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Authors of current volume of Columella

Gergely AMBRUS
Krisztián BALOGH
Mátyás CSERHÁTI
Péter DUDÁS
Márta ERDÉLYI
Csongor GEDEON
Zoltán IZSÁKI
Olga KIRICHENKO
Tamás KISMÁNYOKY
László MENYHÁRT
Miklós MÉZES
Mangesh NAKADE
József PEKLI
Péter PEPÓ
Toleuhkan SADYKULOV
Rubina T. SZABÓ
Tamás SZALAI
Szilvia SURÁNYI
Brigitta TÓTH
Ferenc TÓTH
Mária WEBER
Edit ZAJÁCZ
Erika ZÁNDOKI
Damir ZHARKENOV
Katalin ZSUGA

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Kornél SZALAY
Béla URBÁNYI
Mária WEBER

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PREFACE

VASSALAGE – THE ART OF SUSTAINING SCIENTIFIC RESEARCH AND HIGHER EDUCATION

Science is just like any other craftsmanship; it is profoundly influenced by the participants involved in its activities. There are scholars who render services to the public, and there is the society which highly depends on the output of their contribution. The higher the quality of the scientific community in a country the higher quality of life is there. There is only one problem within this structure. A scientist is not a craftsman and the society is not a consumer. The two sides of this model needs to be implemented by a third party – the Maecenas. Why?

Cover page of the 1550 edition of Machiavelli's Il Principe and La Vita di Castruccio Castracani da Lucca. Source: http://www.storiain.net/arret/num60/artic6.htm.

Simply put, because of the nature of the output of science. If I need a pair of shoes, I go to a shoemaker and he will produce that in accordance with my wishes. However, if I want to solve a scientific problem, like inventing the perpetuum mobile, I cannot simply appoint a scientist to accomplish the problem. Why not? There are two reasons for that; firstly while a scientist may make an honest attempt at achieving your goal, one can never be assured of success. The other reason is that the magnitude of expenses don't always align with the request.

A few hundred years ago count Montecuccoli (1609-1680), a military leader of the Hapsburg Empire formulated his evergreen thesis. War is an activity based on three necessary pillars: money, money and money. And it is true for science as well.

There are various means and ways financing science: support, subsidy, donation, fund, scholarship, grant, gift, present, etc. All these are provided by the Maecenas. The word Maecenas is a widely used byword for the rich generous supporters of arts, science, sports and other activities. Gaius Cilnius Maecenas (cca70-8 BC) was a wealthy Roman nobleman, a friend and advisor of Emperor Augustus. During his life he acted as a sort of quasi-minister of culture and supported such talented persons like Virgil and Horace. His name became an eponym for a patron of arts.

Funding is the act of providing financial resources usually in the form of money, or other values such as effort or time, to finance a need, program, and project by the Maecenas – normally by an organisation or government. Types of funding such as donations, subsidies, and grants that have no direct requirement for return of investment are named as soft funding or crowdfunding.

Up to this point financing of science seems to be a fairy tale. The good, the poor, the clever, the diligent, the honest will be rewarded by the powers that be for their services, so they may act in favour of the society. However, the scheme is much more sophisticated.

Niccolò Machiavelli (1469-1527) an Italian diplomat, scholar and philosopher of the Renaissance period of Tuscany has written his immortal pamphlet book "The prince" dealing with various problems of governing society. This book is sometimes claimed to be one of the first works of modern philosophy, especially for its modern political assessment, in which the effective truth is taken to be more important than any abstract ideal. It was also in direct conflict with the dominant religious and scholastic doctrines of that period concerning politics and ethics.

Macchiavelli postulated that actors of the society in all fields of life are arranged into an autocratic structure, the vassalage that is directed and operated by the decision of the powers that be. The vassal or feudatory, the subject is a person who has entered into a mutual obligation to a lord or monarch in the context of the feudal system in medieval Europe. The obligations often included military support and mutual protection, in exchange for certain privileges, usually including the grant of land held as a fiefdom. However distinctions have to be made within this mutual structure between obligations and challenges. Whenever a state has no power to finance public duties, it turns to be a Maecenas raising funds for the few rather than fulfilling its general obligations.

The life of Columella - the scientific paper - is also highly determined by its financial resources. It would be beneficial to be run on a regular budget rather than on occasional donations depending on the goodwill of the Maecenas.

Katalin Posta
editor-in-chief

Márton Jolánkai
guest editor

EFFECTS OF THE NUTRIENT SUPPLY AND SOWING TIME ON THE YIELD AND THE PHYTOPATHOLOGICAL TRAITS OF WINTER OILSEED RAPE (*BRASSICA NAPUS* VAR. NAPUS F. BIENNIS L.) ON CHERNOZEM SOIL

Péter PEPÓ

University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Crop Science, H-4032 Debrecen Böszörményi út 138., Hungary; E-mail: pepopeter@agr.unideb.hu

Abstract: We have studied the fertilizer response of oilseed rape on the chernozem soil in Eastern Hungary in a three-year experiment (years of 2009/2010, 2010/2011, 2011/2012) in two sowing times (end of August, middle of September). Our research results proved that oilseed rape has high fertilizer (N+PK) demand. In the experiments, N = 210 kg ha^{-1} +PK proved to be the optimal fertilizer dose. The yield increasing effect of fertilization was 800 to 1300 kg ha^{-1}, depending on the cropyear. The maximum yield (5000 kg ha^{-1}) was obtained in the cropyear of the less infection. The excellent natural nutrient providing ability of chernozem soil was confirmed by the high yield level (3000-4200 kg ha^{-1}) of the control treatment (N = 0 kg ha^{-1} +PK). According to our studies, as an effect of the increasing N doses, the specific fertilization yield surplus of oilseed rape declined (from 19-27 kg/1 kg NPK to 11-12 kg/1 kg NPK). On the other hand, fertilization improved the water utilization efficiency (WUE) of oilseed rape in the experimental years (from 4-8 kg/1 mm precipitation + irrigation water to 11-14 kg/1 mm precipitation + irrigation water). The results of our studies confirmed that the hybrid rape had of excellent adaptation to the sowing time; due to this fact, the yield decrease was minimal (0-270 kg ha^{-1}) in the middle September sowing time compared to the late August one. According to the results of the Pearson's correlation analysis, strong correlation (0.6*-0.9**) was found between the spring precipitation and temperature and the most important diseases (Sclerotinia, Alternaria, Peronospora, Phoma) of oilseed rape.

