)	8.101±0.0021 (97.41)	7.959±0.015 (95.70)	7.851±0.016 (94.40)	7.641±0.011 (91.88)	7.432±0.037 (89.36)	7.111±0.031 (85.50)
AChE	0.962±0.0009 (100)	0.903±0.011 (93.86)	0.891±0.0022 (92.61)	0.885±0.031 (91.99)	0.871±0.025 (90.54)	0.857±0.0023 (89.08)	0.841±0.019 (87.42)	0.836±0.0013 (86.90)	0.811±0.008 (84.30)

Values are mean ±SE of three replicates

Table 10: Effect of 40% and 80% of LD_{50} of *Capparis decidua* petroleum ether fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.346±0.003 (100)	2.028±0.0017 (86.44)	1.865±0.016 (79.49)	1.856±0.0019 (79.11)	1.674±0.018 (71.35)	1.629±0.0001 (69.43)	1.612±0.0013 (68.71)	1.595±0.0012 (67.98)	1.583±0.012 (67.47)
ALP	1.853±0.047 (100)	1.511±0.05 (81.54)	1.502±0.036 (81.05)	1.459±0.0022 (78.73)	1.439±.0081 (77.65)	1.421±0.0011 (76.68)	1.401±0.036 (75.60)	1.391±0.015 (75.06)	1.365±0.03 (73.66)
GPT	4.289±0.0046 (100)	3.811±0.007 (88.85)	3.711±0.01 (86.34)	3.632±0.0083 (84.68)	3.412±0.012 (79.55)	3.398±0.0021 (79.22)	3.201±0.0021 (74.63)	3.179±0.002 (74.11)	3.109±0.033 (72.48)
GOT	3.117±0.0012 (100)	2.801±0.0009 (89.86)	2.792±0.03 (89.57)	2.768±0.0019 (88.80)	2.756±0.002 (88.41)	2.702±0.033 (86.68)	2.659±0.001 (85.30)	2.652±0.003 (85.08)	2.414±0.002 (77.44)
LDH	8.316±0.0022 (100)	8.066±0.0019 (96.99)	8.048±0.012 (96.77)	8.035±0.0083 (96.62)	7.788±0.045 (93.65)	7.771±0.016 (93.44)	7.523±0.011 (90.46)	7.369±0.017 (88.61)	7.242±0.011 (87.08)
AChE	0.962±0.0009 (100)	0.811±0.013 (84.30)	0.781±0.0011 (81.18)	0.772±0.031 (80.24)	0.765±0.045 (79.52)	0.731±0.014 (75.98)	0.712±0.019 (74.01)	0.703±0.03 (73.07)	0.687±0.011 (71.41)

Values are mean ±SE of three replicates

Table 11: Effect of 40% and 80% of LD_{50} of *Capparis decidua* methanol fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.346±0.003 (100)	2.118±0.002 (90.28)	2.004±0.04 (85.42)	1.898±0.009 (80.90)	1.787±0.018 (76.17)	1.766±0.0013 (75.27)	1.731±0.0023 (73.78)	1.715±0.002 (73.10)	1.703±0.011 (72.59)
ALP	1.853±0.047 (100)	1.587±0.015 (85.64)	1.546±0.016 (83.43)	1.492±0.005 (80.51)	1.468±.0009 (79.22)	1.454±0.03 (78.46)	1.447±0.016 (78.08)	1.403±0.05 (75.71)	1.391±0.0023 (75.06)
GPT	4.289±0.0046 (100)	3.825±0.006 (89.18)	3.717±0.001 (86.66)	3.641±0.0081 (84.89)	3.417±0.0032 (79.66)	3.401±0.021 (79.23)	3.209±0.003 (74.81)	3.181±0.0032 (74.16)	3.116±0.0013 (72.65)
GOT	3.117±0.0012 (100)	2.813±0.02 (90.24)	2.802±0.015 (89.89)	2.779±0.009 (89.15)	2.764±0.0012 (88.67)	2.711±0.0013 (86.97)	2.676±0.01 (85.85)	2.666±0.034 (85.53)	2.425±0.021 (77.79)
LDH	8.316±0.0022 (100)	8.086±0.0019 (97.23)	8.059±0.032 (96.90)	8.041±0.0031 (96.69)	7.869±0.05 (94.62)	7.848±0.016 (94.37)	7.591±0.002 (91.28)	7.397±0.0009 (88.94)	7.261±0.0021 (87.31)
AChE	0.962±0.0009 (100)	0.887±0.012 (92.20)	0.872±0.0011 (90.64)	0.862±0.005 (89.60)	0.853±0.01 (88.66)	0.846±0.004 (87.94)	0.839±0.009 (87.21)	0.822±0.013 (85.44)	0.801±0.001 (83.26)

Values are mean ±SE of three replicates

Table 12: Effect of 40% and 80% of LD_{50} of *Capparis decidua* hexane fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.346±0.003 (100)	1.877±0.001 (80.00)	1.745±0.006 (74.38)	1.564±0.009 (66.66)	1.446±0.008 (61.63)	1.421±0.0013 (60.57)	1.382±0.0031 (58.90)	1.355±0.001 (57.75)	1.311±0.05 (55.88)
ALP	1.853±0.047 (100)	1.482±0.01 (79.97)	1.442±0.02 (77.78)	1.431±0.0012 (77.22)	1.425±.0001 (76.90)	1.412±0.021 (76.20)	1.391±0.006 (75.06)	1.372±0.03 (74.04)	1.351±0.0023 (72.90)
GPT	4.289±0.0046 (100)	3.802±0.007 (88.64)	3.703±0.009 (86.33)	3.522±0.008 (82.11)	3.401±0.0012 (79.29)	3.359±0.015 (78.31)	3.195±0.003 (74.49)	3.172±0.002 (73.95)	2.979±0.0013 (69.45)
GOT	3.117±0.0012 (100)	2.783±0.032 (89.28)	2.751±0.009 (88.25)	2.658±0.0019 (85.27)	2.639±0.0022 (84.66)	2.621±0.013 (84.08)	2.619±0.04 (84.02)	2.602±0.001 (83.47)	2.401±0.032 (77.02)
LDH	8.316±0.0022 (100)	7.969±0.0001 (95.82)	7.849±0.012 (94.38)	7.735±0.008 (93.01)	7.728±0.006 (92.92)	7.702±0.016 (92.61)	7.513±0.011 (90.34)	7.319±0.0009 (88.01)	7.214±0.0011 (86.74)
AChE	0.962±0.0009 (100)	0.781±0.011 (81.18)	0.774±0.0081 (80.45)	0.766±0.01 (79.62)	0.754±0.015 (78.37)	0.711±0.004 (73.90)	0.703±0.009 (73.07)	0.685±0.0013 (71.20)	0.672±0.021 (69.85)

Values are mean ±SE of three replicates

Table 13: Effect of 40% and 80% of LD_{50} of *Capparis decidua* aqueous fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Tribolium castaneum* (Herbst)

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.346±0.003 (100)	2.240±0.004 (95.48)	2.134±0.0061 (90.96)	2.038±0.002 (86.87)	1.941±0.003 (82.77)	1.938±0.013 (82.60)	1.931±0.023 (82.31)	1.925±0.038 (82.02)	1.913±0.019 (81.54)
ALP	1.853±0.047 (100)	1.792±0.01 (96.70)	1.786±0.006 (96.38)	1.717±0.005 (92.66)	1.686±.0001 (90.98)	1.671±0.0009 (90.17)	1.666±0.04 (89.90)	1.624±0.031 (87.64)	1.619±0.0002 (87.37)
GPT	4.289±0.0046 (100)	4.145±0.007 (96.64)	4.139±0.005 (96.50)	4.131±0.0031 (96.31)	4.127±0.0021 (96.22)	4.115±0.011 (95.94)	4.112±0.0013 (95.87)	4.109±0.002 (95.80)	4.056±0.013 (94.36)
GOT	3.117±0.0012 (100)	3.019±0.002 (96.85)	3.006±0.002 (96.43)	2.949±0.003 (94.61)	2.915±0.005 (93.51)	2.911±0.031 (93.39)	2.906±0.021 (93.23)	2.897±0.0034 (92.94)	2.891±0.012 (92.74)
LDH	8.316±0.0022 (100)	8.301±0.019 (99.81)	8.297±0.012 (99.77)	8.285±0.0083 (99.62)	8.269±0.001 (99.43)	8.256±0.006 (99.27)	8.248±0.03 (99.18)	8.149±0.017 (97.99)	8.041±0.031 (96.69)
AChE	0.962±0.0009 (100)	0.938±0.012 (97.50)	0.926±0.001 (96.25)	0.919±0.031 (65.53)	0.911±0.024 (94.69)	0.909±0.0023 (94.49)	0.901±0.04 (93.65)	0.896±0.0023 (93.13)	0.881±0.006 (91.58)

Values are mean ±SE of three replicates

4 Discussion

The present investigation clearly demonstrates that both solvent and aqueous extracts of *C. decidua* are highly toxic to *T. castaneum*. Each extract has shown very low LD50 value. However, maximum toxicity was obtained in hexane extract of *C. decidua* i.e. 0.3 μg/mg, while acetone, chloroform, petroleum ether methanol and water extracts has shown 1.5 μg/gm, 1.2 μg/gm, 1.2 μg/gm, 1.57 μg/gm and 2.0 μg/gm $LD_{50}50$ value. (Table 1). Similarly, *Artemisia princepi* and *Cinnamomum camphora* (L) have shown potent toxic activity against *Sitophilus oryzae* and *Bruchus rugimanus* [20] having low LD_{50} value. Chemical constituents of Foeniculum vulgare [21] and Japanese mint (Mentha arvensis) [22] have successfully control the damage caused by *S. oryzae, T. castenum* and *Rhizopertha dominica* (F) [23].

Extract from *C. deciduas* has potentially reduced the body content of glycogen highest in hexane extract i.e 34.12%. This may be due to Depletion of glycogen indicates more and more utilization of food reserves to cope up the insecticide induced stress [24]. This decrease in glycogen level may be due to high release of glucagon, corticosteroids and catecholamines which stimulate glucose production to combat energy demand. Normally in the body free glycogen floats in the haemolymph/ blood that after breakdown help to maintain glucose level in hemolymph. These changes provide ample stimulus for glycogenolysis in insect tissues and rapid utilization of glycogen units in response to stress caused by pesticide treatment [25]. Highest reduction in DNA i. e. 41.46%, RNA i. e. 33.33% and protein i. e. 44.19% was also reported in hexane extract (Table 2-7). Similarly protein and nucleic acid synthesis may also block at cellular level and catabolism get increased which results into low availability of proteins and nucleic acid. [26, 27].

Similar results were reported in *Pimpla turionella* wasp when its larvae, pupae and adult females were treated with cypermethrin. Cypermethrin affected the level of glycogen, protein and lipid [28]. Similarly cypermethirn decrease the protein level in Spodoptera litrua larvae in comparison to control [29]. Few organophosphorus insecticides such as chloropyrifos, thiamethoxam, fipronil, and malathion caused significant depletion in total protein in haemolymph and fat body of silk worm Bombyx mori [30]. Normally in the body free glycogen floats in the haemolymph/ blood that after breakdown help to maintain glucose level in blood. These changes provide ample stimulus for glycogenolysis in insect tissues and rapid utilization of glycogen units in response to stress caused by pesticide treatment [25].

In the present investigation hexane extract of C. decidua worked as enzyme inhibitor and check the activity of ACP (55.88%), ALP (72.90%), GPT (69.45%), GOT (77.02%), LDH (85.50) and AChE (69.85) (Table 8-13). Similar effects were obtained in *T. castaneum* treated with sub-lethal concentration malathion and permethrin combinations that have check the activity of activities of acetylcholine esterase, carbohydrate-metabolizing enzyme- lactate dehydrogenase, protein-metabolizing enzymes (GPT and GOT), as well as acid and basic phosphatases (ACP and ALP) [26]. Acid and alkaline phosphatase has been studied as enzymes significant in detoxification. The compound isolated from *C. deciduas* inhibits the phosphatase enzymes and made its body defense week.

Moreover, both GPT and GOT also play an important role in protein metabolism and were inhibited by the C. decidua extracts that affected the level of glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) in treated insects [31]. However, fat body and heamolymph exhibit higher glutamate oxaloacetate transaminase activity than the glutamate pyruvate transaminase. Hence, the level of heamolymph aminotransferase gets significantly decreased. Similarly an increase in glycogenesis causes a significant decrease in free amino acid level [26]. Therefore, a sharp decrease or increase in the level of above enzymes effect oxygen consumption in insects. However, inhibition of phosphatase and lactic dehydrogenase level shows tissue necrosis in insects [32]. However, this imbalance in enzyme level indicates inhibition of important metabolic pathways [33]. Similar effects on phosphatases activity were observed in Pectinophora gossypiella after insecticide treatment [34]. Hence, all significant changes in the level of ALP, ACP, GPT, GOT, LDH and AchE indicate very high insecticidal activity of the *C. decidua* extracts towards the *T. castaneum*. However, it can be concluded that *C. decidua* possess few active ingredients that might be highly effective against stored grain insects. It is proved by the results that these ingredients cause high lethality in *T. castaneum* at a very low dose and caused significant inhibition of metabolic enzymes. Therefore, it is recommended that *C. decidua* active ingredients could be used for preparation of herbal insecticidal formulation to control stored grain insects.

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Assessment of toxic effects of solvent and aqueous extracts of *Capparis decidua* on biochemical and enzymatic parameters of *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

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Abstract. Different extracts of *Capparis decidua* stem was assessed to demonstrate their toxic effects on bio-molecules and certain metabolic enzymes of *Callosobruchus chinensis*. Solvent extracts i. e. acetone, chloroform, petroleum ether, methanol, hexane and water have given very low LD_{50} values 0.580 μg/gm, 0.290 μg/gm, 0.580 μg/gm, 0.370 μg/gm 0.590 μ g/gm and 3.05 μg/gm respectively. After 16 hr of extract exposure of *C. decidua* extracts the body contents of glycogen, protein, DNA, RNA, amino acid and lipid were depleted 26.72%, 40.60%, 37.14%, 30.31%, 29.52% and 58.25% compared to control. However it also suppressed the level of metabolically significant enzymes i.e. ACP (53.08%), ALP (67.18%), GPT (67.81%), GOT (75.75%), LDH (84.19%) and AChE (60.66%). Therefore solvent and aqueous extracts of *C. deciduas* is proved to have strong insecticidal activity against *C. chinensis* and can be used for control of stored grain insects.

Keywords: *Capparis decidua*, *Callosobruchus chinensis*, biochemical changes

1 Introduction

The pulse beetle *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) are far more diversified and are highly destructive stored grain insects in comparison to moths. Both grubs and adult insects attack the stored food material and cause loss of grain [1]. Among all important stored grain pests bruchids mainly the pulse beetle, *Bruchus chinensis* is highly serious pest of stored grains, cowpea, gram, arhar, soybean, moong and urd. It damages food grains, occurs in storehouses and godowns and has a worldwide distribution. The grubs eat the entire content of the grain and leave the shell behind. Adult beetles also reside in the circular holes of the grains. For controlling losses caused by the pulse beetle, farmer apply several biological control method such as treatment with burnt wood ash, sand with high amounts of quartz and crystal of NaCl, but it was not found more successful [2]. In most of the cases to control the stored grain insect synthetic pesticides are being used [3], that imposed certain hazardous and lethal effects on non-tagret organisms and put adverse impacts on the environment. Hence, there alternative and safer formulations should explored in form of bio-organic pesticides [4]. However, present plant species '*Capparis decidua*' selected for investigation that possess very high insecticidal activity and belong to family Capparidaceae and is an indigenous medicinal plant, commonly known as 'Kureel' in Hindi. It is a densely branching shrub with scanty, small, caduceus leaves. Barks, leaves and roots of *C. decidua* have been claimed to relieve variety of ailments such as toothache, cough, asthma, intermittent fever and rheumatism [5]. The powdered fruit of *C. decidua* is used in anti-diabetic formulations [6]. Upadhyay et al., [7] have demonstrated that compound isolated from C. decidua successfully inhibit the ovipositional responses of pulse beetle *Callosobruchus chinensis*. However, in the present study, insecticidal effects of *C. decidua* were observed on biochemical and enzymatic parameters of *Callosobruchus chinensis*.

2 Materials and methods

Insect culture

Adult insects of *Callosbruchus chinensis* L. were collected from the food grain store houses available in local market in Gorakhpur. The beetles were reared on healthy, clean and un-infested wheat seeds in glass jars and capped with muslin cloth for ventilation. Culture was maintained in laboratory under controlled temperature (28±2°C), relative humidity (75±5% RH) and a

photoperiod of 12: 12 (L:D) h in B.O.D. Insects were reared in glass jars on gram seeds and each time early age beetles were used for the experiments.

Collection of plant material

Stems of *Capparis decidua* were collected from different places of western part of India especially from state of Rajasthan. Specimens were identified by applying standard taxonomic key specially by observing inflorescence and family formula with the help of a taxonomic expert. Fresh plant material was used to prepare extracts. Plant material was dried, chopped, grounded and milled to make powder in domestic grinder.

Preparation of extracts

Stem of *C. decidua* was collected and chopped in to small pieces, dried and pulverized to make fine powder in an electric grinder. The powdered stem (200 gm) was then extracted with various solvent according to their polarity. Extracts were allowed to evaporate in a speed vac to get residue. It was dried and weighed and re-dissolved in known volume of different solvents. Dissolved residues were stored in cold at 4°C temperature for experimental purpose.

Toxicity bio-assays

Adults of *C. chinensis* were exposed with various increasing concentrations of each plant extracts separately. For this purpose, separate filter paper strips (1 cm^2) were coated with different concentrations of plant extracts were placed in the glass culture tubes and open ends were plugged with cotton balls. The coated filter paper strips were air-dried before application. Only solvent treated filter papers were strips used to set control. Ten adult insects were released culture in glass culture tubes (10 cm Height $\times$ 4 cm diameter). For each extract, five different concentrations were used and for each concentration six replicates were set. Mortality in *C. chinensis* was recorded after 24 hr in presence and absence of various plants extracts separately. LD_{50} values were determined by Probit method [8]. LD_{50} values were calculated in μg/gm body weight of the insect.

Determination of glycogen

Glycogen contents were measured according to method of Dubois et al., [9]. For this purpose 500 mg of *C. chinensis* were homogenized in 2 ml of 5% Tri-

chloro acetic acid with the help of glass-glass homogenizer and centrifuged. Optical density of the reactant was read at 530 nm. Glycogen contents in unknown (supernatant) were calculated by using standard curve drawn with known amount of glucose. The blank was set by taking 0.50 ml of 5% TCA and 6 ml of concentrate H_2SO_4. The amount of glycogen was expressed in gm/100 gm of body weight of *C. chinensis*. Three treatments were performed at three trials. Data obtained was statistically analyzed by using ANOVA method.

Determination of total free amino acid

Level of free amino acids was determined following Spies, [10]. A total 500 mg of *C. chinensis* were homogeninzed in 2 ml of 95% ethyl alcohol. Homogenate was centrifuged at 15,000 × g for 20 minutes and supernatant was separated. For estimation of total free amino acids 0.1 ml of supernatant was taken and to it 0.1 ml of distilled water and 2.0 ml Ninhydrin reagent were mixed. The reaction mixture was kept in boiling water for 15 minutes. A total of 2 ml of 5.0 % ethyl alcohol was added to the above boiled mixture. A violet color was developed in the reaction mixture which was measured at 575 nm. For calculating the total free amino acid content standard curve was prepared by using known amount of glycine and was expressed in gm/100 gm body weight of *C. chinensis*. Three replicates were used and data is statistically analyzed by ANOVA method.

Determination of nucleic acids

Level of nucleic acids in the whole body extracts of *C. chinensis* was estimated according to method of Scheidner [11]. For this purpose a total 500 mg of *C. chinensis* were fed with 40% and 80% of LD_{50} of different solvent extracts of *C. decidua* separately. Insects were scarified and homogenized in 5%TCA with glass-glass homogenizer at 15,000 × g for 25 minutes.

DNA estimation

For DNA estimation, 0.2 ml of supernatant was taken and it was diluted by adding 3.8 ml of distilled water. Then 4.0 ml of diphenylamine reagent (1 gm of diphenylamine, 100 glacial acetic acid and 2.5 ml of conc. H_2SO_4) were added to it. The mixtures were kept in boiling water bath for 10 minutes. A blue color was developed in the solution which is measured at 595 nm (O.D.).