Keywords: oilseed rape, fertilization, sowing time, yield, diseases

Introduction

The demand for vegetable oils has been constantly growing both in the world and in Hungary during the last decades. This was partially the consequence of the growing demand of population for food and partially for the broadening usage of vegetable oils by the industry. A new kind of demand is the production of biodiesel among the renewable energy sources; its most important base material is currently rape oil. Oilseed rape is the third most important cultivated oil plant all over the world while second in Hungary after sunflower. Its cultivation area has been increasing since 1990; currently it varies between 200 and 250 thousand ha. During the past years, oilseed rape production appeared also on such areas, in farms, which previously have not cultivated this plant. In the past years, the Hungarian variety/hybrid portfolio significantly changed; nowadays new types of hybrids (e.g. semidwarf, IMI, etc.) appeared, whose production technological demands considerably differ from the agrotechnical needs of the older genotypes. Among the technological elements, the appropriate nutrient supply and the optimal sowing time are of especial importance in the oilseed rape production.

Oilseed rape is a field crop that needs large and harmonic NPK supply (*Kádár* and *Márton* 2007, *Pospišil* et al. 2008). The optimal NPK dose was significantly influenced by the soil traits (*Máthé-Gáspár* et al. 2007, *Máthé-Gáspár* et al. 2008). According to the differences of the genotype and the agro-ecological factors, *Gulzar Ahmad* et al. (2011) found N = 120 kg ha^{-1}, while *Boelcke* et al. (1991) N = 240 kg ha^{-1} fertilizer doses as the most favourable ones for the yield of oilseed rape. In their experiment not only the yield maximum was realised but the yield stability was also the most favourable. According to the studies of *Sieling* and *Christen* (1997), the increase of the N doses from 80 kg ha^{-1} to 200 kg ha^{-1} increased the yield from 3.21 t ha^{-1} to 3.84 t ha^{-1}. They have found that the distribution of the N doses did not affect the yield amount

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of oilseed rape. In their studies summarizing the results of several experiments, *Rathke* et al. (2006) emphasized the importance of numerous factors (crop rotation, fertilizer doses, fertilizer splitting, genotype) in the N utilization of oilseed rape.

The optimal selection of the sowing time of oilseed rape is very important for the germination, the development of homogenous stocks and over-wintering. In their experiments, *Risnoreanu* and *Buzdugan* (2011) found the interval between 5 and 10 September as the optimal sowing time. In the studies of *Sharafizadeh* et al. (2012), the sowing time significantly influenced the yield of oilseed rape.

In Hungarian research, we can found only limited amounts of experimental data in connection with the nutrient supply and sowing time of oilseed rape (*Pepó* 2012). Results of pathology studies are almost entirely missing; therefore, we started a research project investigating the individual effects of fertilization and sowing time and their interactions in winter oilseed rape in three different cropyears.

Materials and methods

Our experiments were setup on calcareous chernozem soil in the Hajdúság, 15 km from Debrecen, in three different cropyears (2009/2010; 2010/2011; 2011/2012). The soil of the experiment is characterized by favourable physical, chemical and biological traits. The humus content of the calcareous chernozem soil of the experiment is 2.76%, its AL soluble P_2O_5 value is 133 mg kg^{-1}, its AL soluble K_2O value is 240 mg kg^{-1}. The soil has favourable water management traits. The soil saturated up to the field water capacity can store 578 mm water in the 0-2 m layer, 50% of which is disponable water.

In the experimental years, the fore-crop was winter wheat. Soil preparation was conducted by disc, mulch tiller (loosening) and germinator. In the experimental years, weed control, regulator and insecticide use were done uniformly. In the experimental years, protection against diseases was performed at the beginning of flowering (200.0 g l^{-1} boscalid + 200.0 g l^{-1} dimoxistrobin [0.5 l ha^{-1}]). Before harvest, we made pod sticking and stock desiccation. Harvest was performed at the end of June-beginning of July, depending on the cropyear.

We applied traditional row distance (24 cm) for the sowing of the experiment. The hybrid used in the experiment was Rohan, which we sowed with germ number of 350 thousand per hectare. During the experiment, we applied two sowing dates (August and September):

Cropyear of 2009/2010
26/08/2009 and 10/09/2009

Cropyear of 2010/2011
27/08/2010 and 14/09/2010

Cropyear of 2011/2012
25/08/2011 and 14/09/2011

The applied fertilizer doses and N splitting are listed in *Table 1*.

Table 1. The fertilizer doses in the long-term experiment (Debrecen, 2010-2012 years)

Fertilizer treatments	N kg ha^{-1}				P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Note
	autumn	end of winter	spring	total			
1	0	0	0	0	48	108	
2	40	60	40	140	48	108	
3	40	100	70	210	48	108	
4	40	100	70	210	48	108	sulfur + foliar fertilizer

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The phosphorus and potassium doses during the tillage works were applied by 0:8:18 complex fertilizer. The application of nitrogen was conducted in the form of NH_4NO_3.

During the vegetation period of winter rape we determined the infections of Peronospora (*Peronospora parasitica*) and Phoma (*Leptophaeria maculans*, anamorph: *Phoma lingam*) as the leaf diseases. We detected the infected leaf area (%) on 15-15 crops in each plots. The stalk disease was Sclerotinia (*Sclerotinia sclerotiorum*). If the infection area of stem by Sclerotinia was over 20% we stated as an infected plant (15-15 plants plot^{-1}). The pod disease was Alternaria (*Leptoshaeria napi*, amorf: *Alternaria brassicae*). If the infected number of pods was over 20% we took as an infected plant (15-15 plant plot^{-1}). All phytosamitary measurement were carried out in four replications.

The experiment design originally was split-split-plot. The factors of experiment were: fertilization (4 treatments), sowing time (2 treatments) and plant density (3 treatments). We publish the results of plant density of 350 thousand ha^{-1} (the others were 200 thousand ha^{-1} and 500 thousand ha^{-1}). We used four replicates. The main plots (fertilization) were 216 m^2, the sub-plots (sowing time) were 108 m^2 and the sub-sub-plots (plant density) were 36 m^2. So we used the two-factor variance analysis and correlation analysis by Pearson for statistical analysis our experimental data (split-plot experiment design). During the evaluation of experimental treatments, we have used the yields per 1 kg NPK active ingredient and 1 mm precipitation + irrigation water (WUE = Water Use Efficiency).

For the characterization of the weather of the experimental years, we list the monthly precipitation and temperature data of the whole vegetation period (August to June) in *Tables 2* and *3*. The weather of the experimental years significantly differed from each other. These differences were especially pronounced in the case of the weather conditions at the end of summer-autumn.