RNA estimation

For RNA estimation 0.2 ml of supernatant was taken and it was diluted by adding 4.8 ml of distilled water. Now 2 ml of orcinol reagent (1 gm orcinol, 100 ml conc. HCl and 0.5 gm ferric acid) was added to it. The solution was kept in boiling water bath for 10 minutes, a green color was developed, which was measured at 660 nm. In both cases three replicates were set and data obtained was statistically analyzed by ANOVA method.

Determination of total protein

Total proteins of *C. chinensis* were estimated according to Lowry et al., [12]. For this purpose 500 mg of *C. chinensis* were treated with 40% and 80% of LD_{50} of different solvent extracts of *C. deciduas*. These treated *C. chinensis* were homogenized in 4.0 ml of 10% TCA with the help of glass-glass homogenizer. The obtained homogenate was centrifuged at 15,000 × g for 15 minutes. Each experiment was performed three times. Standard curve was prepared by using 10 μg, 20 μg, 40 μg, 80 μg and 100 μg of Bovine serum albumen. Data obtained was statistically analyzed by ANOVA method.

Determination of Total lipid

Level of total lipid in whole body extracts of *C. chinensis* was estimated according to method of Floch et al., [13]. A total of 500 mg of insects homogenized in 5 ml of chloroform and methanol mixture (2:1 v/v). Total lipid contents were weighted at the end and expressed in gm/100 gm body weight of insect. Three replicates were set and data was statistically analyzed by ANOVA method.

In vivo Determination of enzymatic parameters

To observe the effect on enzymatic parameters 500 mg of *C. chinensis* were provided sub-lethal doses (40% and 80% of LD_{50}) of different solvent extract of *C. decidua* was provided. Insects were sacrificed at the 4 h interval up to 16 h for measurement of various enzyme levels. Insects were homogenized in phosphate saline buffer (pH 6.9) in a glass-glass homogenizer and centrifuged at 4°C for 25 minutes at 15,000 × g. Supernatant was isolated in a glass tube and used as enzyme source.

Determination of acid and alkaline phosphatase

Level of alkaline phophatase level was determined according to the method of Bergmeyer, [14]. For this purpose 500 mg of *C. chinensis* were homogenized in 1 ml of PBS buffer at 4°C and centrifuged at 15,000 × g for 15 min. A 0.2 ml of supernatant was taken in a test tube and 1.0 ml of acid buffer substrate solution was added. Contents were mixed thoroughly and incubated for 30 minutes at 37°C. Now 4.0 ml of 0.10 N NaOH solution was added to the incubation mixture. Similarly, for determination of ALP, 0.10 ml of supernatant was taken in a test tube and 1.0 ml of alkaline buffer substrate was mixed with it. The mixture was mixed thoroughly and incubated for 30 minutes at 37°C. Now 5.0 ml of 0.02 N NaOH was added to the incubation mixture. The reaction was stopped by adding excess of NaOH. The p-nitrophenol formed as result of hydrolysis of p-nitrophenyl phosphate gave a yellow colour with NaOH. Optical density was measured at 420 nm. Standard curve was drawn with the help of different concentrations of p-nitrophenol. Enzyme activity was expressed as μ moles of p-nitrophenol formed /30min/mg protein.

Determination of lactic dehydrogenase

Activity of lactic dehydrogenase was measured according to the method of Annon, [15]. For this purpose, 100 mg of insects were homogenized in 1.0 ml of 0.1 M phosphate buffer (pH 7.5) in ice bath and centrifuged at 10000 × g for 30 minutes in cold centrifuge at 4°C. Supernatant was used as enzyme source. For determination of enzyme activity 0.05 ml of enzyme source was added to 0.50 ml of pyruvate substrate. Now the contents were incubated at 37°C for 45 minutes. Now 0.50 ml of 2,4- dinitrophenyl hydrazine solution was added and the contents were mixture and kept at the room temperature. After 20 minutes, 5.0 ml of 0.4 N NaOH was mixed and left for 30 minutes at room temperature. The optical density was measured at 540 nm and it was converted to LDH unit by drawing a standard curve. Enzyme activity has been expressed as moles of pyruvate reduced/45min/mg protein.

Determination of glutamate pyruvate transaminase and glutamic-oxaloacetic transaminase

GPT and GOT activity was measured according to the method of Reitman and Frankel, [16]. A total of 500 mg *C. chinensis* were homogenized in 2 ml ice cold PBS buffer and centrifuged at 15,000 × g for 15 min at 4°C. For determining the activity of GPT, 0.10 ml of enzyme source was taken and

0.50 ml of GPT substrate. Similarly, for determination of GOT, 0.10 ml of enzyme source was taken and 0.50 ml of GOT substrate was added to it. Now 0.50 ml of 2, 4-dinitrophenyl hydrazine solution was added and contents were left stand for 15 minutes at room temperature. Then 5.0 ml of 0.4 N NaOH was added and mixed well and allowed to stand at room temperature for 20 minutes. The optical density was read at 505 nm after setting the blank. Standard curve was prepared by using oxaloacetic acid as working standard. The enzyme activity was expressed in units of glutamate pyruvate transaminase or glutamate oxaloacetate transaminase activity/ hr/mg protein

Determination of acetylcholinesterase

Acetylcholinesterase activity was determined according to the method of Ellman et al., [17]. For this purpose 500 mg treated *C. chinensis* were homogenized 50 mM phosphate buffer (pH 8) in ice bath and centrifuged at 1000 × g for 30 minutes in cold centrifuge at 4°C. To the supernatant 0.10 ml (5×10^{-4} M) of freshly prepared acetylcholinethioiodide solution, 0.05 ml of DTNB reagent (chromogenic agent) and 1.45 ml of PBS (pH 6.9) were added. The changes in optical density were monitored at 412 nm regularly for three minutes at 25°C. Enzyme activity has been expressed as moles 'SH' hydrolysed per minute per mg protein.

Statistical analysis

The LD_{50} for each extract was determined by using Probit analysis. Mean, standard deviation, standard error and Student t-test were applied [18].

3 Results

Toxicity determination

The solvent extracts of *C. decidua* have shown a higher toxic potency a against the insect *C. chinensis* exhibiting very low LD_{50} i.e. 0.580 μg/gm, 0.290 μg/gm, 0.580 μg/gm, 0.370 μg/gm, 0.590 μg/gm, and 3.05 μg/gm of body weight of *C. chinensis* respectively for acetone, chloroform, petroleum ether, methanol, hexane and water extracts (Table 1).

Determination of bio-molecules

C. chinensis exposed with sub-lethal concentration of acetone, chloroform, petroleum ether, methanol hexane and water fraction of *C. decidua* have shown significant depleted in glycogen content up to 39.85%, 33.05%, 33.24%, 33.33%, 26.72 and 34.86% after 16 hr (Table2-7). The same treatment have retard the protein synthesis and protein content was found 49.79%, 53.73%, 49.11%, 50.24%, 40.27% and 49.60% in comparison to control insects (Table 2-7). It significantly cut down the DNA level up to 46.94%, 44.61%, 37.14%, 43.58%, 38.16% and 43.62%. In a similar consequence RNA content was also found to be decreased 35.62%, 30.31%, 43.15%, 48.28%, 49.27% and 52.82% (Table 2-7). Similarly, a remarkable suppression (49.49%, 43.78%, 40.49%, 48.61%, 29.52% and 53.22%) was reported in amino acid content, while a initial increase was observed in lipid content later it was cut down up to 66.34%, 74.59%, 74.67%, 75.08%, 74.75% and 58.25% compared to control (Table 2-7)

Determination of enzymes

Sub-lethal doses of acetone, chloroform, petroleum ether, methanol hexane and water fractions of *C. decidua* have expressed physiologically little or more toxic effects on certain enzymes in insect body. Concerned to this significant (p ¡ 0.05) deletion in acid phosphatase was recorded 83.97%, 71.83%, 64.37%, 71.65%, 53.08% and 81.38% in comparison to control respectively for the above fractions (Table 8-13). Similarly, alkaline phosphatase was cut down up to 77.28%, 73.15%, 68.58%, 70.25%, 67.18% and 83.53% after 16 hr (Table 8-13). Further, depletion was also noticed in GPT (75.87%, 72.18%, 72.47%, 72.06%, 67.81% and 97.87%) and GOT (89.46%, 77.54%, 76.31%, 76.25%, 75.75% and 86.15%). These extracts caused a slight decrease of 95.30%, 84.19%, 85.78%, 85.83%, 85.37% and 95.30 in lecatic dehydrogenase level (Table 8-13). However it have also caused neurotoxic effects and block AChE activity up to 75.15%, 75.26%, 64.39%, 75.15%, 60.66% and 84.32% in comparison to control (Table 8-13).

4 Discussion

Although stored grain insects are being controlled by synthetic pesticides, it is contaminating the environment and causing food poisoning. However in the present investigation natural extracts isolated from *C. deciduas* have used to observe its lethality in *C. chinensis*. Results obtained in the present investigation clearly demonstrate that both solvent and aqueous extracts of *C. decidua* are highly toxic to *C. chinensis* as each extract have shown high toxicity with very low LD_{50} values. However, maximum toxicity was obtained in chloroform extract i.e. 0.290 μg/mg, while acetone petroleum ether, methanol, hexane and water extracts have shown 0.580 μg/gm, 0.580 μg/gm, 0.370 μg/gm, 0.590 μg/gm and 3.05 μg/gm LD_{50} value. Similarly, active compounds isolated from Piper nigrum have shown strong insecticidal activity *Tribolium castaneum* [19]. Besides this, *Foeniculum vulgare* [20] and Azadirachta indica [21] have been reported to have strong toxic potential against stored grain insects. Fractions of *C. decidu*a have shown significant decrease in glycogen (26.72%), protein (40.27%), DNA (37.14%), RNA (30.31%), amino acid (29.52) and lipid (58.25%) contents after 16 hr of exposure.. Similarly, *Pimpla turionella* wasp treated with cypermethrin have dsisplayed remarkable changes in glycogen, protein and lipid [22]. It is the sign of stressful condition and to meet the energy demand glycogen was broken down [23]. It is evident that glycogen is primary reservoir of energy then protein and lipid compensates the energy demand. Parallel depletion in glycogen, protein and lipid indicates more and more utilization of food reserves to cope up the insecticide induced stress [24]. These changes provide ample stimulus for glycogenolysis in insect tissues and rapid utilization of glycogen units in response to stress caused by pesticide treatment [25]. Similarly protein and nucleic acid synthesis may also block at cellular level and catabolism get increased which results into low availability of proteins and nucleic acid.

More specifically, *C. decidua* extract have shown significant inhibition in certain enzymes i.e. ACP (53.08%), ALP (67.18%), GPT (72.18%), GOT (75.75%), LDH (84.19%) and AChE (60.66%). This reduction indicates the obstruction in their chemical pathways. This led to the formation of abnormal state in the insects and make insects unable to survive. Similarly solvent and aqueous extracts of *Gloriosa superba* [26], *Cassia obtusifolia* [27], *Artemisia annua* [28], *Teucrium royleanum* significantly inhibit certain enzymes like acetyl cholinesterase, lipoxygenase, urease and alkaline phosphatase, amino transferase of insects [29].

To fight with stress insect show significant induction in hydrolytic activities with in the body tissues which cut down the acid and alkaline phosphatase level [30]. Similarly in presence of toxicant transamination of amino acids get increase, hence the synthesis glutamate pyruvate transaminase, glutamate oxalo acetate transaminases get retard [31]. Similarly increase in lactic dehydrogenase level shows tissue necrosis in insects. Therefore, a decrease in the level of above enzymes effect oxygen consumption in insects. Solvent extracts significantly altered phosphatases, transaminase, dehydrogenase and esterase levels, which indicate very high toxic effects on body tissues of the insect. Hence it can be concluded that above plant species can be used for isolation of bio-pesticides to control pulse beetles (*C. chinensis*). For this purpose, constituent's level study along with structure activity relationships of natural products is to be required. Certainly active components from prepared plant species would show wider insecticidal performance and efficacy against *C. chinensis*.

Table 1: LD_{50} of different extracts of *Capparis decidua* against *C. chinensis*

Solvent extract	LD_{50} (μg/gm)	UCL	LCL	Slope function
Acetone	0.580	1.057	0.317	1.99
Chloroform	0.290	0.598	0.140	2029
Petroleum ether	0.580	1.141	0.294	2.17
Methanol	0.370	0.751	0.182	2025
Hexane	0.590	1.10	0.316	2.04
Water	3.05	5.758	1.615	2.07

Table 2: Effect of 40% and 80% of LD_{50} of *Capparis decidua* acetone fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.861±0.0081 (86.63)	1.782±0.0062 (82.95)	1.664±0.01 (77.46)	1.615±0.0048 (75.18)	1.338±0.0062 (62.28)	1.232±0.0046 (57.35)	0.933±0.0035 (43.43)	0.856±0.005 (39.85)
Protein (µg/gm)	10.512±0.0031 (100)	8.327±0.0064 (70.19)	7.366±0.004 (70.05)	7.173±0.0071 (68.21)	6.864±0.0056 (65.28)	6.782±0.0041 (64.49)	6.359±0.002 (60.47)	5.372±0.0069 (51.09)	5.2363±0.0048 (49.79)
DNA (µg/gm)	0.924±0.0042 (100)	0.8458±0.0056 (91.53)	0.7411±0.0059 (80.20)	0.7344±0.0068 (79.48)	0.6424±0.0045 (69.52)	0.5839±0.002 (63.19)	0.5404±0.0007 (58.48)	0.5057±0.0094 (54.73)	0.4338±0.053 (46.94)
RNA (µg/gm)	0.6848±0.0002 (100)	0.48535±0.004 (70.60)	0.4337±0.0053 (63.33)	0.3529±0.005 (51.04)	0.3643±0.007 (53.20)	0.3248±0.0002 (47.43)	0.475±0.002 (46.49)	0.2551±0.007 (37.25)	0.2439±0.004 (35.62)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.707±0.004 (77.58)	0.679±0.004 (74.51)	0.611±0.0035 (67.04)	0.609±0.0063 (66.82)	0.531±0.0023 (58.27)	0.475±0.009 (52.12)	0.47±0.0023 (51.57)	0.451±0.0035 (49.49)
Lipid (µg/gm)	1.212±0.0029 (100)	1.427±0.0145 (117.74)	1.605±0.0017 (132.43)	1.238±0.0019 (102.15)	1.303±0.002 (107.57)	0.803±0.0024 (66.86)	1.017±0.012 (83.91)	0.704±0.002 (58.08)	0.804±0.002 (66.34)

Values are mean ±SE of three replicates

Table 3: Effect of 40% and 80% of LD_{50} of *Capparis decidua* chloroform fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.765±0.0084 (82.16)	1.679±0.0017 (78.16)	1.589±0.0087 (73.97)	1.426±0.0077 (66.38)	1.477±0.007 (68.75)	1.369±0.0063 (63.73)	0.816±0.0049 (37.98)	0.71±0.0032 (33.05)
Protein (µg/gm)	10.512±0.0031 (100)	8.934±0.0058 (84.96)	8.4330±0.0084 (80.20)	7.565±0.006 (71.94)	7.407±0.008 (70.44)	6.986±0.003 (66.44)	6.6883±0.002 (63.60)	5.896±0.0081 (56.07)	5.6497±0.0049 (53.73)
DNA (µg/gm)	0.924±0.0042 (100)	0.8803±0.0056 (95.27)	0.7999±0.0054 (86.56)	0.7653±0.0048 (82.82)	0.6754±0.0037 (73.59)	0.6153±0.0017 (66.59)	0.5517±0.002 (59.70)	0.4830±0.0069 (52.27)	0.4122±0.0051 (44.61)
RNA (µg/gm)	0.6848±0.0002 (100)	0.5037±0.0036 (73.55)	0.4798±0.007 (70.06)	0.4386±0.0071 (64.05)	0.4133±0.0058 (60.35)	0.3476±0.0023 (50.76)	0.3013±0.003 (44.00)	0.2339±0.0094 (34.16)	0.2076±0.0054 (30.31)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.667±0.004 (73.19)	0.571±0.0018 (62.65)	0.598±0.0035 (65.62)	0.518±0.0035 (56.84)	0.526±0.0035 (57.72)	0.512±0.002 (56.18)	0.417±0.0043 (45.76)	0.399±0.0052 (43.78)
Lipid (µg/gm)	1.212±0.0029 (100)	1.507±0.004 (124.34)	1.603±0.002 (132.26)	1.302±0.0014 (107.43)	1.402±0.0012 (115.68)	1.033±0.024 (85.23)	1.002±0.0012 (82.67)	0.803±0.0014 (66.26)	0.904±0.0023 (74.59)

Values are mean ±SE of three replicates

Table 4: Effect of 40% and 80% of LD_{50} of *Capparis decidua* petroleum ether fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.785±0.0054 (83.09)	1.680±0.0046 (78.20)	1.625±0.0065 (75.64)	1.576±0.011 (73.36)	1.397±0.0052 (65.03)	1.203±0.0029 (56.00)	0.816±0.0049 (37.98)	0.714±0.0058 (33.24)
Protein (µg/gm)	10.512±0.0031 (100)	9.713±0.0078 (92.37)	8.095±0.031 (76.98)	8.135±0.0075 (77.36)	7.520±0.0084 (71.51)	6.812±0.0023 (64.78)	6.620±0.0023 (62.95)	5.233±0.0055 (49.76)	5.1642±0.0039 (49.11)
DNA (µg/gm)	0.924±0.0042 (100)	0.9163±0.0063 (99.16)	0.8645±0.0074 (93.56)	0.8287±0.0032 (89.68)	0.756±0.0073 (81.81)	0.5476±0.002 (59.26)	0.4519±0.001 (48.90)	0.3698±0.0053 (40.02)	0.3432±0.0077 (37.14)
RNA (µg/gm)	0.6848±0.0002 (100)	0.6179±0.0013 (90.23)	0.5440±0.0038 (79.44)	0.5532±0.0036 (80.78)	0.5155±0.005 (75.28)	0.4313±0.0018 (62.98)	0.3758±0.0013 (54.88)	0.3089±0.0039 (45.11)	0.2955±0.0058 (43.15)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.845±0.0024 (92.75)	0.817±0.0035 (89.65)	0.737±0.0041 (80.87)	0.716±0.0053 (78.57)	0.614±0.0026 (67.37)	0.593±0.003 (65.07)	0.379±0.0043 (41.59)	0.369±0.0032 (40.49)
Lipid (µg/gm)	1.212±0.0029 (100)	1.304±0.0027 (107.59)	1.409±0.0047 (116.25)	1.105±0.0017 (91.17)	1.205±0.0035 (99.42)	0.904±0.002 (74.59)	1.009±0.0048 (83.25)	0.807±0.0054 (66.58)	0.905±0.0035 (74.67)

Values are mean ±SE of three replicates

Table 5: Effect of 40% and 80% of LD_{50} of *Capparis decidua* methanol fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.731±0.0052 (80.58)	1.626±0.0053 (75.69)	1.635±0.004 (76.11)	1.543±0.01 (71.83)	1.445±0.0041 (67.26)	1.276±0.0035 (59.26)	0.78±0.0023 (36.31)	0.716±0.0049 (33.33)
Protein (µg/gm)	10.512±0.0031 (100)	9.223±0.0047 (87.71)	8.223±0.0059 (78.23)	8.773±0.0069 (83.43)	7.273±0.0081 (69.17)	7.516±0.0023 (71.48)	6.939±0.0017 (65.99)	5.926±0.0053 (56.36)	5.283±0.0075 (50.24)
DNA (µg/gm)	0.924±0.0042 (100)	0.7859±0.0048 (85.05)	0.7338±0.006 (79.41)	0.5803±0.0041 (62.80)	0.5292±0.0056 (57.27)	0.5367±0.0035 (58.08)	0.4846±0.0031 (52.44)	0.4262±0.005 (46.12)	0.4027±0.0052 (43.58)
RNA (µg/gm)	0.6848±0.0002 (100)	0.5528±0.004 (80.72)	0.5113±0.0043 (74.66)	0.4303±0.002 (62.84)	0.3849±0.0055 (56.21)	0.376±0.0011 (54.91)	0.3628±0.002 (52.98)	0.3436±0.0027 (50.17)	0.3306±0.0033 (48.28)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.809±0.0024 (88.77)	0.721±0.0029 (79.11)	0.733±0.0017 (80.43)	0.678±0.0032 (74.39)	0.609±0.0027 (66.82)	0.552±0.0035 (66.57)	0.469±0.0033 (51.46)	0.443±0.0054 (48.61)
Lipid (µg/gm)	1.212±0.0029 (100)	1.305±0.0029 (107.67)	1.406±0.0035 (116.01)	1.202±0.0015 (99.18)	1.303±0.0012 (107.51)	1.104±0.0026 (91.08)	1.105±0.0014 (91.17)	1.006±0.0035 (83.00)	0.91±0.0018 (75.08)

Values are mean ±SE of three replicates

Table 6: Effect of 40% and 80% of LD_{50} of *Capparis decidua* hexane fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.652±0.01 (76.90)	1.533±0.007 (71.36)	1.315±0.004 (61.21)	1.232±0.001 (57.35)	1.182±0.0042 (55.02)	1.071±0.0029 (49.85)	0.653±0.007 (30.40)	0.574±0.0035 (26.72)
Protein (µg/gm)	10.512±0.0031 (100)	8.326±0.005 (79.18)	8.243±0.0068 (78.39)	6.955±0.0068 (66.19)	6.5843±0.0043 (62.62)	5.2187±0.0018 (49.63)	5.143±0.0024 (48.91)	4.363±0.0082 (41.49)	4.2343±0.0063 (40.27)
DNA (µg/gm)	0.924±0.0042 (100)	0.7543±0.0047 (81.63)	0.6595±0.0006 (71.37)	0.7009±0.0004 (75.85)	0.5647±0.005 (61.11)	0.5468±0.0026 (59.17)	0.5158±0.0037 (55.82)	0.3026±0.0005 (42.49)	0.3526±0.007 (38.16)
RNA (µg/gm)	0.6848±0.0002 (100)	0.5513±0.0044 (80.50)	0.4289±0.0043 (62.63)	0.473±0.0048 (69.96)	0.3726±0.0074 (54.41)	0.3944±0.002 (57.59)	0.353±0.0011 (51.55)	0.3551±0.0076 (51.85)	0.3374±0.0019 (49.27)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.706±0.003 (77.47)	0.689±0.0035 (75.60)	0.674±0.0054 (73.96)	0.607±0.0046 (66.61)	0.524±0.0032 (57.50)	0.4913±0.0023 (53.91)	0.316±0.0064 (34.67)	0.269±0.0018 (29.52)
Lipid (µg/gm)	1.212±0.0029 (100)	1.504±0.0023 (124.09)	1.604±0.0023 (132.35)	1.304±0.0023 (107.59)	1.408±0.0023 (116.17)	1.106±0.0023 (91.26)	1.104±0.0031 (91.09)	0.704±0.0023 (58.09)	0.906±0.0021 (74.75)

Values are mean ±SE of three replicates

Table 7: Effect of 40% and 80% of LD_{50} of *Capparis decidua* aqueous fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	2.058±0.01 (95.80)	1.99±0.0081 (89.84)	1.668±0.0081 (77.64)	1.53±0.01 (71.22)	1.237±0.004 (57.58)	1.191±0.0029 (55.44)	0.77±0.0053 (35.84)	0.749±0.0046 (34.86)
Protein (µg/gm)	10.512±0.0031 (100)	8.2733±0.0081 (78.68)	7.978±0.0078 (75.87)	7.231±0.0052 (68.77)	6.533±0.0081 (62.32)	6.71±0.0035 (63.81)	6.484±0.0023 (61.66)	5.2367±0.0066 (49.80)	5.2157±0.0087 (49.60)
DNA (µg/gm)	0.924±0.0042 (100)	0.8417±0.0055 (91.09)	0.7003±0.0067 (75.79)	0.7291±0.0038 (78.90)	0.6272±0.0059 (67.87)	0.5589±0.0029 (60.48)	0.5007±0.0035 (54.18)	0.4186±0.0012 (45.30)	0.4031±0.0056 (43.62)
RNA (µg/gm)	0.6848±0.0002 (100)	0.6736±0.0084 (98.36)	0.6209±0.0025 (90.67)	0.562±0.0044 (82.07)	0.4908±0.0043 (71.67)	0.4933±0.0018 (72.04)	0.4825±0.0044 (70.46)	0.3701±0.0045 (54.04)	0.3617±0.004 (52.82)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.807±0.0035 (88.55)	0.799±0.0055 (87.67)	0.72±0.0038 (79.00)	0.692±0.005 (75.93)	0.608±0.0023 (66.71)	0.578±0.002 (63.42)	0.497±0.0052 (54.53)	0.485±0.0048 (53.22)
Lipid (µg/gm)	1.212±0.0029 (100)	1.304±0.003 (107.59)	1.503±0.0024 (124.01)	1.107±0.0035 (91.34)	1.307±0.0018 (107.84)	0.806±0.0038 (66.50)	1.005±0.0017 (82.92)	0.605±0.0029 (49.92)	0.706±0.0015 (58.25)

Values are mean ±SE of three replicates

Table 8: Effect of 40% and 80% of LD_{50} of *Capparis decidua* acetone fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	2.018±0.0024 (90.08)	1.921±0.057 (85.75)	1.918±0.013 (85.62)	1.917±0.0233 (85.58)	1.915±0.0083 (85.49)	1.913±0.012 (85.40)	1.887±0.01 (84.24)	1.881±0.003 (83.97)
ALP	1.792±0.01 (100)	1.517±0.0057 (84.65)	1.502±.0.0081 (83.81)	1.481±0.011 (82.64)	1.466±0.041 (81.80)	1.424±0.003 (79.46)	1.419±0.0013 (79.18)	1.391±0.0045 (77.62)	1.385±0.0009 (77.28)
GPT	4.145±0.007 (100)	4.031±0.0018 (97.24)	4.027±0.0012 (97.15)	4.005±0.012 (96.62)	3.912±0.003 (94.37)	3.909±0.002 (94.30)	3.886±0.0013 (93.75)	3.166±0.012 (76.38)	3.145±0.024 (75.87)
GOT.	3.019±0.002 (100)	2.849±0.004 (94.36)	2.839±0.0024 (94.03)	2.821±0.031 (93.44)	2.816±0.01 (93.27)	2.807±0.012 (92.97)	2.801±0.022 (92.77)	2.711±0.00081 (89.79)	2.701±0.0017 (89.46)
LDH.	8.301±0.019 (100)	8.281±0.0087 (99.75)	8.259±0.015 (99.49)	8.251±0.024 (99.39)	8.241±0.05 (99.27)	8.131±0.0017 (97.95)	8.001±0.011 (96.38)	7.941±0.0087 (95.66)	7.911±0.0057 (95.30)
AChE	0.938±0.012 (100)	0.909±0.031 (96.90)	0.891±0.035 (94.98)	0.879±0.0014 (93.71)	0.852±0.007 (90.83)	0.846±0.0013 (90.19)	0.831±0.003 (88.59)	0.722±0.031 (76.97)	0.705±0.004 (75.15)

Values are mean ±SE of three replicates

Table 9: Effect of 40% and 80% of LD_{50} of *Capparis decidua* chloroform fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	2.008±0.0019 (89.64)	1.891±0.018 (84.41)	1.788±0.003 (79.82)	1.751±0.0013 (78.16)	1.735±0.001 (77.45)	1.723±0.021 (76.91)	1.621±0.003 (72.36)	1.609±0.031 (71.83)
ALP	1.792±0.01 (100)	1.502±0.0012 (83.81)	1.479±.008 (82.53)	1.471±0.0031 (82.08)	1.455±0.006 (81.19)	1.413±0.05 (78.85)	1.411±0.0023 (78.73)	1.324±0.008 (73.88)	1.311±0.011 (73.15)
GPT	4.145±0.007 (100)	3.661±0.0018 (88.32)	3.437±0.002 (82.91)	3.405±0.012 (82.14)	3.212±0.023 (77.49)	3.189±0.013 (76.93)	3.127±0.0043 (75.44)	3.031±0.0019 (73.12)	2.992±0.002 (72.18)
GOT.	3.019±0.002 (100)	2.809±0.0019 (93.04)	2.793±0.002 (92.51)	2.721±0.013 (90.12)	2.696±0.011 (89.30)	2.687±0.0021 (89.00)	2.485±0.022 (82.31)	2.376±0.018 (78.70)	2.341±0.0019 (77.54)
LDH.	8.301±0.019 (100)	8.101±0.0021 (97.59)	7.959±0.015 (95.88)	7.851±0.016 (94.57)	7.641±0.011 (92.04)	7.432±0.037 (89.53)	7.111±0.031 (85.66)	7.009±0.007 (84.43)	6.989±0.01 (84.19)
AChE	0.938±0.012 (100)	0.885±0.031 (94.34)	0.871±0.025 (92.85)	0.857±0.0023 (91.36)	0.841±0.019 (89.65)	0.836±0.0013 (89.12)	0.811±0.008 (86.46)	0.713±0.031 (76.01)	0.706±0.023 (75.26)

Values are mean ±SE of three replicates

Table 10: Effect of 40% and 80% of LD_{50} of *Capparis decidua* petroleum ether fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	1.856±0.0019 (82.85)	1.674±0.018 (74.73)	1.629±0.0001 (72.72)	1.612±0.0013 (71.96)	1.595±0.0012 (71.20)	1.583±0.012 (70.66)	1.471±0.01 (65.66)	1.442±0.012 (64.37)
ALP	1.792±0.01 (100)	1.459±0.0022 (81.41)	1.439±.0081 (80.30)	1.421±0.0011 (79.29)	1.401±0.036 (78.18)	1.391±0.015 (77.62)	1.365±0.03 (76.17)	1.257±0.0045 (70.14)	1.229±0.018 (68.58)
GPT	4.145±0.007 (100)	3.632±0.0083 (87.62)	3.412±0.012 (82.31)	3.398±0.0021 (81.97)	3.201±0.0021 (77.22)	3.179±0.002 (76.69)	3.109±0.033 (75.00)	3.056±0.013 (73.72)	3.004±0.021 (72.47)
GOT	3.019±0.002 (100)	2.768±0.0019 (91.68)	2.756±0.002 (91.28)	2.702±0.033 (89.49)	2.659±0.001 (88.07)	2.652±0.003 (87.84)	2.414±0.002 (79.96)	2.317±0.05 (76.74)	2.304±0.0083 (76.31)
LDH	8.301±0.019 (100)	8.035±0.0083 (96.79)	7.788±0.045 (93.82)	7.771±0.016 (93.61)	7.523±0.011 (90.62)	7.369±0.017 (88.77)	7.242±0.011 (87.24)	7.154±0.021 (86.18)	7.121±0.033 (85.78)
AChE	0.938±0.012 (100)	0.772±0.031 (82.30)	0.765±0.045 (81.55)	0.731±0.014 (77.93)	0.712±0.019 (75.90)	0.703±0.03 (74.94)	0.687±0.011 (73.24)	0.612±0.04 (65.24)	0.604±0.003 (64.39)

Values are mean ±SE of three replicates

Table 11: Effect of 40% and 80% of LD_{50} of *Capparis decidua* methanol fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	1.898±0.009 (84.73)	1.787±0.018 (79.77)	1.766±0.0013 (78.83)	1.731±0.0023 (77.27)	1.715±0.002 (76.56)	1.703±0.011 (76.02)	1.611±0.004 (71.91)	1.605±0.01 (71.65)
ALP	1.792±0.01 (100)	1.492±0.005 (83.25)	1.468±.0009 (81.91)	1.454±0.03 (81.13)	1.447±0.016 (80.74)	1.403±0.05 (78.29)	1.391±0.0023 (77.62)	1.284±0.0083 (71.65)	1.259±0.009 (70.25)
GPT	4.145±0.007 (100)	3.641±0.0081 (87.84)	3.417±0.0032 (82.43)	3.401±0.021 (82.05)	3.209±0.003 (77.41)	3.181±0.0032 (76.74)	3.116±0.0013 (75.17)	3.005±0.013 (72.49)	2.987±0.002 (72.06)
GOT	3.019±0.002 (100)	2.779±0.009 (92.05)	2.764±0.0012 (91.55)	2.711±0.0013 (89.79)	2.676±0.01 (88.63)	2.666±0.034 (88.30)	2.425±0.021 (80.32)	2.312±0.016 (76.58)	2.302±0.013 (76.25)
LDH	8.301±0.019 (100)	8.041±0.0031 (96.86)	7.869±0.05 (94.79)	7.848±0.016 (94.54)	7.591±0.002 (91.44)	7.397±0.0009 (89.10)	7.261±0.0021 (87.47)	7.154±0.021 (86.18)	7.125±0.017 (85.83)
AChE	0.938±0.012 (100)	0.862±0.005 (91.89)	0.853±0.01 (90.93)	0.846±0.004 (90.19)	0.839±0.009 (89.44)	0.822±0.013 (87.63)	0.801±0.001 (85.39)	0.711±0.007 (75.79)	0.705±0.005 (75.15)

Values are mean ±SE of three replicates

Table 12: Effect of 40% and 80% of LD_{50} of *Capparis decidua* hexane fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	1.564±0.009 (69.82)	1.446±0.008 (64.55)	1.421±0.0013 (63.43)	1.382±0.0031 (61.69)	1.355±0.001 (60.49)	1.311±0.05 (58.52)	1.201±0.0001 (53.61)	1.189±0.024 (53.08)
ALP	1.792±0.01 (100)	1.431±0.0012 (79.85)	1.425±.0001 (79.52)	1.412±0.021 (80.13)	1.391±0.006 (77.62)	1.372±0.03 (76.56)	1.351±0.0023 (75.39)	1.221±0.015 (68.13)	1.204±0.0045 (67.18)
GPT	4.145±0.007 (100)	3.522±0.008 (85.96)	3.401±0.0012 (82.05)	3.359±0.015 (81.03)	3.195±0.003 (77.08)	3.172±0.002 (76.52)	2.979±0.0013 (71.86)	2.854±0.008 (68.85)	2.811±0.015 (67.81)
GOT	3.019±0.002 (100)	2.658±0.0019 (88.04)	2.639±0.0022 (87.41)	2.621±0.013 (86.81)	2.619±0.04 (86.75)	2.602±0.001 (86.18)	2.401±0.032 (79.52)	2.298±0.012 (76.11)	2.287±0.03 (75.75)
LDH	8.301±0.019 (100)	7.735±0.008 (93.18)	7.728±0.006 (93.09)	7.702±0.016 (92.78)	7.513±0.011 (90.50)	7.319±0.0009 (88.17)	7.214±0.0011 (86.90)	7.101±0.0001 (85.54)	7.087±0.0023 (85.37)
AChE	0.938±0.012 (100)	0.766±0.01 (81.66)	0.754±0.015 (80.38)	0.711±0.004 (75.79)	0.703±0.009 (74.94)	0.685±0.0013 (7302)	0.672±0.021 (71.65)	0.585±0.0031 (62.36)	0.569±0.011 (60.66)

Values are mean ±SE of three replicates

Table 13: Effect of 40% and 80% of LD_{50} of *Capparis decidua* aqueous fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	1.941±0.003 (86.65)	1.938±0.013 (86.51)	1.931±0.023 (86.20)	1.925±0.038 (85.93)	1.913±0.019 (85.40)	1.903±0.003 (84.95)	1.841±0.023 (82.18)	1.823±0.021 (81.38)
ALP	1.792±0.01 (100)	1.686±.0001 (94.08)	1.671±0.0009 (93.24)	1.666±0.04 (92.96)	1.624±0.031 (90.62)	1.619±0.0002 (90.34)	1.594±0.0011 (88.95)	1.512±0.024 (84.37)	1.497±0.0045 (83.53)
GPT	4.145±0.007 (100)	4.127±0.0021 (99.56)	4.115±0.011 (99.27)	4.112±0.0013 (99.20)	4.109±0.002 (99.13)	4.056±0.013 (97.85)	3.991±0.02 (96.28)	3.901±0.031 (94.11)	3.891±0.0032 (93.87)
GOT	3.019±0.002 (100)	2.915±0.005 (96.55)	2.911±0.031 (96.42)	2.906±0.021 (96.25)	2.897±0.0034 (95.95)	2.891±0.012 (95.76)	2.671±0.0045 (88.47)	2.611±0.006 (86.48)	2.601±0.0009 (86.15)
LDH	8.301±0.019 (100)	8.269±0.001 (99.61)	8.256±0.006 (99.45)	8.248±0.03 (99.36)	8.149±0.017 (98.16)	8.041±0.031 (96.86)	7.981±0.01 (96.14)	7.914±0.023 (95.33)	7.911±0.05 (95.30)
AChE	0.938±0.012 (100)	0.911±0.024 (97.12)	0.909±0.0023 (96.90)	0.901±0.04 (96.05)	0.896±0.0023 (95.52)	0.881±0.006 (93.92)	0.863±0.003 (92.00)	0.813±0.031 (86.67)	0.791±0.003 (84.32)

Values are mean ±SE of three replicates

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Micrometeorological measurements in orchard and above bare soil

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Abstract. The observation and analysation of the variability and vertical profils of the meteorological parameters in orchard can be useful for choosing of suitable cultivation technologies. Investigation was carried out in Soroksár at the Experimental and Research Farm of the Corvinus University of Budapest. Monitoring was made in apple orchard and at the same time above bare soil surface. Temperature, relative humidity, global radiation and wind velocity were registrated at three different height in September 2010. We analysed the daily characters of the measured parameters at different canopy levels. The vertical gradients were compared at two different spots.

Keywords: temperature, microclimatology, wind speed, relative humidity, apple, bald surface

1 Introduction

The climate in the surface-plant-air level, the microclimate, is the issue of the interactions of the plant community with the radiant energy balance, air temperature, vapour pressure deficit and wind speed. Microclimatical measurements can usually provide some information about crop responses to weather, but even where this is not the case, they can form a vital part of

any study of plant behavior in the field. Knowledge of characteristic of the meteorological factors in intensive cultivation can help the plant protection and irrigation planning, choosing of pruning and treatment techniques. Lot of studies have been made of microclimatology of field crops [1, 2] and orchards [3, 4, 5, 6, 7] to investigate for example the water uptake of the trees [8] or to analyze the temperature of the tree's foliage [9]. The continuous monitoring of variability of meteorological parameters can help the decision about the applied technologies. By comparing of the meteorological elements measured parallel above bare soil surface and among fruit trees, can be understood the modifying effects of the plants. By inquiring of the vertical profiles it can be defined the main energy leader level.

2 Materials and Methods

The investigations were carried out in Soroksár (47°22'N,19°09E, 103 m above sea level) at the Experimental Farm of Corvinus University of Budapest in apple orchard in fourth leaf and parallel above bare soil surface. Detailed data of the apple orchard can be seen in the Table 1.

Table 1: Data of the investigated apple orchard

Planting year	Spring 2007
Species	MR-03, MR-09, MR-10,MR-11,MR-12,MR-13
Controll species	Baujade, Freedom, Florina, Prima, Produkta, Remo
Rootstock	M9
Spacing	4×1 m
Row's orientation	N-S
Trained	to slender spindle
Support system	wire
Repeat	2rows/species, 20trees/controll
Irrigation	dripping below crown level*
Plant protection	integrated, decreased

*during the experiment no irrigation

Rowspaces are grass covered, grass were kept short. Foliage commences at about 0,5 m, with the maximum density at about 1,2 m. About 300 meter to the north there is a forest belt, the crest of which is approximately 10 m above the level of the orchard. On the western side there is a building within 300 meter. On the bare soil there were not any plants expect of some weeds, which were irrelevant for our measurements. The micrometeorological

measurements are made by using 4 meter height columns. Experimental period was 7-15 September 2010, on 8 sample days, on daytime. The two experimental fields are about 300 meters from each other. Temperature, relative humidity and wind speed were registrated parallel at three different heights (1m, 2m, 4m) in the two above mentioned fields. Global radiation was registrated on 4m level. Data were registrated in every minutes and averaged in every 5 minutes. None of the investigated areas got any treatment or irrigation during the experimental period.

3 Results and discussions

In this study we show the daily (7:00 am.-3:00 p.m.) charachteristic of the different meteorological elements at the three different floor on selected day. The daily maximum temperature reached the 25°C, but did not exceed it on 15th September. There was windy day, and sometimes cloudy, that is attested by the daily variability of the global radiation. To see the Fig.1. it can be said, that the temperature in orchard at 1 m exceeded the measured one above bare soil by 1-1,5°C in the monitored hours. In spite of the opened structure of the young plantation the densest foliage level has got heat holding role. On the other hand above bare soil the heat transport was unhampered by the wind. The temperature different decreased after midday, when the global radiation relapsed, because the clouding over. Highest global radiation value was 800 Wm^{-2}.

Wind velocity has got one of the most important roles in the forming of the microclimate, because it affects the exchanging processes in the air (water vapour, heat, substances transport). At flowering time it influences the pollens transport and the insect's flight. Wind speed measured at 2 m is lower in the orchard due to the blocking effect of the plantation (Fig. 2). Above the bare soil there was not any barrier against the free wind run. The highest wind speed value did not exceed 2 ms^{-1} in the apple plantation, while above the bare soil 3,2 ms^{-1} moderate wind was measured in the afternoon at 2 m. Too high humidity level at canopy can cause infection spreading, it flavours for the bacterias rising, at the same time it can result steady water balance in the orchard [9]. Despite of the moderate wind the relative humidity on both spots was between 60-87% measured at 4 m. In the morning according to the increasing temperature the relative humidity falls 10%. Above the foliage level the humidity values are lower than above bair soil (Fig. 3).