There were also differences between the weather of the spring and early summer months. We demonstrate the effects of the meteorological factors in the experimental results section in details. In the case of oilseed rape, the basic criteria of the comparability of the experiments are the adequate plant number and stock homogeneity. In favour of achieving these, we performed irrigation in the years of unfavourable late summer-autumn weather by linear irrigation equipment providing precise

Table 2. Monthly precipitation in the vegetation period of rape (mm) (Debrecen, 2010-2012 years)

Years	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Febr.	March	Apr.	May	June
2009/2010	11.3	21.7	79.3	78.3	54.9	48.8	58.6	14.4	89.9	111.4	100.9
2010/2011	98.3	98.4	22.8	52.9	104.2	19.2	16.8	35.1	15.6	52.3	22.0
2011/2012	42.7	6.2	18.1	0	71.1	28.0	17.8	1.4	20.7	71.9	91.7
Average of 30 years	60.7	38.0	30.8	45.2	43.5	37.0	30.2	33.5	42.4	58.8	79.5

Table 3. Monthly average temperature in the vegetation period of rape (^{o}C) (Debrecen, 2010-2012 years)

Years	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Febr.	March	Apr.	May	June
2009/2010	22.6	18.9	11.4	7.6	2.3	-1.1	0.5	7.6	11.6	16.6	19.7
2010/2011	19.0	14.1	6.9	7.7	-1.7	-1.2	-2.5	5.0	12.2	16.4	20.8
2011/2012	21.4	18.0	8.6	0.6	1.5	-0.6	-5.7	6.3	11.7	16.4	20.9
Average of 30 years	19.6	15.8	10.3	4.5	-0.2	-2.6	0.2	5.0	10.7	15.8	18.7

Table 4. Irrigation system in the vegetation period of oilseed rape (Debrecen, 2010-2012 years)

Years	Planting in August (irrigation time and water, mm)	Planting in September (irrigation time and water, mm)
2009/2010	31/08/2009 – 20 mm 25/09/2009 – 20 mm	25/09/2009 – 20 mm
2010/2011	-	-
2011/2012	24/08/2011 – 25 mm 31/08/2011 – 15 mm 02/09/2011 – 15 mm 14/09/2011 – 15 mm	14/09/2011 – 15 mm 21/09/2011 – 20 mm 28/09/2011 – 20 mm

water application. The irrigation system of the different experimental years are listed in *Table 4*.

The experimental years were significantly different considering the weather at the end of summer-autumn. The August and September of the 2009/2010 cropyear were dry (11.3 mm and 21.7 mm), which made the application of the 40 mm and 20 mm irrigation water reasonable. From October, a favourable rainy weather began to take place, which was proved by the precipitation of the autumn-winter months (August-February, 352.9 mm) that exceeded the 30-year average (285.4 mm). This rainy weather persisted during the spring and early summer (March-June) of 2010 (316.6 mm precipitation, compared to the multi-year average of 214.2 mm).

The weather of the cropyear of 2010/2011 was completely different. Due to the late summer-autumn precipitation of favourable amount and distribution (412.6 mm rainfall in August-February, exceeding the multi-year average by 127.2 mm), there was no need for irrigation. Although the precipitation of the spring-early summer months (125.8 mm between March and June, 88.4 mm below the multi-year average) was low, the autumn and winter precipitation stored in the chernozem soil provided adequate water reservoire for the development and crop formation of the rape stocks.

The highest water shortage of late summer-winter took place in the cropyear of 2011/2012. The precipitation fell between August and February (183.9 mm) was 101.5 mm lower than the multi-year average. In favour of the appropriate development of plant stocks in autumn, we have to apply a sum of 70 mm irrigation water (in four occasions) in the August sowing, while a total of 55 mm in three occasions in the September sowing. The precipitation of the spring-early summer months (March-June, 185.7 mm) was close to the multi-year average of 214.2 mm. The water supply was especially favourable in May (71.9 mm) and June (91.7 mm) favouring for pod development and seed filling.

The aim of our experiments was to determine the influence of increasing N fertilizer doses and different sowing times on the pathological traits and yield of oilseed rape in different cropyears.

Results

During the three-year-long experiment, we have investigated the most important leaf, stalk and pod diseases of oilseed rape (*Table 5*). The precipitation amount during the most important spring period (April-May-June) in terms of the appearance of diseases, significantly influenced the infection. Especially the differences between the values of Sclerotinia infection were considerable among the cropyears. During the spring of 2010, the Sclerotinia infection varied between 3.0 and 11.0%, between 0.2 and 0.8% in 2011, while between 1.1 and 2.1% in 2012, respectively. The Sclerotinia infection was closely related to the precipitation fell in April, May and June. The highest Sclerotinia infection rate was measured in 2010 (302.2 mm precipitation in April, May and June), while that was significantly lower in 2012

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Table 5. Effect of fertilizer and sowing time on the diseases of oilseed rape
(Debrecen, chernozem soil, 2010-2012 years)

Treatments Fertilizers (A) Sowing time (B)	2009/2010				2010/2011				2011/2012			
	Sclerotinia	Alternaria	Peronospora	Phoma	Sclerotinia	Alternaria	Peronospora	Phoma	Sclerotinia	Alternaria	Peronospora	Phoma
	infection%											
Sowing in August												
1	3.0	10	16	11	0.2	5	11	8	1.1	7	12	11
2	8.0	16	22	20	0.5	5	13	9	1.4	8	15	13
3	10.0	19	25	23	0.4	6	15	9	1.4	12	18	15
4	11.0	21	26	24	0.6	6	15	11	2.2	14	22	19
Sowing in September												
1	5.0	10	11	10	0.5	4	9	5	1.4	5	11	9
2	8.0	14	20	16	0.7	3	10	5	1.4	8	14	12
3	9.0	17	23	19	0.8	5	10	7	1.9	10	17	17
4	9.0	18	22	21	0.3	5	12	8	2.1	10	21	18
$LSD_{5\%}$ (A)	**1.2**	**2**	**2**	**1**	**0.1**	**1**	**1**	**1**	**0.2**	**2**	**1**	**2**
$LSD_{5\%}$ (B)	**0.9**	**1**	**3**	**2**	**0.1**	**1**	**1**	**1**	**0.1**	**1**	**2**	**1**
$LSD_{5\%}$ (AxB)	**2.0**	**4**	**6**	**5**	**0.2**	**2**	**2**	**2**	**0.4**	**3**	**4**	**4**

(184.3 mm) and 2011 (89.9 mm). In the case of the most important leaf diseases, similar tendencies were found, but the differences among the cropyears were not that pronounced. The Peronospora infection varied between 11 and 26% in 2010, between 9 and 15% in 2011 and between 11 and 22% in 2012, respectively. The values of the Phoma infection were as follows: 2010: 10-24%, 2011: 5-11%, 2012: 9-19%. The most important pod disease, the Alternaria infection was the highest in the rainy spring of 2010 (10-21%), while the lowest in the dry 2011 (3-6%). In 2012, it varied between 5 and 14%.

As an effect of the increasing fertilizer doses, the infection values of the diseases significantly increased, while between the sowing times of August and September, there were no significant differences between the infection values.

The results of our traditional, non-long-term experiments confirmed the favourable natural nutrient fertility of the chernozem soil. In the case of appropriate phosphorus and potassium supply (P_2O_5 = 48 kg ha^{-1} and K_2O = 108 kg ha^{-1}), the yield of oilseed rape varied between 3010 and 3102 kg ha^{-1} in 2010, between 4157 and 4218 kg ha^{-1} in 2011, while between 3139 and 3722 kg ha^{-1} in 2012, respectively, without nitrogen fertilizer, depending on sowing time (*Table 6*). The sowing time did not effect yields significantly in 2010 and 2011, but we did not found significant differences in the case of all fertilizer levels in 2012 between the sowing times. The application of nitrogen fertilizer increased yield up to the dose of N = 210 kg ha^{-1} +PK. The adequate sulphur content (favourable humus content) and sulphur providing ability of the chernozem soil was confirmed by the fact that there were no significant differences between N = 210 kg ha^{-1} +PK (3rd treatment) and N = 210 kg ha^{-1} +PK+sulphur (4th treatment). In a traditional experiment, the maximum yield surplus achieved by N fertilization was 1300 kg ha^{-1} in 2010, 800 kg ha^{-1} in 2011, and 1000 kg ha^{-1} in 2012, respectively. The maximum yields

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Table 6. Effect of fertilization and sowing time on the yields of oilseed rape (Debrecen, chernozem soil, 2010-2012 years)

Treatments Fertilizers (A) Sowing time (B)	2009/2010		2010/2011		2011/2012	
	Yield kg ha^{-1}	Yield surplus kg ha^{-1}	Yield kg ha^{-1}	Yield surplus kg ha^{-1}	Yield kg ha^{-1}	Yield surplus kg ha^{-1}
Sowing in August						
1	3102	0	4157	0	3722	0
2	3932	830	4714	557	4181	459
3	4361	1259	4950	793	4311	589
4	4390	1288	4796	639	4140	418
Sowing in September						
1	3010	0	4218	0	3139	0
2	3709	699	42642	424	3875	736
3	4241	1231	4996	778	4042	903
4	4273	1263	4654	436	4111	972
$LSD_{5\%}$ (A)	**210**	**-**	**196**	**-**	**154**	**-**
$LSD_{5\%}$ (B)	**168**	**-**	**243**	**-**	**268**	**-**
$LSD_{5\%}$ (C)	**516**	**-**	**508**	**-**	**547**	**-**

were obtained in August sowing time during all three years (2010: 4390 kg ha^{-1}, 2011: 4950 kg ha^{-1}, 2012: 4311 kg ha^{-1}). The difference between the two sowing times was the lowest in 2011, the most favourable cropyear for the vegetative and generative development of oilseed rape (46 kg ha^{-1}), but slight differences were found in 2010 (117 kg ha^{-1}) and 2012 (269 kg ha^{-1}) too. This confirmed that in the case of the applied oilseed rape hybrid, the optimal sowing interval is very wide (from the end of August to the middle of September). According to the results of our experiments, although the increasing fertilizer doses increased the infection of different diseases, the higher N doses resulted in higher yields. In 2011, when the leaf, stalk and pod infections were the lowest, we have achieved the highest yield (~5000 kg ha^{-1}) among the three studied cropyears.

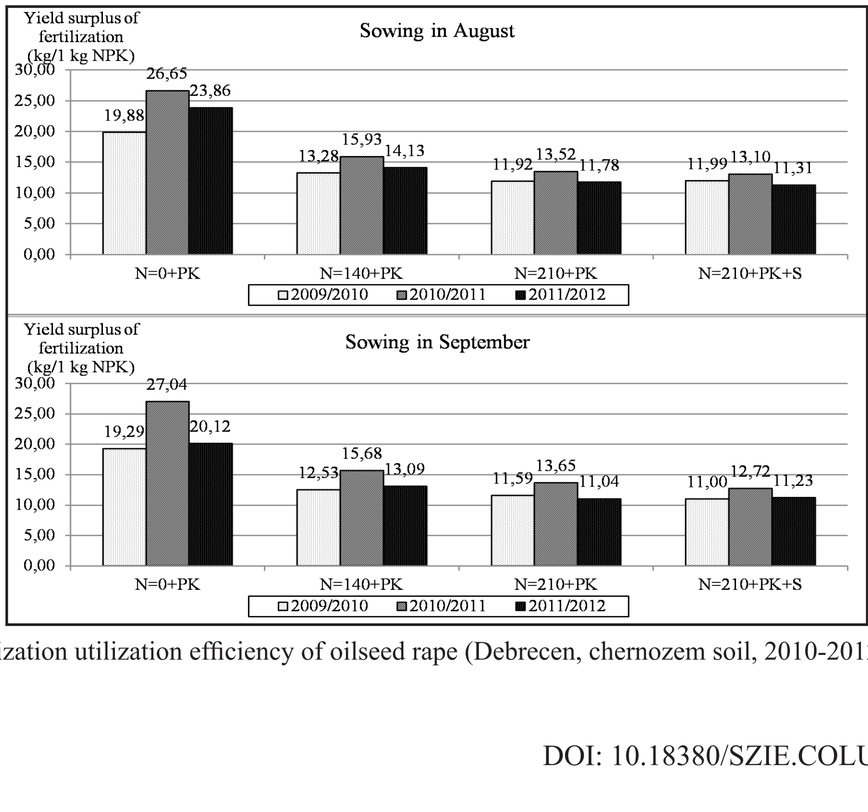

Figure 1. Fertilization utilization efficiency of oilseed rape (Debrecen, chernozem soil, 2010-2012 years)

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In the experiment, we characterized the efficiency of fertilization by yield per 1 kg NPK fertilizer (*Figure 1*). Our research results confirmed that the increasing N doses resulted in decreasing efficiency. In the case of the control (1st treatment, N = 0 kg ha^{-1}) 19.29-27.40 kg/1 kg NPK, while in the case of the highest N dose (3rd treatment, N = 210 kg ha^{-1}) 11.04-13.65 kg/1 kg NPK was the efficiency of fertilization despite the yield maximums were achieved in the case of the latter treatment (N = 210 kg ha^{-1}) in both sowing times, all three experimental years.

We have experienced considerable difference between the cropyears in terms of water utilization (*Figure 2*). During the calculation of water utilization, the precipitation fell during the vegetation period and the amount of applied irrigation water during the given sowing time were considered jointly. The worst water utilization was found in the year of the highest precipitation and infection (2010, 4.37-6.20 kg/1 mm precipitation + irrigation water, respectively). There were no significant differences found between the water utilization values of 2011 and 2012 (2011: 7.72-9.28 kg, 2012: 7.39-9.81 kg/1 mm precipitation + irrigation water). We have not found significant difference between the two sowing times in terms of water utilization. As an effect of the increasing N doses, the water utilization of oilseed rape improved.