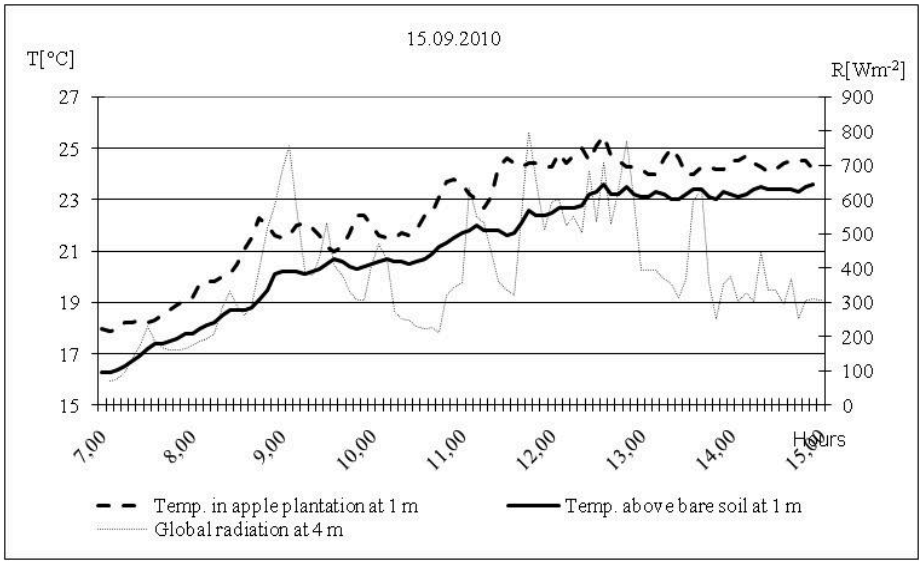

Figure 1: Daytime temperature at 1 m in orchard and above bare soil

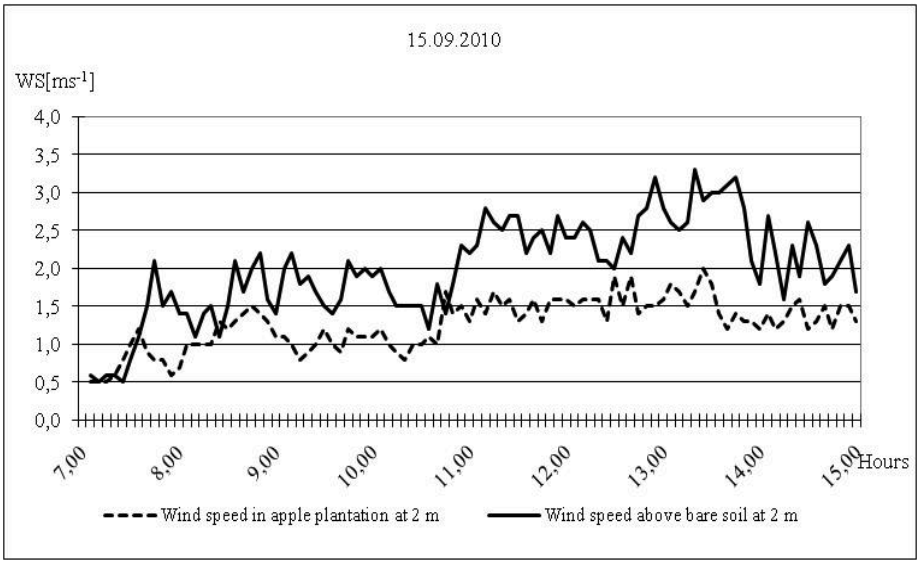

Figure 2: Daytime wind speed at 2 m in orchard and above bare soil

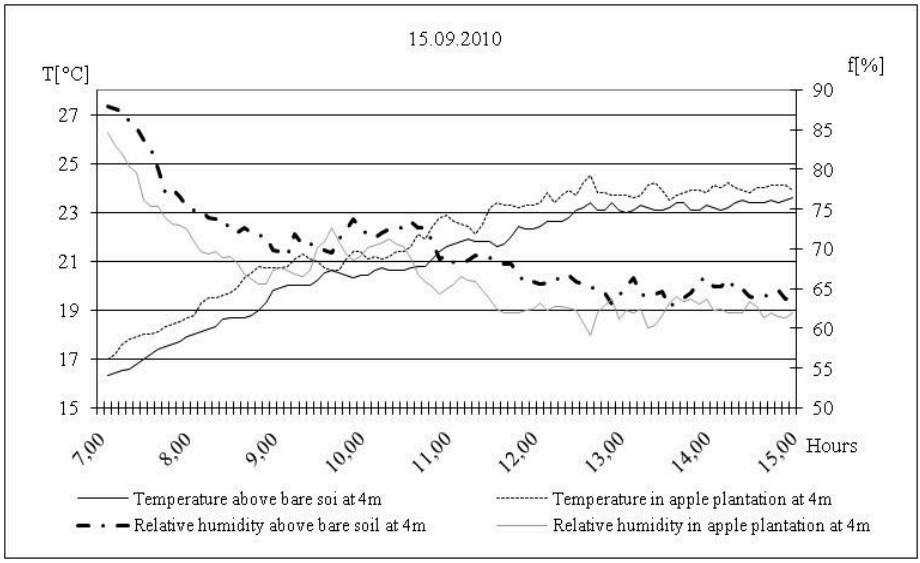

Figure 3: Daytime temperature and relative humidity at 4 m in orchard and above bare soil

Vertical profiles are shown by the Figures 4, 5, 6. By the vertical change of the temperature (Fig. 4) can be seen that the warmest level in the plantation is close to the soil, which is the active zone. Soil is heated by the radiance and than heat has being transported to the above levels. The foliages of the trees do not cover fully the rowspaces due to the opened canopy structure. Air temperature decreases exponentially by the height. The dropping is higher between 1 and 2 m than between 2 and 4 m. The relative humidity change according to the temperature explained of their relation (Fig 6.), the changing by the hight is logarithmic. To see the wind speed diagram (Fig 5.) on the selected day the wind velocity measured at 1 m above bare soil is almost the same as wind speed measured at 2 m in the orchard. The relation is logarithmic in the orchard and exponential above bare soil.

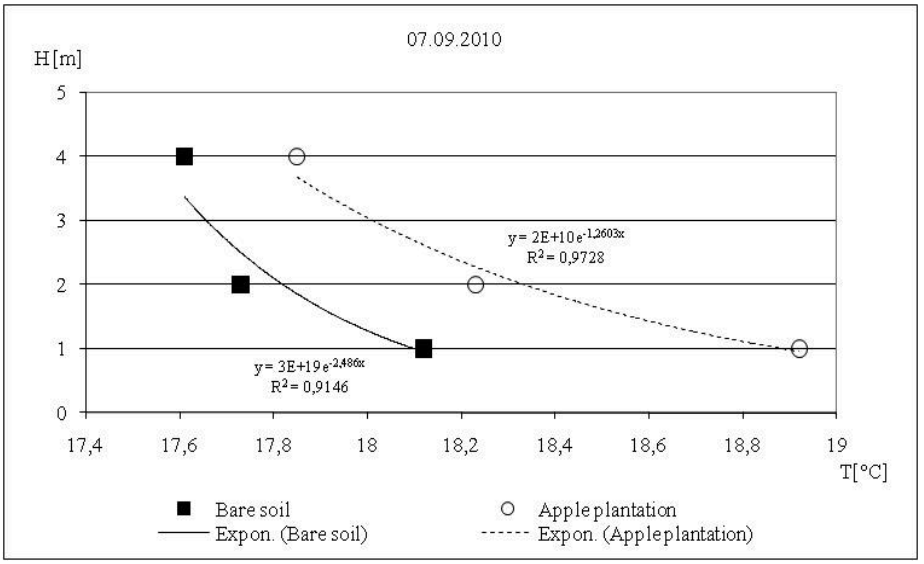

Figure 4: Vertical profil of temperature

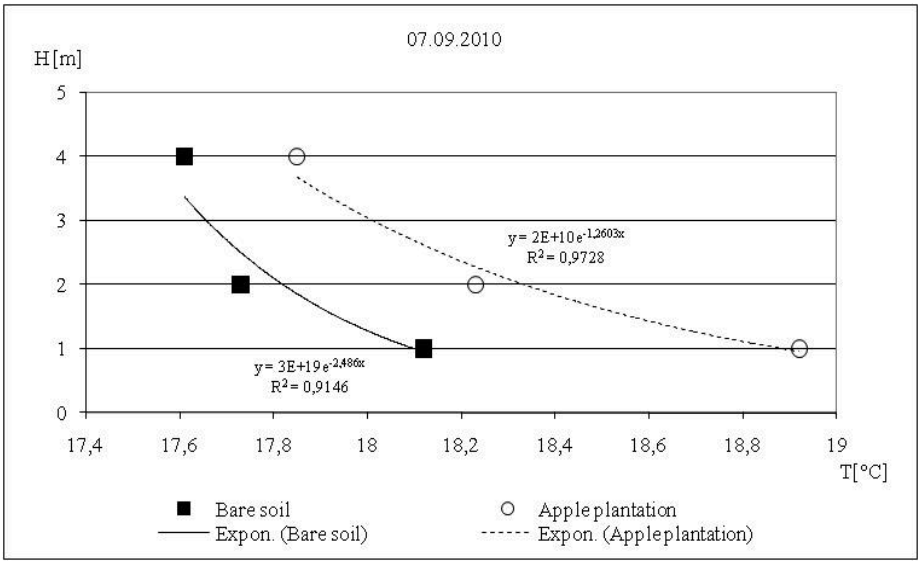

Figure 5: Vertical profil of wind speed

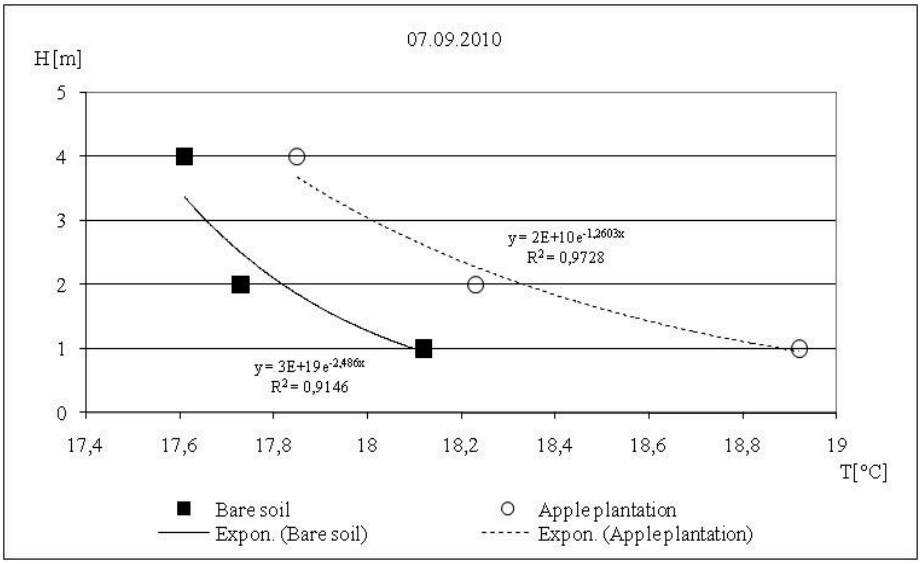

Figure 6: Vertical profil of relative humidity

4 Conclusion

The observation and analisation of the variability and vertical profiles of the meteorological parameters in orchard can be useful for choosing of suitable cultivation technologies. Knowledge of characteristic of the meteorological factors in intensive cultivation can help the plant protection and irrigation planning, choosing of pruning and treatment techniques. Our expedition measurements were carried out in Soroksár at the Experimental and Research Farm of the Corvinus University of Budapest. Investigation was carried out in young apple plantation with opened canopy structure and at the same time above bare soil surface. Temperature, relative humidity, global radiation and wind velocity were registrated at three different height (1m, 2m, 4m) in the above mentioned fields in September 2010. We have made daily (7:00 a.m.-03:00 p.m.) averages for illustrating the vertical profiles of the relative humidity, wind speed and air temperature on the two investigated areas. On both spots the surface was the prime energy - and stuff transport leader. The heating process started from the surface and than the upper layers pass each other the heat. On daytime the hottest zone was closed to the soil. It was also revealing for the orchard, because the canopy has been opened structured yet and the micrometeorolo-

gical transport processes were ruled by the surface. The relative humidity set also according to temperature (inversely proportional). Average wind speed was higher above the bare soil at 1m and 2m height than in the orchard. It increased also at 4m on both fields, but probably due to some local blow at 4m it could be stronger above the apple plantation.

Acknowledgements

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Spatial differences of night temperature in hilly regions and its horticultural importance

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Abstract. In the Carpathian Basin freezing injury is one of the main yield limiting factors in fruit production. In our research the microclimatic differences of night temperature values were studied. On the northern side of Bükk Mountains, at two nearby (app. 1 km distance) locations, parallel temperature measurements were conducted from 17th April until 4th December 2010 (HOBO UA-002 logger). The measuring points represent the fruit gardens located in valleys (260 m a.s.l) and on slopes with medium cold air drainage (330 m a.s.l), respectively.

The critical frost events occur mainly (in the vegetation period almost exclusively) at clear, calm nights. In this type of weather the valley is generally 3-5°C colder than the slope position 70 m above it. These values hint to the enormous importance of site selection of orchards which is probably the simplest and most effective way to reduce frost risk.

Keywords: minimum temperature, frost risk, microclimate, orchard, topography, site selection

1 Introduction

The importance of low temperature is well known in horticulture. In the Carpathian Basin – similarly to many temperate regions – freezing injury is

one of the main yield limiting factors in fruit production Most species are likely to suffer a serious loss due to spring frosts. One frost event is able to completely destroy the yield in some orchards, caused by the irreversible injury of reproductive organs. The losses can occur widespread in the country, or sometimes concentrated only to smaller regions. The yield-loss of particular species exceeds 50% on country-level in some years.

The autumn and winter frosts can also limit the production of some species (cultivars). In cold and temperate climates, low winter temperatures can damage the xylem, bark, roots and buds perhaps resulting the death of the trees. This limits the areal distribution of a particular crop relative clearly. However, the fruit trees that are tolerant to low winter temperature, may be very sensitive to spring frost injury [10].

The climate change probably will not reduce these risks, especially because of increasing temperature anomalies. Though, the forecasted milder winters and early springs may enhance the frost risk effecting the buds, flowers and developing fruits, because of the earlier beginning of the vegetation period [4].

Breeding of new cultivars for frost hardiness could be a possible way to handle this problem. However, these efforts are only slightly effective in reducing frost injury [10].

Different ways of frost protection are widely used in fruit growing [6, 9]. The methods show large differences in effectiveness and in costs. Generally, establishing and operating an effective protection system can take a significant part of the total costs of fruit production. Micrometeorological studies of frost events can help to develop the most suitable protection practices [11].

Low minimum temperature (different degree of frost) affects the production of most species of vegetables, as well. In this case there is an extra tool in hands of growers to reduce the risk of damage. They can minimize the damage by choosing proper sowing date [5].

The critical frost events occur mainly (in the vegetation period almost exclusively) at clear, calm nights. In this weather type the cold air layer developing due to longwave outgoing radiation of surfaces “flows down” according to the local terrain. Cold air accumulates in valleys, on low plains and in basins, while places with good cold air drainage remain relatively mild [2].

The nighttime temperature distribution in hilly areas was studied and modeled usually in case of forest or heterogeneous land cover [1, 8, 12]. Huge differences in temperature (5-10°C) can develop even within short distances (¡100 m) in case of some terrain forms [3, 7]. Against the different vegetation type, these results highlight the importance of microclimate. The microclimatic characteristics are usually considered in site selection of orchards, but until

now usually without quantitative information about temperature differences or about risk levels.

In our research we compared the nighttime thermal characteristics of two sites close to each other, representing orchards in an "average good" and in an "average unfavorable" position. Differences in daily minimum values and in courses of night temperature were analyzed according to weather type.

2 Material and methods

On the northern side of Bükk Mountains in Hungary (48°09'N, 20°30'E), at two nearby (app. 1 km distance) locations, parallel temperature measurements were conducted from 17th April until 4th December 2010. Temperature loggers (HOBO UA-002, accuracy within 0.2°C according to our tests), were placed in shelter at 2 m height, and installed to logging interval of 10 minutes. The measuring points represent the fruit gardens located in valleys (260 m a.s.l) and on slopes with medium cold air drainage (330 m a.s.l), respectively.

Hourly data of the nearest (20 km) synoptic meteorological station of the Hungarian Meteorological Service, Miskolc were used to categorize the nighttime weather. Data of temperature, air humidity, wind, cloudiness and actual weather event were read out from archived synop reports (www.ogimet.com).

The nights were chosen into weather type "calm-clear" when the minimum of temperature was developed typically under the following circumstances:

- cloudiness $\leq 2/8 (\leq 4/8$ in case of Cirrus clouds),
- wind speed ≤ 3 m/s.

As opposite, also the overcast, rainy night were studied, when the longwave radiation plays no role in forming the temperature distribution. The minimum temperature of each day (from 8 PM till 8 AM), and their differences between the two locations were calculated using Excel. The nighttime courses of temperature were displayed and compared using Hoboware program and Excel.

3 Results and discussion

In the study period 69 nights were clear and calm, according to our classification. In every occasion the minimum temperature in the valley was at least 2°C lower than that registered at the upper station. The difference was in average 3.7°C.

It was not possible to detect seasonal variation, because it was hidden by the specific, usually moist weather of this year. Instead of this we found that the differences are smaller when air humidity is higher (which is in connection with previous precipitation).

Distribution of the differences shows that in the "clear and calm" weather type the valley is generally 3-5°C colder than the slope position 70 m above it. The temperature difference was in this interval in 55 from 69 cases (80%), and it showed a maximal probability between 3.5 and 3.9°C (Figure 1).

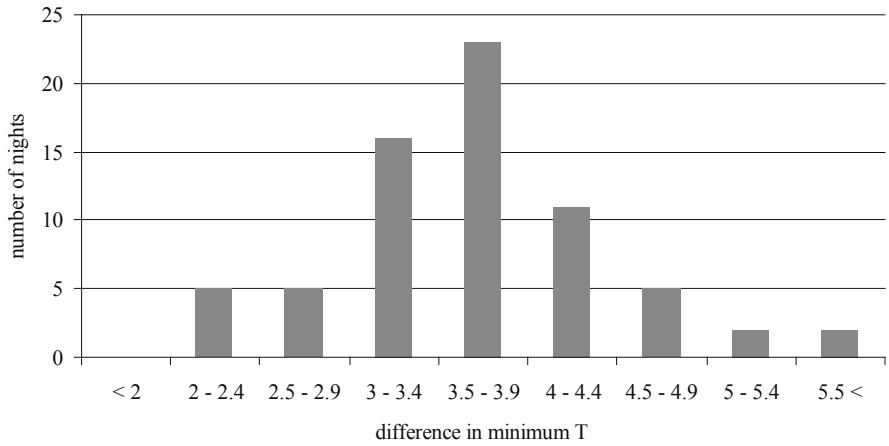

Figure 1: Distribution of the differences between the minimum temperature measured at the station on the slope and in the valley, at calm-clear nights

The largest difference in minimum temperature reached 7.3°C at an April night. This could occur after/at the end of a cool period, when warmer air in the higher air layers helped develop a very strong inversion. The dry air (low value of dewpoint) made an undisturbed cooling possible at the valley.

It was found that at overcast nights with precipitation the temperature decreases with elevation nearly according to the wet adiabatic gradient of 0.6°C/100 m. So, the differences are very small compared to the differences in inversion of clear-calm nights. Figure 2 shows that the differences are limited to a very narrow interval.

This stability in differences is valid not only for the minimum values, but also for any time of the night. During rainy nights the temperatures in the valley and on the slope run almost parallel (Figure 3).