The data of the Pearson's correlation analysis (*Tables 7* and *8*) confirmed that due to the favourable nutrient management features of the chernozem soil, medium and tight correlation was detected between the fertilization and the yield of oilseed rape both in the case of the August (0.609*) and September sowings (0.602*). Among the meteorological factors, the yields were primarily negatively influenced by the temperature of March-June (-0.638* and -0.683*, respectively), i.e. lower yield belonged to the higher temperature. We have found unambiguous and tight correlation between the weather factors and oilseed rape diseases. Especially tight, positive correlation was detected between the precipitation and temperature of the spring months (March-June) and the infection values measured at both sowing times (August and September). The correlation coefficient values varied between 0.638* and 0.943* in the case of Sclerotinia, between 0.761** and 0.889** in the case of Alternaria, between 0.652* and 0.742** in the case of Peronospora, while between 0.701*

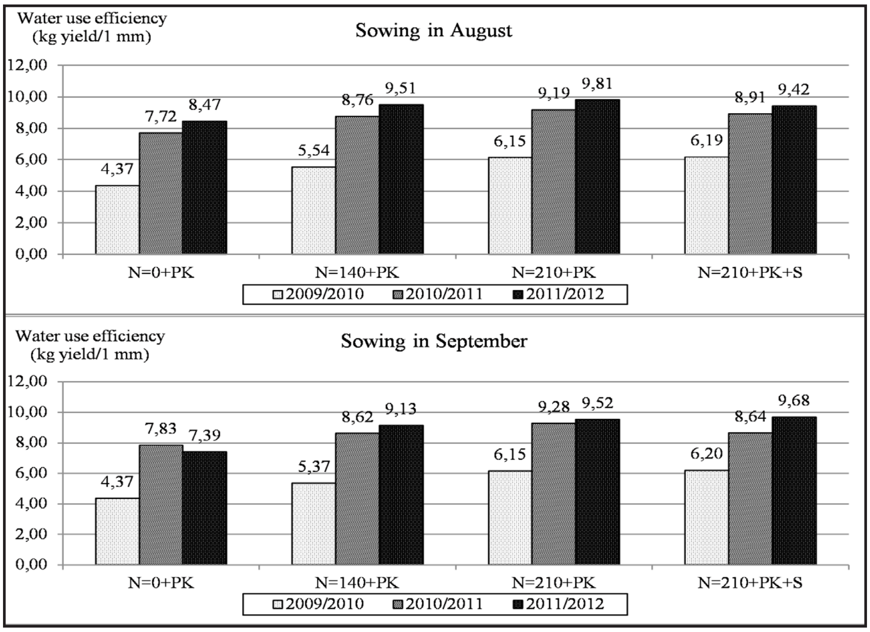

Figure 2. Effect of fertilization and sowing time on the water use efficiency of oilseed rape (Debrecen, chernozem soil, 2010-2012 years)

Table 7. Evaluation of meteorological parameters, yield and infections of diseases by Pearson correlation analyses in winter rape (Debrecen, sowing time in August, chernozem soil, 2010-2012 years)

	Fertilization	Rainfall in Aug.-Febr.	Mean temp. in Aug.-Febr.	Rainfall in March-June	Mean temp. in March-June	Rainfall in Aug.-June	Mean temp. in Aug.-June
Fertilization	1	0.000	0.000	0.000	0.000	0.000	0.000
Yield	0.609*	0.276^{NS}	-0.450^{NS}	-0.546^{NS}	-0.638*	-0.215^{NS}	-0.631*
Sclerotinia	0.281^{NS}	0.244^{NS}	0.884**	0.871**	0.649*	0.777**	0.743**
Alternaria	0.451^{NS}	-0.30^{NS}	0.780**	0.831**	0.761**	0.575^{NS}	0.810**
Peronospora	0.614*	0.025^{NS}	0.709**	0.742**	0.652*	0.546^{NS}	0.704*
Phoma	0.528^{NS}	-0.098^{NS}	0.687*	0.750**	0.725**	0.474^{NS}	0.759**

**Correlation on LSD1% level; Correlation on LSD5% level; ^{NS} Non significant

Table 8. Evaluation of meteorological parameters, yield and infections of diseases by Pearson correlation analyses in winter rape (Debrecen, sowing time in September, chernozem soil, 2010-2012 years)

	Fertilization	Rainfall in Aug.-Febr.	Mean temp. in Aug.-Febr.	Rainfall in March-June	Mean temp. in March-June	Rainfall in Aug.-June	Mean temp. in Aug.-June
Fertilization	1	0.000	0.000	0.000	0.000	0.000	0.000
Yield	0.602*	0.479^{NS}	-0.379	-0.508^{NS}	-0.683*	-0.044^{NS}	-0.651*
Sclerotinia	0.171^{NS}	0.150^{NS}	0.953**	0.943**	0.710**	0.795**	0.810**
Alternaria	0.376^{NS}	-0.074^{NS}	0.847**	0.889**	0.785**	0.602*	0.846**
Peronospora	0.576^{NS}	-0.257	0.590*	0.670*	0.700*	0.316^{NS}	0.715**
Phoma	0.539^{NS}	-0.388	0.589*	0.701*	0.797**	0.249^{NS}	0.794**

**Correlation on LSD1% level; Correlation on LSD5% level; ^{NS} Non significant

and 0.797** in the case of Phoma infection with the March-June precipitation and March-June temperature values.

Discussion

Our oilseed rape experiments conducted on chernozem soil in different cropyears confirmed that the cropyear influenced the yield of oilseed rape due to the autumn germination and stock settlement and the differences between the infection of the diseases occurring during spring. The unfavourable effects of the dry late summer-autumn weather were compensated by irrigation during the autumn periods of the 2009/2010 and 2011/2012 cropyears. Therefore, in all three experimental years, complete, homogenous stocks developed making the precise comparison and evaluation of the experimental results possible.

Due to the favourable autumn weather of the 2010/2011 cropyear, no irrigation was conducted. Our experimental results confirmed that the infection of leaf, stalk and pod diseases were determined by the weather of the spring period (April-May-June) and the amount of precipitation. In the case of the rainy spring weather (302 mm precipitation in April-May-June), the Sclerotinia infection was especially high (3.0-11.0%). As an effect of the lower precipitation in spring, in 2012 (184 mm) we have found the varied between Sclerotinia infection 1.1-2.1%, while in 2011 (90 mm) 0.2-0.8%, respectively. Same tendencies but lesser differences were found in the cases of the Peronospora, Phoma and Alternaria infections. The increasing N fertilizer doses significantly, while the sowing time (lower infection in the August sowing) non-significantly influenced the infection values of oilseed rape.

The most favourable yields were achieved in 2011, the year of the lower infections. The maximum yield was obtained in the case of the N = 210 kg ha^{-1} +PK treatment (5000 kg ha^{-1}), which confirmed the high nutrient demand of oilseed rape. With its widespread, intensive rootsystem, oilseed rape could utilize

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the natural nutrient supply of the chernozem soil to a great extent. In the case of the P + K fertilizer treatment, the yield was between 3000 and 4200 kg ha^{-1}, while in the N = 210 kg ha^{-1} +PK treatment, it varied between 4000 and 5000 kg ha^{-1}. As an effect of sulphur fertilization, the yield of oilseed rape did not changed, this proved that the chernozem soil could provide the high sulphur demand of rape. As an effect of the increasing N doses, the specific fertilization yield surplus of rape decreased (in the case of N = 0 kg ha^{-1} +PK treatment 19-27 kg/1 kg NPK, in the N = 210 kg ha^{-1} +PK treatment 11-12 kg/1 kg NPK). Our studies confirmed that N fertilization conversely improved the water utilization of oilseed rape in all three experimental years (in the case of the N = 0 kg ha^{-1} +PK treatment 4-8 kg/1 mm precipitation + irrigation water, in the case of the N = 210 kg ha^{-1} +PK treatment 11-14 kg/1 mm precipitation + irrigation water).

According to the data of our research – under irrigated circumstances –, the hybrid oilseed rape has favourable adaptive capacity to the different sowing times. Although in each year, comparing the late August and middle September sowing times, the August one was more favourable, the yield decreasement was only minimal in the middle September one (0-270 kg ha^{-1} depending on the cropyear). Similarly to the experiments of *Boelcke* et al. (1991) (optimal N = 240 kg ha^{-1}), our research results confirmed the high nutrient demand of oilseed rape (in our experiments the optimum was N = 240 kg ha^{-1}). Due to the traditional, non-long-term experiment, the yield increasing effect of fertilization varied between 800 and 1300 kg ha^{-1}, depending on the cropyear. These values considerably exceeded the yield increase obtained by *Sieling* and *Christen* (1997) in their studies. While in the experiments of *Risnoveanu* and *Buzdugen* (2011), the optimal sowing time interval was relatively narrow (5 to 10 September), in our studies we obtained comparatively broad optimum sowing time interval (25 August-15 September) in the case of the hybrid of new genotype (Rohan). There was moderate yield difference (0-270 kg ha^{-1}) between the August and September sowing times in the studied cropyears.

The results of the Pearson's correlation analyses confirmed that the correlation between fertilization and the yield of oilseed rape was medium (0.6*). The weather factors, especially the precipitation and temperature of the spring-early summer months (March-June) were in strong positive correlation (0.6*-0.9**) with the Sclerotinia, Alternaria, Peronospora and Phoma infections.

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CATCH CROP (OIL RADISH) FUNCTIONS IN LONG TERM CEREAL CROP ROTATION

Tamás KISMÁNYOKY – Brigitta TÓTH

University of Pannonia, Georgikon Faculty, Department of Crop Production and Soil Science, Festetics u. 7, 8360 Keszthely, Hungary, E-mail: kis5556@ella.hu

Abstract: The aim of our research work was to study the role of the oil radish catch crop (Raphanus sativus var oleiformis Pers) as second crop or stubble crop for green manuring in winter wheat-winter barley-maize crop rotation. The oil radish was grown after the harvest of winter barley in the crop sequence. The green biomass (t ha^{-1}), nitrogen (N) content in dry material (g kg^{-1}) of catch crop and the N (kg ha^{-1}) taken up by the catch crop were investigated for six years (1997-2002). The main goal was to measure the amount of remained N in the soil left by the main crop (WB) and the amount of N taken up by the oil radish catch crop. The maximum N uptake was about 120 kg ha^{-1} calculated on the average of the years but in the rainy 1998 the N removal reached the 200 kg ha^{-1} N. On the basis of the N balance calculation it is proved that incorporation of the catch crop in the crop sequence, the risk of N leaching diminishes, or can be minimal even if the level of N input is high, because the N removal of green biomass retains N in the soil and this way N turnover of the crop rotation remains balanced.

Keywords: oil radish catch crop, N fertilization, N loss, N uptake, N balance.

Introduction

In many regions of the world, soils are managed intensively during a certain period of the year mostly for the production of a particular crop. Following that period, little is done to keep the soil in optimal conditions. The problem is that major degradation processes, such as erosion, leaching and compaction are often most intense during the period without crop cover. A key question for crop production sustainability in the 21th century will be the extent to which agriculture will develop special agronomic practices to manage the uncovered period so that to minimize undesirable material flows from the agroecosystem. For example, cover crops can increase the net primary productivity, can have advantageous effect on the food chain quality and enhance the organic portion of nutrient cycle (Lal and Pierce, 1991).

To compile a proper crop rotation it is important to know how long time is available between the harvest of pre-crop and the sowing time of the next crop and how appropriate this period is for the following crop. In this term it is possible to strengthen the positive effects of fore crop and diminish the unfavourable effects of the inadequate pre-crop (Kismányoky, 2013). According to Kemenesy (1961), the positive and negative effects of green manure as a second crop are as follows: increasing the amount of labile humus, ameliorating soil structure, mobilizing soil nutrients, bonding nutrients in organic forms, N-fixing by legume crops, increasing the effects of fertilizers, loosening the soil by deep rooting, weed control, overshadowing the soil surface, improving and maintaining the biological activity of the soil, erosion control. On the other hand, the water and nitrogen reserve in the soil can be decreased. Experiments of Westsík (1965) performed in crop rotation on sandy soils is outstanding. He focused on the agro-technique of N-fixing crops such like lupines as a main and second crop.

Green manures improve soil quality not merely because of the incorporated organic crop parts, rich in nutrients, but owing to the strong root structure developed, too. The roots increase the organic carbon (OC) content in the deeper soil layers, and pave the way for the roots of the next crop as well. The roots of the following crop penetrate into the deeper soil layers and this way they will be able to resist the dry periods (Láng, 1962). Kahnt (1986) summed up in 12 points the favourable effects of green manuring, and emphasizes the importance of the organic matter and nitrogen

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accumulation, furthermore the mitigation of nutrient losses, first of all the N leaching. Further advantages and disadvantages of green manuring are summarized in the papers of Kismányoky (1996) and Tóth (2006).

According to the field experiments of Ujj (2006) the cultivation of oil radish as stubble crop for green manure is advisable to grow but the hazards should be taken into consideration such as sowing time, water demand of the next crop, weed control etc.

Oil radish as green manure and winter barley straw residues (plus added N) increased the OC content of soil by 0,2% in a long run compared to the control NPK treatments in crop rotation, but this increase didn't reach that of by farmyard manure application. The continuous OC input is important for humus dynamics and humus balance, which is more effective than adding a higher amount of organic manure occasionally (Kismányoky, 2013).

There is a proverb from Arizona which says: "It does not matter to the horse how much water is in the tank as long as replenishment is continuous". Organic matter feeds microorganisms that release nutrients from organic matter and minerals that improve soil structure and increase water-holding capacity. Green manure and cover crops are planted to support soil protection, to pick up soluble nutrients that might otherwise be leached. Therefore the principle in soil management is that soils should never be allowed „rest", something should always be grown on them (Cook and Ellis, 1987). The cruciferous summer crops with N fertilization shadow the soil surface, this way the decomposition of straw is accelerated under humid circumstances, while, on the other hand, slows down in dry conditions (Kahnt, 1986).