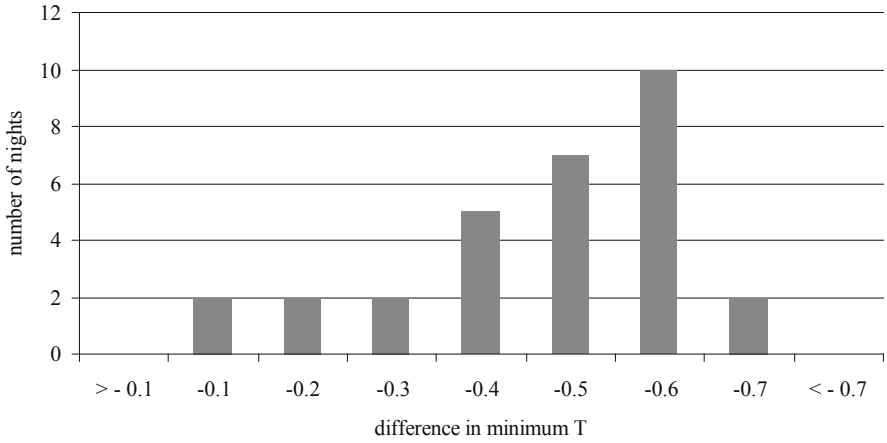

Figure 2: Distribution of the differences between the minimum temperature measured at the station on the slope and in the valley, at rainy nights

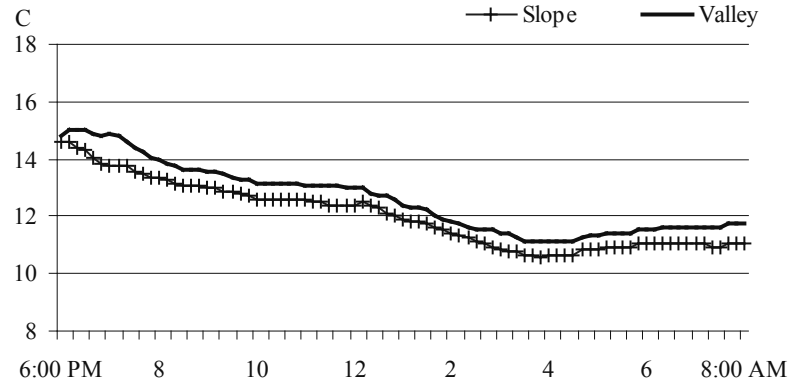

Figure 3: The course of temperature at a rainy night (31th May, 2010) at the station in the valley (260 m) and on the slope (330 m)

At calm-clear nights the course of the temperature in the valley differs from the course of the temperature on the slope. Typically, the temperature difference increases rapidly in the first part of the night (the valley is colder). The air cools down near to its minimum relatively early in the valley (compared to the slope). In some cases the temperature on the slope remains relatively high except a short period around the time of minimum (Figure 4).

Thus in a significant part of the night the temperature difference between the two locations can be larger than the differences in minimum values. This phenomenon also has a horticultural importance.

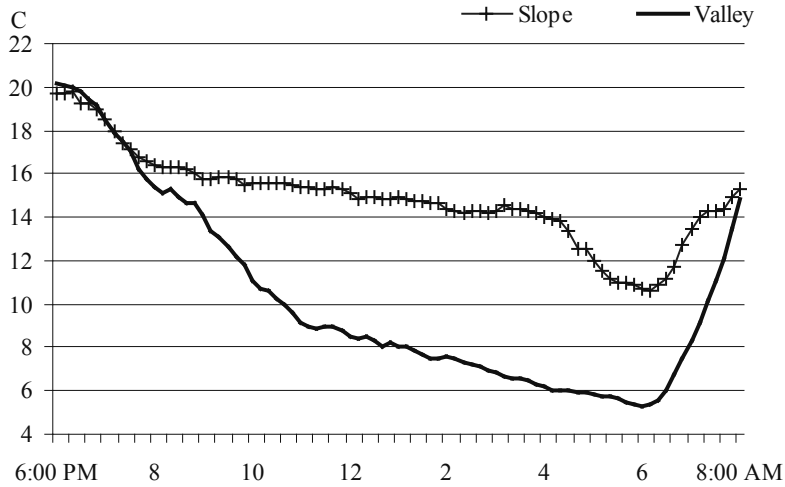

Figure 4: The course of temperature at a calm-clear night (April 24-25, 2010) at the station in the valley (260 m) and on the slope (330 m)

Figure 5 shows the temperature 22nd April, 2010. In the valley the minimum reached nearly -3°C and temperature was subzero for about 6 hours. At the same time the slope with cold air drainage remained frost-free. In such situations high variations in frost injury can evolve according to the local topography.

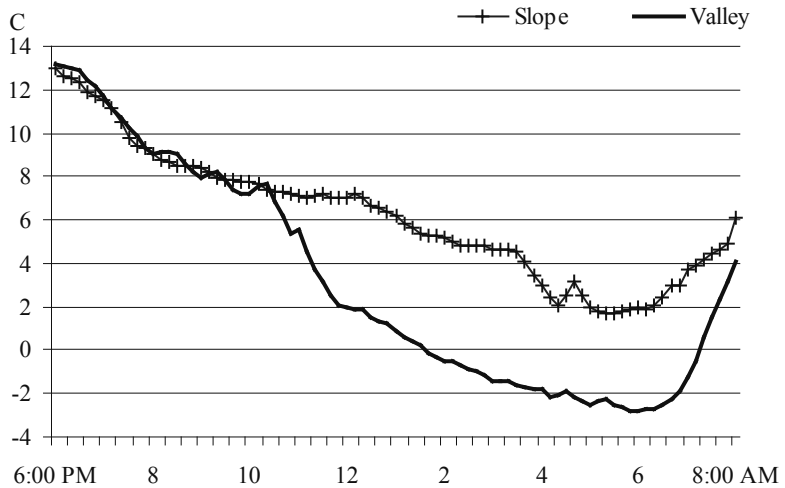

Figure 5: The course of temperature at a calm-clear night (April 22-23, 2010) at the station in the valley (260 m) and on the slope (330 m)

4 Conclusion

Our results hint to the enormous importance of the site selection of orchards. Favorable locations can be warmer by near 5°C at critical cold nights. This difference can mean an advance of nearly one month in time of the last damaging frost. Probably, the site selection based on detailed microclimatic studies is the simplest and most effective way to reduce frost risk. The cooperation of meteorologists and horticultural engineers can give the best results both in the research and the operative phase of the work.

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Andosols of the East Carpathian volcanic range

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Abstract. According to WRB (World Reference Base for Soil Resources) [39] andosols are dark soils with high organic matter content formed on young volcanic rocks. Their characteristics are determined primarily by allophanes, imogolite, ferrihydrite and Al/Fe-humus complexes. Their macro-structure is very loose with low value for volume mass, thixotropy, high cation exchange capacity and phosphorous retaining capacity are characteristic for them.

Geographical distribution, upper and lower limits of andosols in Romania are still to be made clear. Limited publications give controversial data in relation to the latter. Their appearance on maps published in recent years is controversial and hard to interpret especially in the cases of the Harghita and the Gurghiu Mountains.

According to Romanian pedological literature andosols can be found above 1200 m a.s.l., however, on soil maps of the scale 1:200.000 [35, 36, 37] covering the Călimani-Gurghiu-Harghita volcanic chain as well they extend up to only 1500 m.

Researches carried out in the region detected initial spodosolization processes in soil profiles described in the Harghita and Călimani Mountains that, however, cannot be corresponded to the current criteria for andosols set by the WRB.

Primary aim of our research therefore is to make clear, at least partially, these two issues.

In order to study the two aspects presented above, detailed soil investigation was carried out along a reference profile between the heights of 1780 m and 700 m a.s.l. in our study area. Ten major profiles are located so that the most characteristic landforms are crossed. Apart from the major profiles a control profile network was set as well. Disturbed and undisturbed soil samples taken along the major profiles were analysed in the laboratory.

Based on the results, it can be stated that specific andosols (non allophonic aluandic soils) can be found in the Eastern Carpathians. In most of the studied profiles traces of spodosolization (podsolization) can be detected. This is suggested by laboratory analyses revealing the inwash of Al/Fe humus complexes not mention in literature elsewhere only in Romania. As characteristics of andosols are present in all of the studied profiles, joint activity of two processes, andosolization and podsolization or spodosolization can be suggested. It is only a conjecture that the process runs from andosolization towards podsolization, obtaining proofs, however, requires further research.

Based on the above we suggest the introduction of the above soils into the WRB system under the name Spodic andosol or Podzic andosol at one of the lower levels of the Andosoil soil type.

The results do not support the limitation of andosols in the narrow height section between 1200 and 1500 m a.s.l. as seen on Romanian soil maps, they shall be corrected.

Keywords: pyroxene andesite, thixotropy, andosolization, aluandic soil, Spodic andosol, Podzic andosol

1 Introduction

According to WRB (World Reference Base for Soil Resources) andosols are dark soils with high organic matter content formed on young volcanic rocks. Their characteristics are determined primarily by allophanes, imogolite, ferrihydrite and Al/Fe-humus complexes. Their macro-structure is very loose with low value for volume mass, thixotropy, high cation exchange capacity and phosphorous retaining capacities are characteristic for them. Due to their high organic matter and amorphous clay mineral content they are suitable for binding heavy metals, micro-elements (cations and anions) and organic

components. Their detailed analysis is generally performed in countries where volcanic activity is currently experienced or where very young volcanic rocks can be found. Professionals in the Carpathian Basin started the studying of andosols in the 1970s. Detailed studies have been published by Slovakian scientists recently [4, 5, 6], however, scientific attention has been drawn to these soils nowadays in Hungary as well, even though natural conditions in Hungary are not the best for andosol formation. Papers related to andosols were published first in Romania in 1974 [8]. The category andosol appeared first on a soil map in 1979 [17], however, the Research Institute for Pedology and Agrochemistry (Institutul pentru Cercetări de Pedologie şi Agrochimie - ICPA) uses the category on maps published only after 1987. Before these soils were regarded to be acid brown forest soils, podsol brown soils or brown podsols (cryptopodsols).

In Romania the longest, relatively young, continuous volcanic range is found comprising the western belt of the Eastern Carpathians. This is an excellent location for the forming of andosols from several aspects. Despite this only a few scientists considered them for research due to their difficult accessibility. Romanian andosols were studied in detail only by Vasu A. [29, 30, 31, 32], Perepeliţă [23, 25] and Perepeliţă and al. [24]. They detected initial spodosolization processes in soil profiles described in the Harghita Mountain that, however, cannot be corresponded to the current criteria for andosols set by the WRB. This has to be made clear by future research. Geographical distribution, upper and lower limits of andosols in Romania are still to be made clear. Limited publications give controversial data in relation to the latter. Their appearance on maps published in recent years is controversial and hard to interpret especially in the cases of the Harghita and the Gurghiu Mountains. Primary aim of our research therefore is to make clear, at least partially, these two issues.

Environmental conditions determining the formation of andosols

Geological and relief conditions

The 160 km long and 60 km wide youngest western range of the Eastern Carpathians is composed of andesite tuff, breccia and andesite lava rocks formed by intense volcanic activity at the end of the Tertiary extending into the Pleistocene. The southern youngest segment of the longest volcanic chain in Europe can be dissected into three major units: Călimani Mountains, Gurghiu Mountains and Harghita Mountains. Our study area, the Saca and Harghita

Mădăraşului forms the central block of the central unit, and the northern block of the southern one It is composed mainly of lava rocks. The outer gentler slopes of the shield-like volcano extending across 20 km are dissected radially by rivers. Volcanic cone of the 1780 m high Saca Mare and the 1800 m high Harghita Mădăraşului are composed of pyroxene andesite lava flows with subordinate amount of pyroclasts. The central crater cones are encircled by an upland of volcanic agglomerates at the height of 850-1000 m.

According to the K-Ar age determination of three independent laboratories – [20, 22, Seghedi et al. (in print) – the age of the rocks is around 7.2 million years, i.e. upper Pontus – Dacian [28].

The entire mass of the Gurghiu Mountains, including the Saca Mare as well can be regarded rather uniform considering both petrology and geochemistry. Apart from some small andesite-basalt and dacite sections the whole mountain is composed of pyroxene andesite. Material of the rock consists of 29-32% phenocrysts, its major part is amorphous glassy matrix. Based on its chemical composition it is a typical K-calcalkaline rock (Table 1).

Table 1: Chemical composition of the pyroxene andesite

	%
SiO_2	57.00
Al_2O_3	17.64
Fe_2O_3	4.19
FeO	3.18
MnO	0.15
MgO	3.52
CaO	7.35
K_2O	1.22
Na_2O	3.53
TiO_2	1.11
P_2O_5	0.2
H_2O^+	0.53
S	0.17
Total	99.80

Presence of volcanic glass and feldspars together with great amount of silicon and aluminium – if climatic conditions are suitable – helps andosolization in the course of soil formation (characteristic for volcanic rocks).

Climatic conditions

Specificly cool, humid climate mountain climate characterizes our study area. Annual precipitation varies between 800 and 1200 mm depending on height. At the Bucin meteorological observatory at the height of 1280 m 20 years average of annual mean temperatures is 3.8°C while the 20 years average of annual precipitation is 951 mm. These climatic data are perfect for andosol formation.

Vegetation

Vegetation in the lower region between 600-1000 m is composed mainly of beech- Fagetum carpaticum (Soó 1935). After the beech mixed spruce transitional belt the bilberry spruce forest – Piceetum myrtilletosum – is dominant. Above the forest line on the gentle sloping plateau of Mezőhavas between 1650 and 1780 m almost completely continuous dwarf mountain pine – Pinus mugo – is found. One-or-two sporadic spruce may still occur. Herb layer consists of bilberry – Vaccinium myrtillus, bog bilberry – V. uliginosum, rough small-reed – Calamagrostis arundinacea, red fescue – Festuca rubra, mat- grass – Nardus stricta. Due to low temperatures decay in the soil is slow producing large amount of raw humus.

Forests are interrupted by clearings and mountain pastures of various sizes. In the narrow valleys of the lower region vegetation inversion is frequent: beech is above pines; pine forests may extend down to 800 m. Shrub layer of the woodlands is composed of a few species. Characteristic species are blackberry, raspberry, germander meadowsweet, black-berried honeysuckle and bilberry. Cover of the grass level reaches not 50%. Developed moss level is also formed in the wet, shaded spruce forests with closed canopy.

Soil of areas covered by forests is mostly undisturbed; covered primarily by 5-8 cm thick fresh and decaying fallen leaves that were not mixed with the mineral layer and by raw humus. In the case of traditional forestry, areas of forest clearings show disturbed soils, however, significant soil degradation was not experienced. At these locations, felling plants appear right after the termination of forestry indicating the first step of forest regeneration. Herbs appearing after felling are generally high, requiring light and enduring disturbance. Most of them have tiny seeds that are moved by wind. Shrubs also start to grow. Raspberry is generally amongst the first ones to appear together with red-berried elder (Sambucus racemosa), goat willow (Salix caprea), rowan (Sorbus aucuparia) and blackberry species. Amongst the herbs of the initial stages

rosebay willow herb (Chamaenerion angustifolium), Alpine ragwort (Senecio nemorensis subsp. fuchsii) are the most frequent forming continuous cover with associated grama-grass species. This vegetation provides the source of high amount of organic carbon accumulation characteristic for andosols.

2 Material and methods

According to Romanian pedological literature andosols can be found above 1200 m a.s.l., however, on soil maps of the scale 1:200000 covering the Călimani-Gurghiu-Harghita volcanic chain as well they extend up to only 1500 m. In higher regions only brown podsols (podzoluri brune) and podsol brown soils (soluri brune feriiluviale podzolice) are depicted on the maps of the Pedological and Agrochemical Research Institute published in 1988 and 1994 (Harta Solurilor României, scara 1:200000, foile 11 Bistriţa 1994, 12 Gheorgheni 1988, 20 Odorhei 1994).

Perepelită et al. [24] limits the conditions of andosol formation to the narrow belt between 1000 and 1300 m in the Harghita.

Our observations revealed that both the upper and lower limits of andosol distribution mentioned above need revision. In order to study the issue detailed soil investigation was carried out along a reference profile between the heights of 1780 m and 700 m a.s.l. in our study area. Ten major profiles are located so that the most characteristic landforms are crossed. Apart from the major profiles a control profile network was set as well. Disturbed and undisturbed soil samples taken along the major profiles were analysed in the laboratory of the Department of Soil Science and Agricultural Chemistry, Szent István University, Gödöllő. Thin-sections were analysed in the Pedological and Agrochemical Research Institute (Institutul de Cercetări pentru Pedologie şi Agrochimie).

Apart from analyses for determining basic characteristics the following analyses were performed in order to investigate ando characters:

Volume mass (Ts) (g/cm^3)
NaF reaction $pH_{(NaF)}$
Organic C content Corg (in mass%);
Phosphate retaining capacity (P_{ret}%);
Oxalate reaction (Al_o; Fe_o; Si_o);
Dithionitic extraction (Al_d; Fe_d; Si_d);
Pyrophosphate extraction (Al_p; Fe_p; Si_p).

3 Results and discussion

In situ measurements and laboratory analyses – Ts ¡ 0.9 g/cm^3, Pret ¿ 70%, (Al_o+0.5Fe_o) $\geq$ 2, pH_{NaF} $\geq$9.4 – prove andosol character of the soil formed on pyroxene andesite in the region of the Gurghiu Mountains above 950-1000 m up to the 1780 m top of Saca Mare (Table 2). Distribution of andosols is the same as that of beech mixed spruce, pure spruce and dwarf mountain pine and mountain pastures.

Table 2: Soil parameters relevant for andic properties

Section hight a.s.l.	Layer	Depth cm	pH_{NaF}	C_{org}	Si_o	Al_p/Al_o	Al_o+1/2 Fe_o%	Ts g/cm^3	P_{ret} %	Andic properties
1775	Ah ABw	5-17	8,9	13.0	0,07	1,18	2,00	0,51	82	+++
	Bw	17-21	10,1	10,1	0,13	1,20	3,84	Nd	94	++++
		21-35	9,7	6,8	0,15	1,20	4,05	0,62	94	++++
1500	Ah	0-15	7,5	14,63	0,11	1,03	1,10	Nd.	68	?
	ABws	15-18	8,0	10,06	0,08	1,33	2,68	Nd.	92	++
	Bws	18-26	10,7	10,37	0,12	1,11	3,14	0,46	95	++++
	Bw	26-60	10,6	2,47	0,04	0,48	2,08	0,86	83	++++
1250	Ah	4-14	8,2	17,2	0,06	1,38	1,27	0,35	71	++
	Bws	14-21	10,8	8,6	0,19	1,28	3,58	0,50	94	++++
	Bw	30-50	10,2	2,8	0,43	0,57	2,65	0,65	85	++++
1050	Ah	5-20	9,3	22,8	0,18	1,03	1,90	0,31	83	++
	Bws	20-25	11,3	9,8	0,21	0,44	5,74	0,40	85	++++
	Bw	25-45	10,2	3,8	0,07	0,45	3,33	0,67	90	++++
850	Ao	0-20	7,6	4,2	0,06	0,60	0,67	Nd.	36	–
	AB	20-30	7,6	2,3	0,06	0,61	0,62	Nd.	31	–
	Bw	30-60	7,7	0,08	0,08	0,50	0,54	Nd.	32	–

Andic properties: Al_o+1/2Fe_o% $\geq$ 2; P_{ret} >70%; pH_{NaF} $\geq$ 9,4; Ts < 0,9
Strength: ++++ (4/4), +++ (4/3), ++ (4/2)

It is a special characteristic that transition towards lessivage brown forest soils or acid brown forest soils is very sharp at the lower boundary of andosols. A transitional ando-like acid brown forest soil (andic acrisol or andic cambisol) belt is missing. If it would be present it was so narrow that could be detected only by a very detailed in situ survey. One explanation for this can be that lava rocks are replaced by pyroclasts at this height that mix with large amount of non igneous material.

Lower limit of andosol distribution is indicated by the rapid decrease (by 5-6 times) of organic carbon and humus.

Although older volcanic rocks disintegrate easier weathering is markedly stronger in the case of soils in the Gurghiu Mountains compared to andosols

on rocks of similar age in other landscapes of the World. This is verified by the high values of aluminium and silicon determined by oxalate extraction.

Although the current soil cover was formed entirely in the Holocene it has to be noted that prior to soil formation in the Holocene periglacial conditions "prepared" the rocks for soil formation disintegrating and weathering the surface rocks of the volcanic chain of Transylvania.

It is interesting that no clear difference can be detected in the intensity of ando characters related to height despite the almost 1000 m height difference between the lowermost and uppermost parts of the study area. Ando characters appear strongest in the ABw and Bw horizons of all studied profiles.

Nanzyo et al. [21] separated two types of andosol environments considering active aluminium: non allophanic when Al_p/Al_0 gives values between 0.8 and 1.0, and allophonic when this ration is between 0.1 and 0.4. Considering this, soils of the Gurghiu and Harghita Mountains are non allophanic andosols. This is also proved by the low pH value and high organic matter content of our soils. These make amorphous clay minerals subordinate and Al-humus complexes dominant. Also based on the low pH values and high organic matter content (Table 2) together with silicon determined by oxalate extraction and Al_p/Al_0 ratio soils of the Gurghiu and Harghita Mountains can be regarded as aluandic soils.