With joint application of green manure and fertilizers the efficiency of fertilizers improves (Kismányoky, 1993). The green crops sown in the stubble take up the soluble nutrients remaining after the previous crop that might otherwise be leached (El Titi and Landes, 1990). This is the so called retaining effect, which is very important under rainy conditions (Gyuricza, 2008). Non-leguminous cover crops, although they do not fix N, can provide substantial N amounts as "sanitary crops", taking up the leftover N, or, in rare cases, the N fertilizer applied for the cover crop. With the catch crops we can prevent the leaching of soluble nutrients from the soil, e.g. the most part of nitrogen which develops during the summer season (Gyárfás, 1951). Without their use, some of these surplus nutrients may be lost due to leaching or erosion and under intensive fertilisation and humid climate conditions, agro-environmental problems would increase (Wolf-Snyder, 2003).

Management of nutrients by including a green manure crop is a rationale method to prevent nitrate losses, mostly under intensive fertilisation, and humid climate conditions. For groundwater protection purposes, farmers in Baden-Würtenberg (Germany) must, by law, maintain the water soluble nitrate fraction of the soils bellow 45 kg ha^{-1}. Catch cropping is a practical way to mitigate nitrogen leaching in an environmentally sound way. In integrated and organic farms green manure crops were grown to fix nitrate that had been left behind by the main crop or had been mineralized after harvest.

Taking into consideration the above mentioned principles, the aims of the research work was to study the role of oil radish (Raphanus sativus var oleiformis Pers) catch crop as second crop or stubble crop, for green manuring in cereal crop rotation. The crop rotation consisted of winter wheat-winter barley (WB)-maize. The oil radish came after the harvest of WB in the rotation. The green biomass (t ha^{-1}), nitrogen (N) content of oil radish based on in dry material (g kg^{-1}) and the amount of N (kg ha^{-1}) taken up by catch crop were analysed in the period of 1997 to 2002. The main goal of setting up the trial was to measure the amount of N remained in the soil after the main crop

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(WB) and the amount of N taken up by the catch crop. It was supposed that as an effect of the different fertilizer treatments of the pre-crop the amount of N remaining in the soil following the harvest would be different as well.

Material and methods

The study was conducted in the international mineral and organic nitrogen fertilization trial (IOSDV) located in Keszthely in the west part of Hungary (46°44’ N 17 13 E, 112 m above sea level). The long term field experiment was set up in the autumn of 1983. The experiment has been maintained since that time. In this study the data derived from the period of 1997 to 2002 are analysed.

The soil type of the study site was Eutric Cambisol (IUSS, 2014), which is a Ramann –type brown forest soil according to the Hungarian soil classification system (Stefanovits et al., 1999). The texture of the plough layer is loam, containing 410 g kg^{-1} sand, 320 g kg^{-1} silt and 270 g kg^{-1} clay. The soil of the experiment showed low phosphorus supply (ammonium-lactate /AL/ soluble P_2O_5: 60-80 mg kg^{-1}), medium potassium supply (AL-K_2O: 140-160 mg kg^{-1}) and the humus (H) content fairly low (16-17 g kg^{-1}), with a pH_{KCl} value of 7.1. The bulk density is 1.53 g cm^{-3}. The 100 year average annual precipitation was 683 mm, but its distribution was often unequal, most average rainfall occurring in June (79 mm) while the lowest amount in January (35 mm). The long term annual mean temperature was 10.8 °C.

The factorial experiment has a strip-plot design with three replications. The size of the subplots is 48 m^2. The factorial treatments were mineral N fertilization (5 rates) combined with organic fertilizers (3 levels). Treatments were applied with three-year cereal crop rotation (maize-winter wheat-winter barley) system. The mineral N fertilizer rates were 0, 70, 140, and 280 kg ha^{-1} in case of maize, 0, 50, 150, and 200 kg ha^{-1} for winter wheat and 0, 40, 80, 120, and 160 kg ha^{-1} for WB. N rates are referred to as N0, N1, N2, N3, N4 indicating N doses hereinafter. Supplemental P and K fertilizers at rates of 100 kg ha^{-1} P_2O_5 and K_2O, resp. were applied on all experimental plots (even on the N control /N0/ plots).

Organic fertilizer treatments were applied as complementary fertilization with the mineral NPK fertilizers having three different variants: (I) no organic fertilizer application (control), (II) application of 35 t ha^{-1} farmyard manure (FYM) in every third years before maize, (III) straw incorporation (St) completed with 10 kg ha^{-1} mineral N for each t of straw/ stem. After WB, on the „St” plots oil radish (Raphanus sativus var. Oleiformis) was sown into the WB stubble for green manuring (Gm).

In this paper the effect of the oil radish catch crop (Figure 1.), incorporated in the crop rotation is presented, based on the (III) St+Gm variant block including N0-N4 treatments in the 5th, 6th and 7th crop rotation periods (1997 to 2002). It was grown after WB main crop


a)


b)

Figure 1. Study field (a)) and flowering of oil radish (b)).

as second crop (stubble sowing). Following the harvest, WB straw was chopped and incorporated into the soil at the same time with the sowing of catch crop. The sowing was carried out at the beginning of August with15-20 kg ha^{-1} seeds of the oil radish variety, without any fertilization. At the end of October green samples were taken from the oil radish biomass (one sample from 2x1 m^2/ plots) for plant analysis. Dry matter content was determined in exsiccator. The N content of dry matter was measured by Kjeldahl method (Buzás, 1988).

For the calculation of N balance, on the input side we took into consideration the N rates of fertilization (N0-N4) before WB, the N content of straw of the fore crop wheat, which was chaffed and ploughed into the soil before WB, and the complementary amount of N fertilizer - 1 kg N for 100 kg dry material - , which was applied to secure the degradation of the straw manure. In the output side of N balance we considered the removed N by WB grain and straw, and the amount of N taken up by the catch crop biomass.

To define significant differences between different nitrogen fertilization rates Tukey tests were performed on the green biomass of oil radish, N uptake and N content by years and all year. Tukey HSD test at the 5% significance level was applied using the R package agricolae (de Mendiburu, 2014). Statistical analysis and graphs were prepared with R statistics (R Core Team, 2013).

Results and discussion

Green biomass production

The green biomass of oil radish increased continuously with increasing N fertilization rates in every year (Table 1). There is a strong correlation (0.876-0.999) between the N fertilization rates of previous main crop (WB) and the amount of remaining N fertilizer in the soil. The green biomass production varied between 10-40 t ha^{-1} according to the N fertilization of WB. Antal (1999) produced 15-25 t ha^{-1} green material of oil radish on sandy soil. The effect of the cropping years (rainfall) were even more determinate, defining the growing intensity of oil radish's vegetative parts.

On the other hand, according to the analysis of Ujj (2006), the green mass of catch crop as second crop was not always directly proportional to the intensity of nitrate uptake. Gyuricza (2008) found similar results in field trials, the mass of green manure crops was about 25-45 t ha^{-1}. He highlighted that green manure usually decreases the water content of topsoils, but during the winter time it becomes balanced. In the 2nd and 3rd variants the soil N significantly increased the green production of oil radish in every year compared to the control plots and to the previous variant, except in 1998. The variant 4th did not significantly increase the green biomass compared to variant 3, except in 1998. There was no significant difference between variant 4 and 5.