In the course of in situ analysis a barely detectable spodic (podsol) horizon was found in some of the andosol profiles. Laboratory analyses also supported the presence of this horizon as $Al_0+0.5Fe_0$ content increased suddenly in layer ABw or/and Bw below layer A. Based on these -ü although WRB do not recognise it -ü we consider the introduction of the term spodic andosol justified classifying them into the sub-units of andosols. Initial spodosolization processes were detected by Conea and Ghinea [8] and Perepelită et al. [24] in soils of the Gurghiu Mountains and the Harghita respectively. Comprehensive clearing of the issue justifies the continuing of in-depth research.

4 Conclusions

On the pyroxene lava flows of the Gurghiu Mountains andosols form dominantly the soil cover from the elevation of 950-1000 m a.s.l. up to the highest point of Saca Mare (1780 m). This coincides to the distribution of beech mixed spruce, spruce and dwarf mountain pine.

At the lower limit of andosols the soil turns suddenly to acid brown forest soil without a transition belt where the brown forest soil would have any ando

character. Here the rapid decrease of organic carbon and humus quantity is detected in the soil profiles while the volume mass of the soils increases from 0.3-0.6 g/cm^3 to 1 g/cm^3. Based on high organic matter content, low pH_{H2O}, relatively low value of Si_0 and large quantity of aluminium forming complexes with organic acids, non allophonic aluandic soils prevail in the Gurghiu Mountains.

Based on the results, it can be stated that specific andosols can be found in the Eastern Carpathians. In most of the studied profiles traces of podosolization (podsolization) can be detected. This is suggested by laboratory analyses revealing the in-wash of Al/Fe humus complexes not mention in literature elsewhere only in Romania [8], [24], [32]. As characteristics of andosols are present in all of the studied profiles, joint activity of two processes, andosolization and podsolization or spodosolization can be suggested. It is only a conjecture that the process runs from andosolization towards podsolization, obtaining proofs, however, requires further research.

Based on the above we suggest the introduction of the above soils into the WRB system under the name Spodic andosol or Podzic andosol at one of the lower levels of the Andosoil soil type.

The results do not support the limitation of andosols in the narrow height section between 1200 and 1500 m a.s.l. as seen on Romanian soil maps, they shall be corrected.

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Examination of the arsenic accumulating capacity of lettuce growing in aggregate hydroponics under the influence of arsenic polluted nutrient solution

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Abstract. The arsenic polluted sprinkling water might appear in the southern regions of Hungary. Arsenic levels sometimes exceed the 200 μg/l limit, allowed in underground water in Hungary.

In the teamwork of Soil and Plant Testing Laboratory and the Institute of Vegetable Growing (Kecskemét College, Faculty of Horticulture) we studied some of the effects of sprinkling water containing arsenic pollution on different vegetables since 2006.

In this work, lettuce in hydro-culture was used as an indicator plant. The aim of our examination was to clear up the effect of arsenic on the degree of arsenic accumulation. We used 25, 50, 75, 100, 200, 400 and 600 μg/l arsenic pollution doses.

Keywords: arsenic pollution, lettuce, greenhouse, hydro-culture, hidroponically, ICP-AES

1 Introduction

Arsenic is a well known toxic element can be found in some waters and foods. High arsenic levels contribute to the development of serious disorders. According to laws in Hungary, drinking water may contain 10 μg/l arsenic [1], whereas our food is able to have 200 μg/kg maximal arsenic concentration [2].

Arsenic polluted drinking and sprinkling water appears at the southern parts of the country, in counties Bács-Kiskun, Békés, Csongrád and Szolnok. In these areas plants can accumulate arsenic easily and in high quantity. Based on the teamwork of faculty, we studied the effects of nutrient solution containing arsenic pollution on the growing of lettuce in hydro-culture.

Arsenic (As) is a well known toxic element found in Hungarian well waters due to natural geological conditions [3]. Underground waters in the southern and south-eastern parts of the Great Plain are polluted with 30-150 μg/l arsenic concentration [4].

Due to these measures the impact of polluted water on the population can be reduced, but it must not be forgotten, that in the southern and south-eastern parts of the country fresh vegetables irrigated with arsenic water can threaten the consumers directly.

It is clearly known from geological research [4], that the southern and south-eastern parts of the Great Plain contain high arsenic water concentration. This area represents 80% of the irrigated vegetables territory.

The inorganic forms of arsenic are dangerous poisons noxious to the whole human body, reducing the activity of the nervous system, kidneys, respiratory organs and the liver, also resulting in reproductive and genetically anomalies and cancer [5].

Trial series were started in cooperation between the Ornamental Plant and Vegetable Crops Institute and Soil and Plant Analysis Laboratory of the College for Horticulture (Hungary, Kecskemét) to determine the concentration of this toxic element in some important vegetables irrigated with polluted water. Leaf-vegetables, pepper, tomato, carrot and parsley have been tested from 2006 onwards followed by hydropone lettuce in 2009 and 2010. Lettuce is grown on about 2000 ha, half in the open and half in forcing. The water used for irrigation or for nutrient solutions is obtained from wells, 30-100 m deep [6].

Trial series aimed at finding out the effect of arsenic water characteristic of the region on the arsenic content of lettuce leaves grown in hydroculture when polluted water is used for the nutrient solution. Arsenic doses of 25, 50, 75, 100, 200, 400, 600 μg/l were tested. The first five doses represent

concentrations found in nature, the extreme values (400-600 μg/l) served for scientific observations or modelled extreme conditions.

2 Materials and Methods

Trials included lettuce in hydropone culture in the greenhouse of the Ornamental Plant and Vegetable Crops Institute. There were three tables each containing three nutrient channels made of plastic plates, 4.3 m long, 15 cm high and 30 cm large (Fig. 1).

Figure 1: Hydroculture with nutrient channels

In each channel 25 l standard solution was circulated by a pump controlled by a time switch. An upper container (feeder) and a bottom container (collecting) facilitated the storage of the solution. The slight sloping of the channels furthered the solution flow. In the hydroculture roots developed in the solution and plants were fixed in a neutral agent, rock-wool, and cubes. Fig. 2 shows the cross section of the nutrient channel.

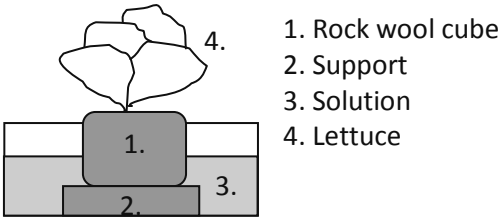

Figure 2: Cross section of the nutrient channel

The nutrient solution was prepared according to a recipe of the Ornamental Plants and Vegetable Crops Institute consisting of Ferticare IV fertilizer complex with 1.6-1.8 mS/cm EC-values and pH 5.5-6.5.

The arsenic solution used in the trials consisted of an arsenic stock solution and the nutrient solution describe above, in 250 mg/l concentration in the stock solution. The stock solution was prepared under laboratory conditions of the Soil and Plant Analysis Laboratory. Increasing doses of the stock solution (2.5; 5; 7.5; 10; 20; 40; 60 ml) were added to the containers (25 l).

The initial compound, arsenic acid (H_3AsO_4) was made of arsenic trioxide. Thus, after dilution arsenic was present in the solution in form of arsenate ($H_2AsO_4^-$). In the naturally polluted waters of the concentrated regions the same ion forms are found.

The hydroculture started 1st September 2009 and 29th March 2010. Two-four leaf lettuce was pricked into rock-wool cubes. The growing period lasted 6 weeks in both years. The nutrient solution was changed once a week. When adding the fresh solution great care was taken of precise dosing.

Evaporation required the replacement of the solution even during the weeks; great care was taken to maintain the initial concentration. Samples were taken and checked by the Laboratory.

At the end of the trial period the lettuce heads were removed from rock-wool cubes and weighed. Random samples were taken on the whole length of each channel (total 17 heads), fully developed healthy leaves were taken from the middle of the heads in four repetitions. Root samples were also collected by lifting the rock-wool cubes and disentangling the roots carefully (Fig. 3).

Figure 3: Rock-wool cube lifted at the end of the trial

The solids content in leaves and roots were determined by drying (70°C) and homogenizing samples in a mill in air dry stage. Samples were digested

in a microwave device by means of concentrated nitric acid and hydrogen peroxide using high pressure teflon bombs at 40-60 bar pressure, at 210°C for 20 minutes. For dilution pure, ion-free water was used and samples were filtered through quantitative filter paper.

Element contents were evaluated in an ICP-AES spectrometer, with radial plasma set, 12 l/min argon flow, 1000 W generator output, at 193.695 nm wavelength, 1 ml/min samples flow. Detector: High Dynamic Detection System (HDD). Limit of quantification: 0.300 mg/kg arsenic referring to samples solids. For quality control all samples were run in duplicates with blanks and certified IPE plant (International Plant Analytical Exchange, Wageningen University). Results from the certified samples were within ± 10% of the known value.

3 Results and discussions

According to classical analytical methods the arsenic content of samples was determined from the solids content. It must not be forgotten, however, that parts of vegetables (in lettuce the whole foliage) have very high water content. In our solids calculations the solids content of the samples varied between 3.05 and 5.82 m/m% with an average of 4.06 m/m%.

Relevant rules [2] allow 0.200 mg/kg arsenic in vegetables for fresh consumption at original water content. The value of arsenic concentration measured in lettuce solids should be divided by 25 to obtain the arsenic concentration of the plant at original water level.

The following two Figures represent arsenic concentrations in the two years and average of repetitions.

As shown by Fig. 4 the 200 μg/l dose in 2009 did not result in measureable As-content in lettuce leaves. Doses 400 and 600 μg/l increased As-content in leaves referring to control and the 200 μg/l dose. Scattering among repetitions is high. The highest As value – 2.67 mg/kg – was found in the third repetition of the 400 μg/l dose. Repetition averages in 400 and 600 μg/l doses were contradictory as the mean of the 400 μg/l dose surpassed that of the 600 μg/l dose (1.56 and 0.952 mg/kg, respectively).

According to valid food decrees [2] the As content of vegetables with original water content can, at most, be as high as 0.200 mg/kg. Due to causes mentioned above the arsenic values of solids are to be divided by 25 to obtain the arsenic concentration of a sample with the original water content.

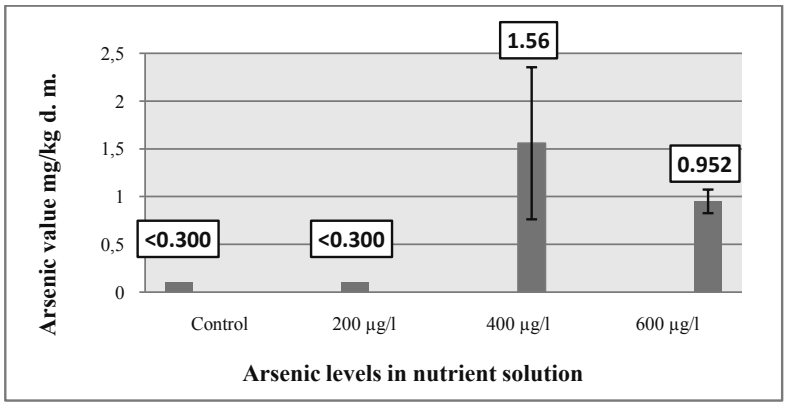

Figure 4: Arsenic levels in leaves referring to solids mg/kg (2009)

When the measured highest value, 2.67 mg/kg was divided by 25 we got the value 0.107 mg/kg which was nearly 50% lower than 0.200 mg/kg. That is, even the highest applied doses did not surpass the limit. Fig. 5 shows our results in 2010.

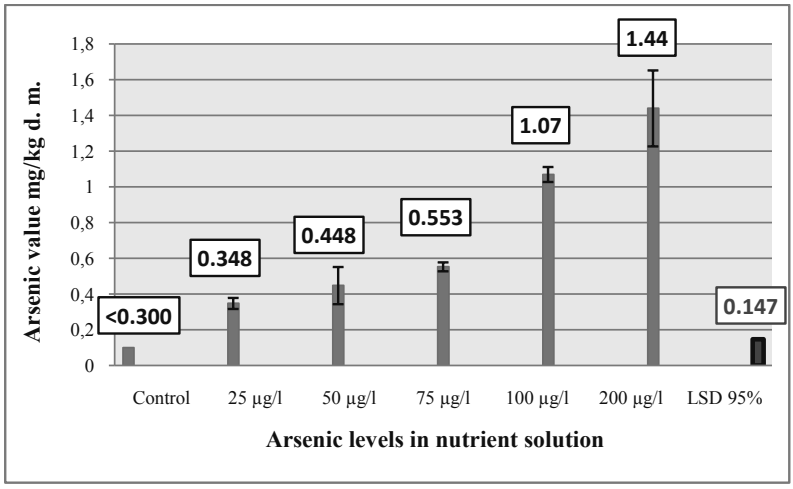

Figure 5: As levels in leaves referring to solids, mg/kg (2010)

Repetitions showed much less scattering than in 2009. Trials in 2010 indicated a more precise execution of trials. Between the same doses of the two years (200μg/l) there was considerable difference despite similar conditions. To clear up the situation trial is going to continue in 2011 involving all the doses.

In 2010 increasing doses increased arsenic concentration in the leaves. Variance analysis [7] showed significant As content increase when applying 100

and 200 μg/l doses referring to control and doses below 100 μg/l. They also differed significantly from each other LSD 95% (Fig. 5).

The highest value was measured in the first repetition of the 200 μg/l dose (1.62 mg/kg). When it was calculated back to the original samples the value of 0.1 mg/kg did not reach half of the limit.

Similar trends were observed in the increase of As content in roots in both years. Figs 6 and 7 represent As values in root samples.

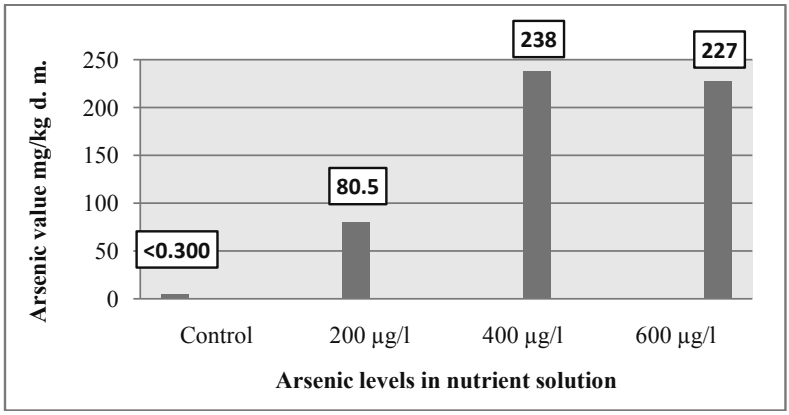

Figure 6: As levels in roots referring to solids (2009)

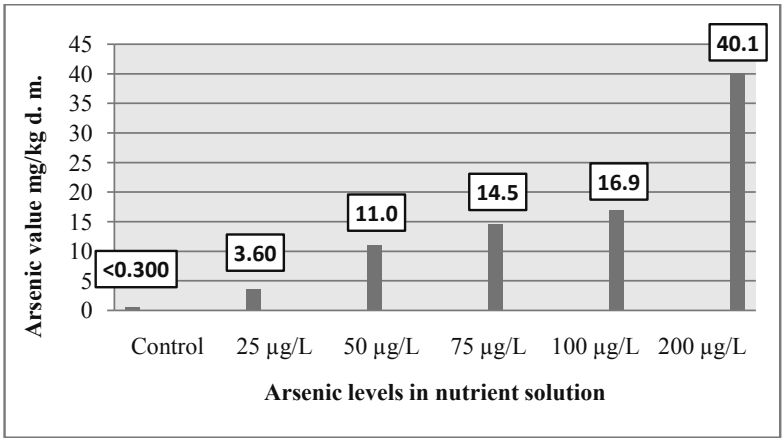

Figure 7: As levels in roots referring to solids (2010)

Increasing As doses increased As concentration in the roots, the low quantities of root samples did not allow repetitions and statistical analysis, yet the

physiological filtration effect of the roots is well expressed.

The ratio of As concentration between roots and foliage as affected by increasing doses was: 10.3; 24.6; 26.2; 15.8; 27.8; 152.6; 238.4. The widening As ratio may indicate the important accumulation function of the roots as affected by high doses of toxic elements. The As content in roots increased more rigidly than in leaves.

The accumulation rate in roots is also expressed by the As concentration in root solids which was 100-600 times higher than in the nutrient solution.

4 Conclusion

Trials show that the arsenic concentration of the nutrient solution affects the As content in the vegetative parts of lettuce. Even slight doses (200μg/l) increased As level in the test plant.

According to [8] As poisoning symptoms in plants are as follow: Reddish-brown, necrotic spots on older leaves, brown discoloration on roots, developmental anomalies in the whole plant. In our trials no such symptoms could be observed. They might have been caused by higher doses than those applied by us.

Bowen stated that in nutrient solutions As belonged to the moderately toxic elements, hindering plant development between 1 and 100 mg/l concentration [9].

The As doses applied in our trials increased As concentration in lettuce leaves significantly from 75 μg/l upwards. The highest As concentration, 2.67 mg/kg in the leaf solids was caused by the 400 μg/l dose.

Increasing As doses increased As concentration in the roots as well but the accumulation was more accentuated. In some doses As content in roots was 10-238 times higher than in leaves. Results are parallel to those of [10] who found 30 mg/kg in roots and 1-5 mg/kg in stems and leaves of the test plants, as affected by As doses.

Rofkar et al. proved in different phytoremediation trials that when comparing plant parts the highest As concentration was found in the roots both in soil and soilless cultures. They also found values between 200-600 mg/kg As in roots of test plants. Our trials confirmed the importance of roots in filtering toxic elements [11].

Smith et al. also studied lettuce in hydroculture adding 2 mg/l arsenic concentration to the nutrient solution. They found 278 μg/kg As in the roots and 3.18 mg/kg in the leaves of the test plant which agrees with our results [12].

Summarizing it can be stated that the arsenic content of lettuce of original water content, grown in hydroculture, increases as affected by As application but it does not surpass the 0.2 mg/kg limit. According to our results even three times higher values than 200 μg/l found in natural well water do not increase the As level above the limit in lettuce.

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Production and quality of fennel

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Abstract. Changes in our life conditions require healthier nutrients of vegetable origin which are rich in vitamins and fibres. Fennel seems to be suitable as it contains sweet, aromatic volatiles, vitamins A, B, C, P and anethole. In horticulture, the production of exotic, hardly known crops, like fennel, should be taken into consideration. Our farm trials in 2009 proved unambiguously that acceptable yield can be obtained even under adverse weather conditions. In the tuber of fennel (Váza and Tauro varieties) the percentage of macro-, mezzo- and micro-elements was found to be between those of the root and the foliage. In the tuber 30-52% of N, P, K was found.

Keywords: macro-, mezzo- and micro-elements of fennel, anethole

1 Introduction

On the threshold of the 21st century horticulture, similar to numerous other spheres, needs changes in our general attitude to life. Our joining the European Union challenged Hungarian growers. Exotic or hardly known crops like, fennel, could be introduced considering, of course, research and empirical experiences as expressed by Lajos Kreybig in the middle of the past century: "every crop should be cultivated according to its requirements." That means,

efficient farming can only be carried on if local conditions are taken into account. The ecological conditions of Hungary favour fennel production.

In 1980 at the Vegetable Crops Research Institute Imre Cserni started research on introducing and working out cultivation technologies for an exotic (new) crop – cultivated in the Mediterranean region and well known in the western countries, but almost completely unknown in Hungary. Results involving three decades and made public at numerous conferences, congresses and in publications seem to bear fruit now.

Today attention is turning towards healthy nourishment. Changes in our attitude to life require more food of vegetable origin rich in vitamins and fibres and poor in fat. Such a vegetable could be the fennel [1, 2, 3, 4].

Fennel in the Mediterranean area

Foeniculum vulgare was cultivated and known by the ancient Greeks and Romans who attributed certain magic power to it. The name Foeniculum first appeared by Plinius. In Italy, Spain and France it was cultivated in the 14th-15th centuries. In France it was very popular in the 16th century and highly appreciated in the court of Louis XIV [4].

At present it has its renaissance. In numerous European countries it seems to gain popularity again. Cultivation is mostly concentrated on the Mediterranean area but it is well known in the Far East as well. In the Muslim countries it belongs to the staple vegetables, like cabbage in Hungary. That is quite natural as in the Muslim world alcohol is prohibited and such aromatic plants are consumers' goods.

In 1980 Italy produced 350000 t/year, France nearly 7000 t/year and Switzerland 3000 t/year [5]. In France mean consumption reached 0,5 kg/head. Today production is gaining ground in North-America, too. Production level is the highest in the Netherlands.

Botanical description

Fennel belongs to the family Apiaceae (Umbelliferae). It was taxonomically ranged by Keller et al. [in 5]. The root is white, spindle-like and deep penetrating. The stem is erect, cylindrical, green. The basic part of the peduncle is bulb-like thickened; it is the tuber (Fig. 1).

Inflorescence is double compound, umbel. Flowers are tiny, yellow.

Fruit is 4-5 mm long, 1-2 mm wide, elongated at the top, twin achene, greenish or light brown-grey. Thousand seed weight is 4-5 g.

Figure 1: Fennel

It requires warm and long day periods. However, there are variants which are indifferent to day length.

Every part of the plant contains sweet, aromatic volatiles [6]. Beside the tuber the foliage is also edible or can be used to decorate warm or cold dishes. Due to its anethole content it is used for soft and strong drinks. It has important physiological effects: stimulates appetite, digestion, intestinal activity, milk secretion, besides diuretic and carminative effects. The curative effect has been known since Hippocrates. Besides these favourable effects it can cause some flatulence. The cosmetics industry also shows interests.

In addition to sugar it contains considerable quantities of P and Ca, as well as A, B_1, B_2, B_5, B_6, C and P vitamins. Carotene and vitamin C contents are considerable. Seeds not usable for cultivation are used as bait by anglers. Foliage and stem residues are favoured by rabbits. In bio-farming root and stem residue compost is especially useful due to its favourable C/N ratio improving the recycling of organic residues. By-products on field (stem and foliage) are useful green manure. Their total N-content can reach 82.1% as related to air dry matter content [7].

Fennel is mostly cultivated for its tuber prepared in various dishes (Fig. 2).

Hungarian people prefer layered fennel (also layered savoy or cauliflower), or with mayonnaise [7, 8].

Figure 2: Different servings: layered, in Greek fashion, with mayonnaise

Leaves and roots can also be consumed fresh or dried [9].

The tuber can be blanched, deep frozen, dried or pickled [9, 10, 11]. No part of the plant should be discarded.

2 Materials and Methods

To test fennel quality 2 varieties Váza (of Cserni) and Tauro (Clause French Seed Company) were sown in the nursery garden of the Horticultural College in the spring of 2010. Roots, tuber and foliage (leaves + stem) were tested separately.

Total N-content was determined by Kjeldahl method (MSZ-08-1783-6:1983) and total P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, B and Mo by ICP spectrometer (MSZ-08-1783-28:1985 and MSZ-08-1983-29:1985, respectively).

The data cited are based on farm trial results performed in Kaba at BonFreeze Co. in 2009. Prior to soil cultivation the nutrient supply of the soil had been tested to guarantee the proper supply of the crop according to requirements.

Fennel was grown on field A/1 of BonFreeze Co., on a loess ridge of Debrecen, a moderately heavy chernozem soil. One part of the field had good humus content, the other part was poor in humus. AL-P_2O_5 and K_2O contents were partly good and partly moderate. Following the nutrient uptake dynamics of the crop 400 kg/ha 8:20:30 Power fertilizer was applied as base. During the growing period 51 kg/ha N active agent (150 kg/ha ammonium nitrate) was given as side-dressing.

Seeds were sown on 1st July 2009 at 75-84 cm row and 10 cm plant distance, corresponding theoretically to 13 plants/m^2. This agrees with former research and farm experience and means 3 kg/m^2 yield in case of 250 g/plant marketable yield.

At present, however, the yield of marketable plants was unimportant as only the tuber (without stem residues), blanched and deep frozen was wanted by the costumer. It meant at least 25% less yield. The ideal marketable yield is 1.5-2.5 kg/m^2, in present case it meant 1.55 kg/m^2, that is 15.5 t/ha (without stem residues). During the growing period weather did not favour growth. During the growing period of 98 days mean temperature was 23.6°C with 74 mm natural precipitation. 300 mm had to be supplied by irrigation (water cannon). During the growing period hand hoeing was performed twice causing some loss of plants. Later hares damaged the crop. No plant protection was needed.

3 Results and discussions

Values

As found in former trials N and K fertilization increased the N and K % in every part of the plant. No considerable change was found in P while N and K surplus affected Na, Ca, Mg and Fe contents negatively. Increasing K doses diminished Na in plant parts. There was a negative correlation between K and Na contents in tuber [12, 13, 14].

According to present analytical data nearly half or even more of the N, Ca, Mg, Na, Mn, Zn, B and Mo contents accumulated in the foliage in both varieties, Váza (registered by Cserni) and Tauro (Clause) (Tables 1a, 1b, 1c, 1d and 2a, 2b, 2c, 2d) in agreement with literary data confirming that green plant parts (stem + leaves) represent best surplus or lack of nutrient elements.

In the tuber the percentage of macro-, mezzo- and micro-nutrients were between those of the root and the foliage. The tuber contained 30-52% of N, P, K (see Tables).

The poorest N, P, K, Ca, Mg, Na, Mn, Zn, B and Mo quantities were found in the root. On the contrary, the root contained the highest Fe content as also experienced by former tests [12, 13]. Present test showed similar results for Cu.

Table 1a: N, P, K content of plant parts of fennel variety Váza

Items	Mean green mass %	Váza						
		Solids g/100g plant*	Measured			Calculated		
			N	P	K	N	P	K
			m/m%			%		
Root	12	1.81	1.65	0.501	2.57	14	22	15
Tuber	52	3.43	2.59	0.616	4.72	41	49	52
Foliage**	36	3.05	3.18	0.412	3.37	45	29	33

*: air dry weight, g/100g plant of average size; **: foliage (leaves+stem).

Table 1b: Ca, Mg and Na content of plant parts of fennel variety Váza

Items	Mean green mass %	Váza						
		Solids g/100g plant*	Measured			Calculated		
			Ca	Mg	Na	Ca	Mg	Na
			m/m%			%		
Root	12	1.81	0.465	0.184	0.458	6	14	20
Tuber	52	3.43	1.070	0.276	0.423	29	37	32
Foliage**	36	3.05	2.730	0.403	0.694	65	49	48

Table 1c: Fe, Mn and Zn content of plant parts of fennel variety Váza

Items	Mean green mass %	Váza						
		Solids g/100g plant*	Measured			Calculated		
			Fe	Mn	Zn	Fe	Mn	Zn
			m/m%			%		
Root	12	1.81	183	17.2	17.5	46	9	9
Tuber	52	3.43	38.7	16.6	32.1	18	15	32
Foliage**	36	3.05	85.2	55.0	41.0	36	76	59

Table 1d: Cu, B and Mo content of plant parts of fennel variety Váza

Items	Mean green mass %	Váza						
		Solids g/100g plant*	Measured			Calculated		
			Cu	B	Mo	Cu	B	Mo
			m/m%			%		
Root	12	1.81	16.0	23.7	0.247	50	12	5
Tuber	52	3.43	32.3	33.9	0.318	43	31	12
Foliage**	36	3.05	7.60	41.9	1.630	7	57	84

Table 2a: N, P, K content of plant parts of fennel variety Tauro

Items	Mean green mass %	Tauro						
		Solids g/100g plant*	Measured			Calculated		
			N	P	K	N	P	K
			m/m%			%		
Root	12	1.44	1.75	0.425	3.66	11	14	12
Tuber	52	2.85	2.38	0.645	6.19	30	41	41
Foliage**	36	5.36	2.48	0,381	3.68	59	45	47

*: air dry weight, g/100g plant of average size; **: foliage (leaves+stem).

Table 2b: Ca, Mg and Na content of plant parts of fennel variety Tauro

Items	Mean green mass %	Tauro						
		Solids g/100g plant*	Measured			Calculated		
			Ca	Mg	Na	Ca	Mg	Na
			m/m%			%		
Root	12	1.44	0.541	0.240	0.342	4	11	15
Tuber	52	2.85	0.772	0.245	0.188	11	22	15
Foliage**	36	5.36	3.260	0.402	0.439	85	67	70

Table 2c: Fe, Mn and Zn content of plant parts of fennel variety Tauro

Items	Mean green mass %	Tauro						
		Solids g/100g plant*	Measured			Calculated		
			Fe	Mn	Zn	Fe	Mn	Zn
			m/m%			%		
Root	12	1.44	319	24.4	20.7	49	9	10
Tuber	52	2.85	36.6	15.3	30.8	10	10	25
Foliage**	36	5.36	73.3	62.1	38.8	41	81	65

Table 2d: Cu, B and Mo content of plant parts of fennel variety Tauro

Items	Mean green mass %	Tauro						
		Solids g/100g plant*	Measured			Calculated		
			Cu	B	Mo	Cu	B	Mo
			m/m%			%		
Root	12	1.44	55.9	22.0	0.213	34	7	2
Tuber	52	2.85	29.7	42.5	0.379	34	21	6
Foliage**	36	5.36	14.4	68.4	3.030	32	72	92

Cultivation

Fennel will never be an important crop in Hungary. Home consumption can be covered by home varieties.

Large-scale production (maybe for export) can only be recommended when reliable markets are available. In 2009 there was a chance in Kaba to compensate for sugar beet by fennel to secure work for local growers.

Adaptation may be a very important criterion for sustainable agriculture, that is, the accommodation to altered conditions in such a way as to preserve our values. Efforts should be made to utilize and take care of our fields to the best of our knowledge.

Production trials helped us learn the most important requirements of fennel. Suitable production technologies for the variety Váza were worked out by Cserni and coworkers [15]. Its requirements for nutrients, water and soil are almost entirely known as well as the ecological production possibilities and economy [16, 17, 18, 19, 20].

Under our climatic conditions, as stated by trials, end of June, beginning of July favour sowing. There is no danger of bolting as days are getting shorter. Harvest time begins in October, the growing period lasts for 90-100 days.

Fennel favours well cultivated soils of loose structure, free of hard pan [21, 22, 23, 24, 25]. It responds positively to bioproducts of high organic matter [15]. For an average yield (20 t/ha) 58-26-194 kg/ha N-P_2O_5-K_2O is taken up by plant parts above the ground. Depending on the nutrient supply of the soil it requires 80-120 kg/ha N, 70-100 kg/ha P (P_2O_5) and 120-160 kg/ha K (K_2O) active agents. P and K are applied as basic fertilizers. On sandy soils N loss can be considerable (40-70 kg/ha) and when irrigated frequently K can also be leached (10%) [25].

To reduce stress on environment on loose soils N can be applied as side-dressing in several portions. On moderately heavy soils N can be given in one portion. On neutral or slightly alkaline soils ammonium nitrate should be substituted by ammonium sulphate. Fennel tolerates monoculture as well as found by Cserni.

Considering a 95-105 day growing period field fennel production requires 200-350 mm natural precipitation or irrigation depending on soil type and soil water supply.

This requirement can best be satisfied by the cooler and rainier climate of Transdanubia.

The recommended area in direct seeding is 13 plants/m^2 (0.13 $\times$ 0.57 m). Denser or looser crops favour bolting in varieties sensitive to day length. Air

dryness can also cause negative effects hindering the development of tubers. Transplants are only recommended for second crops. Forcing is not dealt with in this article.

There are hardly any pests. Occasionally mole-crickets (Gryllotalpa gryllotalpa) and hares can cause some damage. No plant protection was needed.

Marketable I. class tubers have 250-350 g. Tubers of II. class are smaller but shapely and marketable. Elongated but still young tubers can be dried or pickled (2:2:4 % salt: vinegar: sugar). It can be stored at about 5°C for 50 days with 15-25% loss [10]. After blanching it can be kept frozen for 1-2 years.

Former approximative calculations gave 1 million Ft income for 1 ha fennel [19, 20].

Large-scale production experiences in 2009

The variety Tauro bred and propagated by Clause Seed Co. France was produced successfully for an Italian costumer by BonFreeze Co. on 1.7 ha in 2009.

Good results were obtained despite adverse climatic conditions (Fig. 3) due to the harmonized cooperation between research and production [26, 27].

Figure 3: Large-scale production of fennel

4 Conclusion

In horticulture, the production of exotic, hardly known crops, like fennel, should be taken into consideration. Our farm trials in 2009 proved unam-

biguously that acceptable yield can be obtained even under adverse weather conditions. In the tuber of fennel (Váza and Tauro varieties) the percentage of macro-, mezzo- and micro-elements was found to be between those of the root and the foliage. Good results were obtained despite adverse climatic conditions.

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Establishment of Planting Period for Chinese Cabbage (*Brassica campestris* var. *pekinensis* (Lour.) Olson) Early Crops in Open Field, in Transylvanian Tableland Specific Conditions

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Abstract. In the spring of 2010 was organized a research in the experimental field of Vegetable Growing Department, from Horticulture Faculty of University of Agricultural Sciences and Veterinary Medicine Cluj - Napoca. The main subject of this experiment was the cultivation of Chinese cabbage (Brassica campestris var. pekinensis (Lour.) Olson) in Transylvanian Tableland specific conditions.

The results allowed establishing the optimum planting period for this species, so the achieved production to be adequate from the point of view of plants growth and development, the quality and quantity of obtained yield, and obtaining a low bolting percentage.

From the results can be concluded that to avoid the Chinese cabbage bolting and allow the head formation, the seeds have to be seeded in the second decade of March, and the 36 days old seedlings have to be planted in the third decade of April.

Keywords: bolting, planting period, seedlings age

1 Introduction

Chinese cabbage (*Brassica campestris* var. *pekinensis*) is a least known and used vegetable in Romanian gastronomy, but lately has begun to appear more frequently on the shelves of the markets in our country to.

This plant has its origin in Eastern Asia and Japan, and it was taken in culture since the eighteenth century [1].

Chinese cabbage has a very delicate taste and flavor. Some varieties tend to sweetness, others are starchier, some are more watery than others. Plants from this variety need only a light cooking or else their particular flavor is destroyed.

Although the *pekinensis* variety is almost exclusively grown for mature heads, it can be harvested in seedling stage, young plants or semi-mature heads, for leaves or for flowering steams and branches, which have a sweet taste and can be consume.

There is only a little waste with Chinese cabbage: the outer wrapper leaves of a mature head may have to be discarded if they are tattered and weather-beaten, but of the rest, the leaf blade, the midrib and the white leaf bases are all very tender and edible [2].

2 Materials and methods

The culture of Chinese cabbage, which had as main purpose the determination of planting period of this species, in early spring crops, took place from February to June, in 2010. The place of this experiment was the experimental filed of Vegetable Growing Department, which belongs to Horticulture Faculty from the University of Agricultural Sciences and Veterinary Medicine Cluj - Napoca.

In this culture it was used Granat variety, commercialized by Agrosel Company.

This variety has a vegetation period of approximately 70 days. The cabbage heads have a dark green color and a long and cylindrical shape. The heads can reach a weight of 1.5-2 kg. The leaves have a delicate flavor, which recalls the aroma of chicory, turnip and cabbage. The heads can be consumed raw in salads or cooked in different ways. Harvesting takes place from 2 to 3 months after sowing.

To establish the Chinese cabbage optimum planting period in open field culture, in Transylvanian Tableland specific conditions, it was realized a bi-factorial experiment which involved the following factors:

- Factor A: planting period, with 4 graduations:
  - a1: - second decade of April
  - a2: - third decade of April
  - a3: - first decade of May
  - a4: - second decade of May
- Factor B: seedlings age at planting, with 2 graduations:
  - b1: - age I: 46 days
  - b2: - age II: 36 days

By combination of this factors 8 variants resulted, which are presented in the next table (table 1).

Each variant was placed into three repetitions; the surface of one experimental plot was 2.25 m^2.

The research began when the seeds were sown, which took place from 10 to 10 days (first sowing was realized in 27 February), and ended with harvesting, in June. The sowing was made in cells, plants raised in approximately 2 to 4 days, after 15 days there were transplanted in pots of 10×10 cm. The seedlings were planted when they had 36, respectively 46 days.

Table 1: Experimental variants

Variant	**Planting period**	**Seedling age**
1.	Second decade of April (April II)	Age I
2.	Second decade of April (April II)	Age II
3.	Third decade of April (April III)	Age I
4.	Third decade of April (April III)	Age II
5.	First decade of May (May I)	Age I
6.	First decade of May (May I)	Age II
7.	Second decade of May (May II)	Age I
8.	Second decade of May (May II)	Age II

The main purpose of the research was the determination of planting period so that the production to be high and of best quality. During growing season observations were made regarding plants growth and development (these were made at planting, at one month after planting and at harvesting), but on obtained production to.

3 Results and discussions

Before planting, 10 seedlings from each variant were analyzed and measurements were made upon the following characteristics:

- Seedlings height
- Seedlings diameter
- Leaves weight
- Roots weight
- Total weight

Table 2 contains the obtained average dates.

Table 2: Measurements made at planting

Variant	Planting period	Seedling age	Height (cm)	Rosette diameter (cm)	Number of leaves	Weight (g)		
						leaves	roots	total
1	April II	Age I	32.83	35.33	12.67	41.00	3.67	44.67
2	April II	Age II	27.17	29.33	10.00	21.67	2.30	23.97
3	April III	Age I	23.83	37.00	11.00	31.33	3.00	34.33
4	April III	Age II	20.83	33.00	9.67	19.33	1.70	21.03
5	May I	Age I	23.00	37.00	12.00	36.67	3.00	39.67
6	May I	Age II	19.67	35.00	11.00	30.33	2.30	32.63
7	May II	Age I	25.33	37.33	12.67	36.00	3.00	39.00
8	May II	Age II	24.67	32.00	11.33	30.00	2.00	32.00

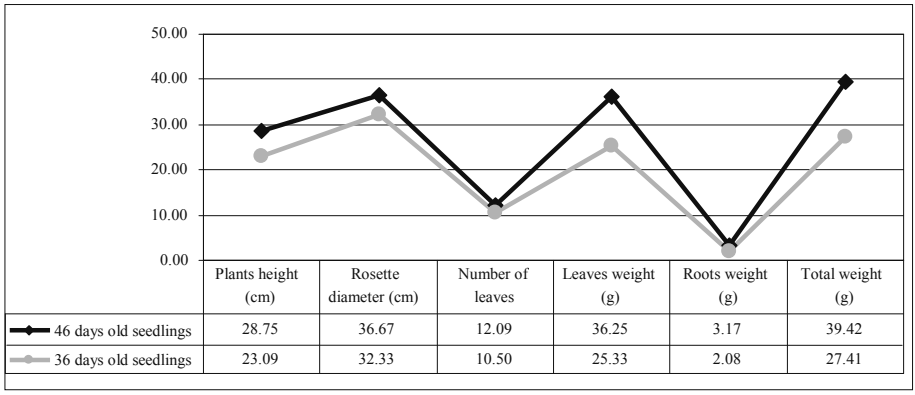
Regarding the average plants height, the highest (32.83 cm) were those from first variant, while at variant 6 were registered those with lowest average height (19.67 cm).

From point of view of rosette diameter, the plants from variant 7 were the most developed (with a diameter of 37.33 cm), while the least developed (with a diameter of 29.33 cm) the plants from variant 2.

Most leaves were formed at seedlings from the first and sixth variant, an average of 12.67 leaves, while at variant 4, were registered only 9.67 leaves.

To analyze plant weight, were measured both: leaves and roots weight, the sum of them gave the total weight. Leaves average weight varied between 19.33 g at variant 4 and 41 g at first variant, while roots weight between 1.70 g at variant 4 and 3.67 g also at variant 1.

In Fig. 1 it can be observed that the older seedlings, which had 46 days, compared to the youngest, the 36 days old ones, had higher values at all measured characteristics. Thereby, the age I seedlings average height was higher with 5.66 cm, their diameter was higher with 4.34 cm, and they had more leaves to. Age difference was observed best when the plants weight was analyzed. Thus, even if the differences between the roots weight were not very significant, the leaves and total weight of the older seedlings were much higher in case of the older ones.



	Plants height (cm)	Rosette diameter (cm)	Number of leaves	Leaves weight (g)	Roots weight (g)	Total weight (g)
46 days old seedlings	28.75	36.67	12.09	36.25	3.17	39.42
36 days old seedlings	23.09	32.33	10.50	25.33	2.08	27.41

Figure 1: Measured characteristics comparison at planting, from the two categories of seedlings

One month after planting were conducted again a series of measurements, which included the following features:

- Plants height
- Rosette diameter
- Number of leaves

The measurements were made at 10 plants of each repetition of the eight variants, after that were calculated the averages, which are presented in table 3.

Regarding plants height at one month after planting, the highest values were recorded at variant 2 (35.55 cm), followed by variant 6 (35 cm), both variants were established with 36 days old seedlings. These variants registered higher values, than the first and fifth ones, where the planting was made at the same planting period, but with older seedlings.

Table 3: Measurements made at one month after planting

Variant	Planting period	Seedling age	Height (cm)	Rosette diameter (cm)	Number of leaves
1	April II	Age I	34.33	50.67	21.33
2	April II	Age II	35.55	50.00	19.67
3	April III	Age I	34.17	53.00	22.17
4	April III	Age II	32.33	51.67	20.00
5	May I	Age I	33.00	43.83	20.67
6	May I	Age II	35.00	41.33	20.33
7	May II	Age I	27.33	33.17	23.17
8	May II	Age II	24.83	35.17	16.33

The lowest average height was measured at last variant, where the 36 days old seedlings were planted in the last planting period. In this case the plants average height was only 24.83 cm.

The rosettes diameter varied from 33.17 cm (at variant 7) to 53 cm (at variant 3).

An other very important characteristic is the number of leaves, because plants with a higher number of leaves can form a bigger head, respectively have a higher weight. From this point of view, plants belonging to variant 3 formed in average 22.17 leaves, while on opposite pole is the last variant, with only 16.33 leaves.

Although at measurements made at planting there were significant differences at all analyzed characteristics, in favor of older seedlings, this has changed a month after planting. Data registered in this period showed that the differences between the two seedling ages have been reduced. The average height of plants was higher at 46 days old seedlings with only 0.34 cm, the rosette diameter with 0.63 cm and they have more leaves formed, in average with 2.67 (Fig. 2).

At one month after planting some of the plants started to emit flower stems, to bolt, before passing through the stage of head formation. The main elements which can cause this physiological diseases are: low temperatures during seedling production, day lengthiness, different genetic elements, in addition there can be some stress factors like: the shock of transplantation, water shortages, excess humidity or sudden temperature changes [2]. Until harvest a large part of plants flowered, which couldn't be harvested or valued as headed cabbages. It is known that between head formation and flower buds differen-

tiation there isn't a certain relationship, so the differentiation can start before or after the start of head formation [3].

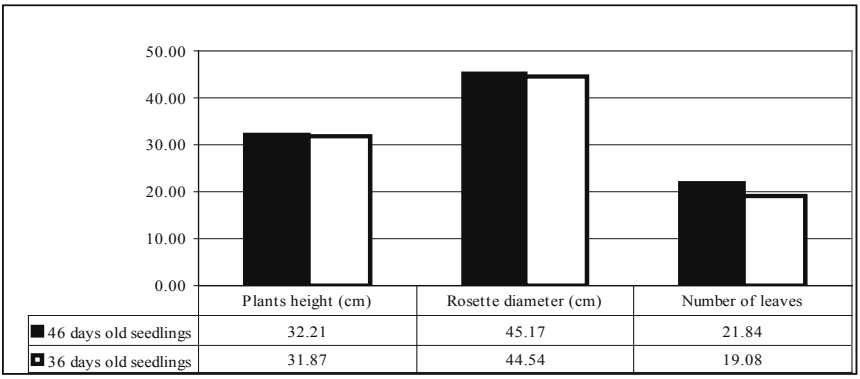


Figure 2: Measured characteristics comparison at one month after planting, from the two categories of seedlings

Fig. 3 presents the percentage of bolted plants from the total planted ones. It can be observed that in case of variants 1, 2 and 8, all the plants have flourished, while at variant 6 the bolting percentage was only 20%.

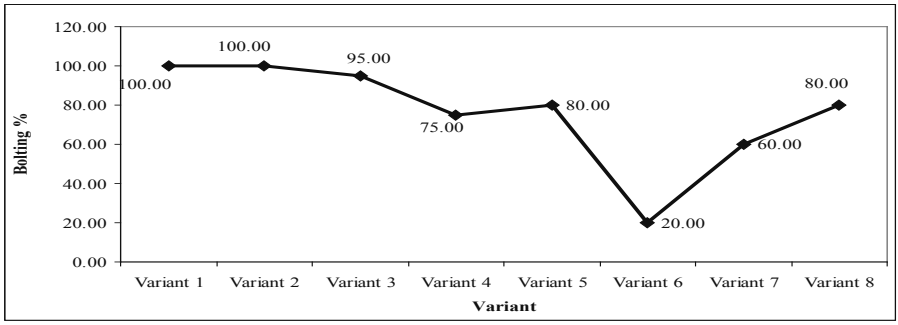


Figure 3: Bolted plants percentage

At variants, in which all the plants have formed flowering stems, the harvesting couldn't be realized, and measurements couldn't be done.

Best variant regarding the plants height, total weight, heads weight and number of leaves in rosette is variant 4 (table 4). The average plants height in this case was 58.30 cm, the average total weight of plants was 1.21 kg and the average heads weight was 0.93 kg, while the plants had in average 13.17 leaves. The third variant pointed the highest rosette diameter (an average of 63 cm), and the longest heads (an average of 60 cm). The highest head

diameter (32.33 cm) and highest number of leaves in heads (in average 20.83 leaves) were registered at variant 6.

The lowest values of analyzed characteristics were measured at variant 7.

Table 4: Measurements made at harvest

Var.	Planting period	Seedling age	Plants height (cm)	Rosette diameter (cm)	Total weight (kg)	Number of leaves in rosette	Head			
							Length (cm)	Diameter (cm)	Weight (kg)	Number of leaves
1.	April II	Age II	-	-	-	-	-	-	-	-
2.	April II	Age II	-	-	-	-	-	-	-	-
3.	April III	Age I	58	63.00	0.8	12	60	30.00	0.62	20.00
4.	April III	Age II	58.30	58.50	1.21	13.17	55.17	29.00	0.93	18.67
5.	May I	Age I	42.00	57.00	0.48	8.25	38.50	23.00	0.40	20.00
6.	May I	Age II	39.33	50.33	0.57	7.33	37.50	32.33	0.49	20.83
7.	May II	Age I	38.00	46.75	0.45	4.75	40.75	32.25	0.39	18.00
8.	May II	Age II	-	-	-	-	-	-	-	-

4 Conclusions

For setting up a Chinese cabbage culture it is recommended to sow in the second decade of March, and the seedlings at planting should be approximately 36 days old.

The optimal planting period of Chinese cabbage culture is the third decade of April, with seedlings which are produced in greenhouses since the middle of March.

It is recommended to avoid sowing to early or planting to late, because of high risk of plants bolting, or using bolting resistant varieties or hybrids.

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Research Concerning the Bolting of Chinese Cabbage (*Brassica campestris* var. *pekinensis* (Lour.) Olson) in Early Crops in Polyethylene Tunnels

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Abstract. The research regarding the bolting of Chinese cabbage (*Brassica campestris* var. *pekinensis* (Lour.) Olson) in early cultures was realized from January to May in 2010. The culture took place in the polyethylene tunnel of Vegetable Growing Department from University of Agricultural Sciences and Veterinary Medicine from Cluj - Napoca.

The main purpose of this experiment was to establish the optimum cultivation technology to obtain a high production of best quality.

The results showed that to avoid Chinese cabbage bolting, before head formation, the culture setting up must be done in the first decade of April with 36 days old seedlings.

Keywords: headed Chinese cabbage, planting period, seedling age

1 Introduction

In parallel with the evolution of white headed cabbage in Europe, some cabbage species have developed in China to, which are part of the same family, *Brassicaceae*, together with the well known and used cabbage in the western kitchens [1]. One of this species is the Chinese cabbage (*Brassica campestris* var. *pekinensis* (Lour, Olson), which looks rather like a well developed lettuce, not at all like the typical rounded or flat western cabbage. It forms an elongated head, with very tight, overlapping leaves, sometimes a looser head. Heads vary enormously in shape and size and when well grown can weight from 1.4 kg to 4.5 kg [2].

The importance of this vegetable is the fact that all plant parts are edible, the leaves, the flowering stems and flowers can be all consumed raw or prepared in different ways.

Although, currently, in our country is a least known and used vegetable, Chinese cabbage is very appreciated in Western Europe, where the cultivated surface and the consumption are growing very fast. In this area is being used increasingly as a substitute for the white headed cabbage in recipes like stuffed cabbage, or is prepared using Chinese methods and recipes [2].

2 Materials and methods

In the spring of 2010 it was realized a Chinese cabbage culture in the polyethylene tunnel of Vegetable Growing Department which belongs to the Faculty of Horticulture from University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca. The experiment took place from January to May.

It was used a single variety, Granat, commercialized by Agrosel Company, which belongs to *pekinensis* variety of *Brassica campestris* species, and has a short vegetation period (70 days). The heads are compact and cylindrical; leaves are not overlapping on the top of the head. Green leaves belonging to this variety have a fine aroma, a taste which recalls the aroma of chicory, turnips and cabbages. They can be eaten raw, in salads or cooked.

Harvesting occurs at 2-3 months after seeding.

This experiment was bifactorial, involving the next factors:

- Factor A: planting period, with 4 graduations:
  - a1: - first decade of March

- a2: - second decade of March
- a3: - third decade of March
- a4: - first decade of April

- Factor B: seedlings age at planting, with 2 graduations:
  - b1: - age I: 48 days
  - b2: - age II: 38 days

By this factors combination were obtained 8 variants, which are presented in table 1.

Table 1: Experimental variants

Variant	Planting period	Seedling age
1.	First decade of March (March I)	Age I
2.	First decade of March (March I)	Age II
3.	Second decade of March (March II)	Age I
4.	Second decade of March (March II)	Age II
5.	Third decade of March (March III)	Age I
6.	Third decade of March (March III)	Age II
7.	First decade of April (April I)	Age I
8.	First decade of April (April I)	Age II

Each variant was placed into three repetitions; the surface of one experimental plot being 3 m^2.

The seeds were individually sown in pots. The plants arisen in 3-4 days and after approximately 15 days they were transplanted in pots of 10 × 10 cm. The sowing started on 18^{th} January, and was done from 10 to 10 days, to obtain seedlings of different ages for the mentioned four planting periods. Last sowing was made in 27^{th} February. The seedlings were planted when they reached 38, respectively 48 days.

The culture was established in the polyethylene tunnel, where was used a plant density of 4.66 plants/m^2, the distance between rows was 75 cm, while between plants was used a distance of 30 cm.

During the vegetation period were made observations regarding plants growth and development (this were made at planting, at one month after planting and at harvest) and regarding the production. In the same time was registered the number of bolted plants, which had a negative role by decreasing the yield.

3 Results and discussions

Flowering marks the transition from vegetative to reproductive stages in seed plants. It is, thus, a crucial event in the life cycle of plants, particularly from the standpoint of seed production.

There is no definite sequence or relationship between head formation and floral bud differentiation, floral buds could differentiate after or before the onset of heading. If floral buds differentiate prior to heading, loose and unmarketable heads are formed [3].

Chinese cabbage varieties differ considerably regarding the predisposition of bolting, especially if they are exposed to low temperatures while seedling is produced.

The causes of bolting are complex and interrelated. To a grater or lesser extent the following factors all play a part:

- Low temperatures in the early stages of growth: this is thought to be the single most important factor. Low temperatures, both when seeds are germinating and in the early stages of growth, lead to the initiation of flowers instead of leaves. Put very simply, the young plants need to "clock up" a certain number of heat units to prevent bolting. Once this has been done they can be subjected to lower temperatures without damage.
- Day length: with many species there is a grater tendency to bolt in long days (more than 12-14 hours of daylight), than in shorter days. In the northern hemisphere the bolting risk is therefore highest in spring and early summer.
- Genetic factors: some types of Brassica are inherently less prone to bolting than others. This makes it possible to select and develop varieties with improved bolting resistance.
- Stress: various kinds of "stress" such as the "shock" of transplanting, lack of water, overwatering or sudden temperature changes can exacerbate the tendency to bolt [2].

To avoid the negative effects of low temperatures, of stress, which is caused by the impropriate soil humidity, and to provide optimum growth and development conditions, measurements regarding: air temperature, soil temperature, relative air humidity and light intensity were made during the seedlings production. These were made three times a week, in morning hours.

Fig. 1 shows the oscillation of air and soil temperature. It can be observed that the air temperature varied between 16-28°C, most temperatures exceeding 20°C. Regarding soil temperature, the lowest temperature recorded was 13.1°C, while the highest 18.2°C.

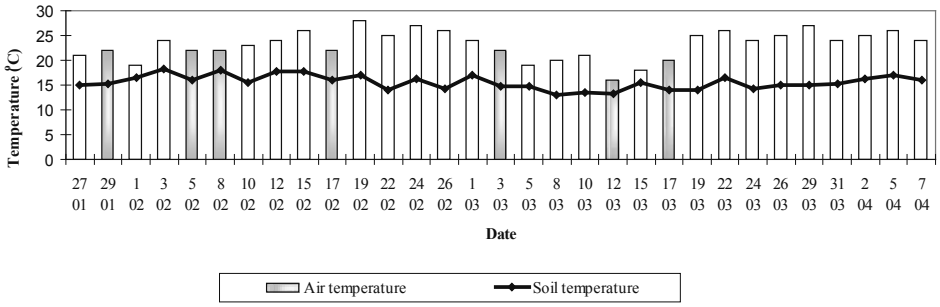

Figure 1: The evolution of air and soil temperatures during seedlings production

Relative air humidity varied between 38 and 69%, this factor was easily controllable by watering when low values were recorded (Fig. 2).

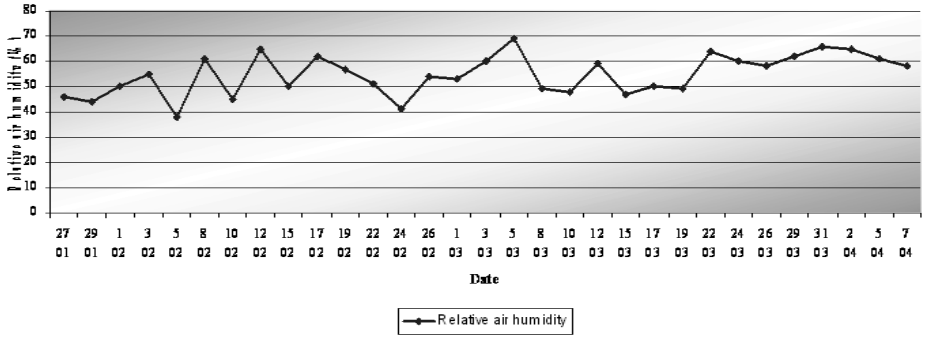

Figure 2: Evolution of relative air humidity during seedling production

For measuring the light intensity, the device was placed always at plants level, so the recorded data could correctly reflect the specific culture conditions.

The lowest light intensity was registered in 15th March (1540 lx), while the highest in 22nd February (23521 lx) (Fig. 3).

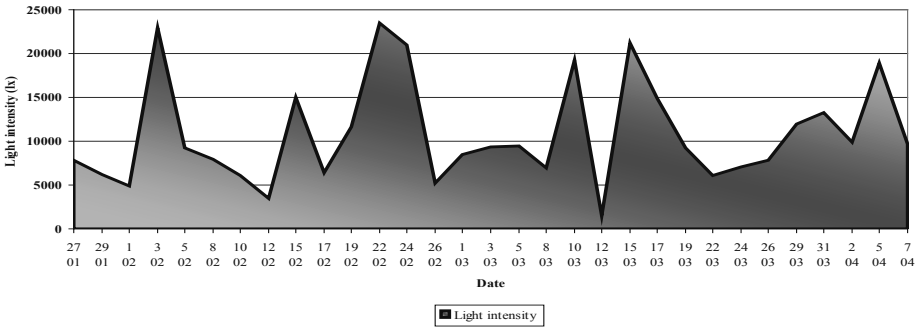

Figure 3: Evolution of light intensity during seedlings production

Observations regarding the number of bolted plants were made at one month after planting, and at harvest.

It was observed that, although at the beginning, bolted plants appeared only sporadically, with the passage of time at more and more plants appeared the flowering stems.

Table 2 presents the number of bolted plants at one month after planting. By analyzing the data from the table, it can be observed that from the 224 plants which were taken in culture, 20 plants emitted flower stems already at one month after planting, which represents 8.93% from the total plants. Most bolted plants (5 plants) were from variant 5, while from variant 8 no plant showed signs of flowering, the bolting percentage being 0.

Table 2: The bolted plants situation at one month after planting

Variant	**Total plants\ variant**	**Number of bolted plants**	**% of bolted plants**
1	28	4	14.29
2	28	4	14.29
3	28	1	3.57
4	28	1	3.57
5	28	5	17.86
6	28	3	10.71
7	28	2	7.14
8	28	0	0.00
Total	224	20	8.93

During harvest a series of observations were made regarding number of

bolted plants, plants which emitted flower stems before head formation. The registered data are presented in table 3. Most bolted plants at harvest were numbered at variant 1, where more than half (53.57%) of the plants have flourished prematurely; from the total of 28 plants of this variant 15 turned to flower too early. The lowest bolting percentage (10.71%) was registered at last variant, where only 3 plants bolted.

Table 3: Bolted plants situation at harvest

Variant	**Total plants\ variant**	**Number of bolted plants**	**% of bolted plants**
1	28	15	53.57
2	28	14	50.00
3	28	11	39.29
4	28	9	32.14
5	28	14	50.00
6	28	8	28.57
7	28	11	39.29
8	28	2	10.71
Total	224	85	37.95

In Fig. 4., which presents the evolution of average bolting percentage, it can be seen that the lowest increasing (10.71%) which regards this character was recorded at variant 8, while the highest increasing (39.28%) at variant 1.

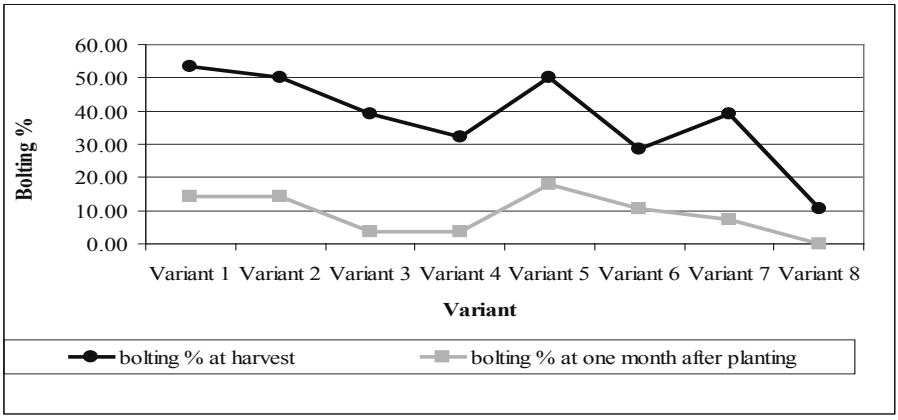

Figure 4: Evolution of average bolting percent

The Chinese cabbage bolting had negative effects regarding the obtained total yield. In Fig. 5. is presented a graphical representation of the achieved production in comparison with the theoretical production, which could be obtained if the plants wouldn't bolted. The theoretical production was calculated with the aid of average weight of the plants.

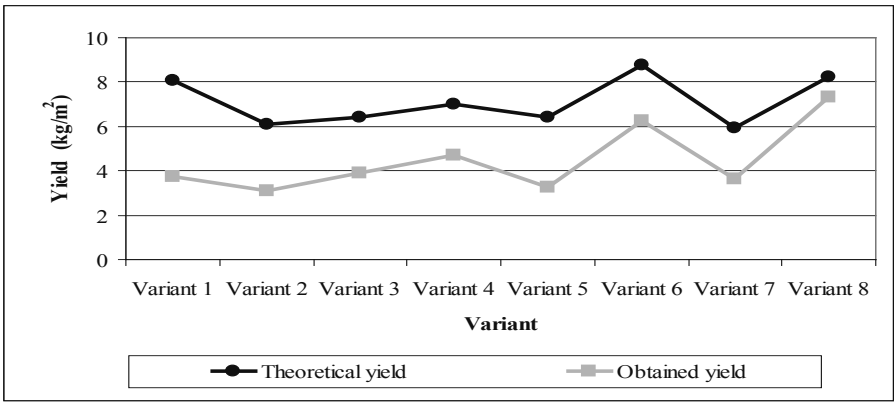

Figure 5: Comparison between obtained and theoretical production

It can be observed that at most variants the obtained production is half of the theoretical one.

Also in this figure can be observed that the highest yield was realized in case of last variant, the obtained production being 7.31 kg/m^2, while the lowest in case of variant 2, with a production of only 3.11 kg/m^2.

4 Conclusions

The lowest percent of bolted plants was registered at the variant which was established in first decade of April, with 38 days old seedlings.

The highest percent of bolted plants was recorded in case of first variant, which was established in the second decade of March, with seedlings of 48 days.

By decreasing the number of bolted plants, the obtained production could increase much, even to a double value.

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