Table 1. Mean green biomass of oil radish (t ha^{-1})

Green biomass by year(t ha^{-1})	N fertilization rates (kg ha^{-1})					Mean green biomass (t ha^{-1})
	0	40	80	120	160	
1997	10.20^{d}	16.53^{c}	18.33^{bc}	20.43^{ab}	21.67^{a}	17.43
1998	10.33^{c}	16.20^{c}	26.73^{b}	35.90^{a}	42.87^{a}	26.41
1999	18.53^{d}	22.87^{cd}	27.70^{bc}	31.53^{ab}	36.53^{a}	27.43
2000	16.00^{c}	28.07^{b}	35.23^{a}	38.07^{a}	38.20^{a}	31.11
2001	10.77^{d}	14.30^{cd}	23.40^{bc}	31.67^{ab}	34.23^{a}	22.87
2002	21.27^{c}	31.53^{b}	34.73^{ab}	36.33^{a}	36.50^{a}	32.07
Mean green biomass (t ha^{-1})	14.52^{d}	21.58^{c}	27.69^{b}	32.32^{ab}	35.00^{a}	26.22

Between different N fertilization rates significant differences at 0.05 level are marked with different letters

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Table 2. Mean nitrogen content of oil radish based on dry material (g kg^{-1})

N content by year (g kg^{-1})	N fertilization rates (kg ha^{-1})					Mean N content (g kg^{-1})
	0	40	80	120	160	
1997*	18.10	18.60	23.00	20.50	25.40	21.12
1998	24.00^{b}	26.57^{b}	34.37^{a}	33.43^{a}	39.10^{a}	31.49
1999	10.30^{a}	11.37^{a}	11.70^{a}	10.83^{a}	11.87^{a}	11.21
2000	20.30^{a}	21.80^{a}	24.57^{a}	27.60^{a}	25.70^{a}	23.99
2001	37.40^{a}	34.30^{a}	39.53^{a}	45.57^{a}	48.23^{a}	41.01
2002	22.17^{a}	20.37^{a}	21.47^{a}	23.20^{a}	26.63^{a}	22.77
Mean N content(g kg^{-1})*	22.04^{a}	22.16^{a}	25.77^{a}	26.85^{a}	29.49^{a}	25.78

*For year 1997 only the mean values were available, therefore in Tukey test data of year 1997 could not be considered due to missing values.

Between different N fertilization rates significant differences at 0.05 level rates are marked with different letters.

It is proved by the green biomass production of catch crop that the amount of the remaining soil N is in accordance with the N rates given by the fertilization of the previous main crop – which was WB in our study. Eichler et al. (2004) considered the cover crops as a good solution to avoid the N leaching. In their field experiments the cover crops decreased the N losses by 240 kg ha^{-1} year^{-1}. The order of their N use efficiency was the following: oil seed radish, rapes, phacelia.

Correlation between green biomass of oil radish and N fertilizer dose is between 0.876 and 0.999 varying with years. Different levels of the green production by the studied years indicate the influence of the cropping years upon the success of second cropping. Successful growing of catch crops depends on many factors, among others the water consumption of main crop, the amount and distribution of summer rainfall, the quality of seed bed and the proper time of sowing are determinant.

N content of catch crop

The N content of oil radish increased parallel with the N fertilizer rates of the previous main crop (WB) and with the presumed higher soil N content from the zero plots to the high rates of N treatments (N4). The N content of oil radish biomass increased from 20 to 30 g kg^{-1} as an average. Actually, the lower limit was 10 g kg^{-1} N but the upper limit was almost 50 g kg^{-1} N in the whole dataset (Table 2.).

The variability of N concentration in the green plant tissue presumes that also the total N uptake (kg ha^{-1}) of catch crop would be variable, further to that the total biomass production (t ha^{-1}) is an independent and determinant variable factor, as well. Correlation coefficient between N content of catch crop's dry material and N taken up by catch crop is 0.759-0.974 varying with years.

N removal by catch crop

From the environmental and sustainable land use point of view the N uptake is a very important indicator in the crop rotation considering the whole productivity of the rotation and the efficiency of N fertilization.

Based on the average of the studied cropping years it can be observed that the N removal by the catch crop is increasing with increasing N fertilizer doses of the preceding crop (Table 3 and Figure 2). Correlation coefficient between catch crop's green biomass and N uptake by catch crop is 0.880-0.994 varying with years. This connection is mostly linear on average and annually, as well. The lowest value was 22 kg ha^{-1} and the highest amount of removed N was about 200 kg ha^{-1}. It is evident that the quantity of the removed N varied by years, as the amount of green biomass (Table 1.) and the N content of dry material (Table 2.) altered as well, however these are the main components of the final amount of N taken up.

Many other researchers published analogous

Table 3. Mean nitrogen uptake by catch crop oil radish (kg ha^{-1})

N uptake by year (kg ha^{-1})	N fertilization rates (kg ha^{-1})					Mean N uptake (kg ha^{-1})
	0	40	80	120	160	
1997	22.13^{d}	36.90^{c}	50.63^{b}	50.27^{b}	66.03^{a}	45.19
1998	29.87^{c}	51.83^{c}	110.37^{b}	143.33^{b}	201.23^{a}	107.33
1999	23.00^{c}	31.17^{bc}	41.30^{ab}	41.00^{b}	51.93^{a}	37.68
2000	39.00^{c}	73.47^{bc}	103.73^{ab}	126.33^{a}	117.90^{a}	92.09
2001	48.27^{c}	58.37^{c}	111.53^{bc}	173.87^{ab}	197.87^{a}	117.98
2002	55.50^{a}	77.00^{a}	82.50^{a}	100.83^{a}	116.93^{a}	86.55
Mean N uptake (kg ha^{-1})	36.29^{d}	54.79^{cd}	83.34^{bc}	105.94^{ab}	125.32^{a}	81.14

Between different N fertilization rates significant differences at 0.05 level rates are marked with different letters.

results. Boguslawski (1981) found that the oil radish as stubble crop, without N fertilization, yielded 3-5 t dry matter (31-51 t ha^{-1} green biomass) containing 20-40 kg ha^{-1} N. Mikó (2009) carried out field trials with a couple of catch crops, in which green material of the oil radish was 20-150 t ha^{-1}, providing 60-325 kg ha^{-1} N uptake without and with fertilization. The source of N residue in the soil is manifold but mostly it is due to the positive N balance of the previous main crop. Catch crops tend to improve the soil nutrient status by maintaining slow release of nutrients, in this way they are not readily lost to leaching or denitrification, contributing to the mitigation of environmental risks. The key factor seems to be the green manure (e.g. oil radish) sown in the stubble field as catch crop, therefore their role is increased nowadays. For ground water protection the water-soluble nitrate

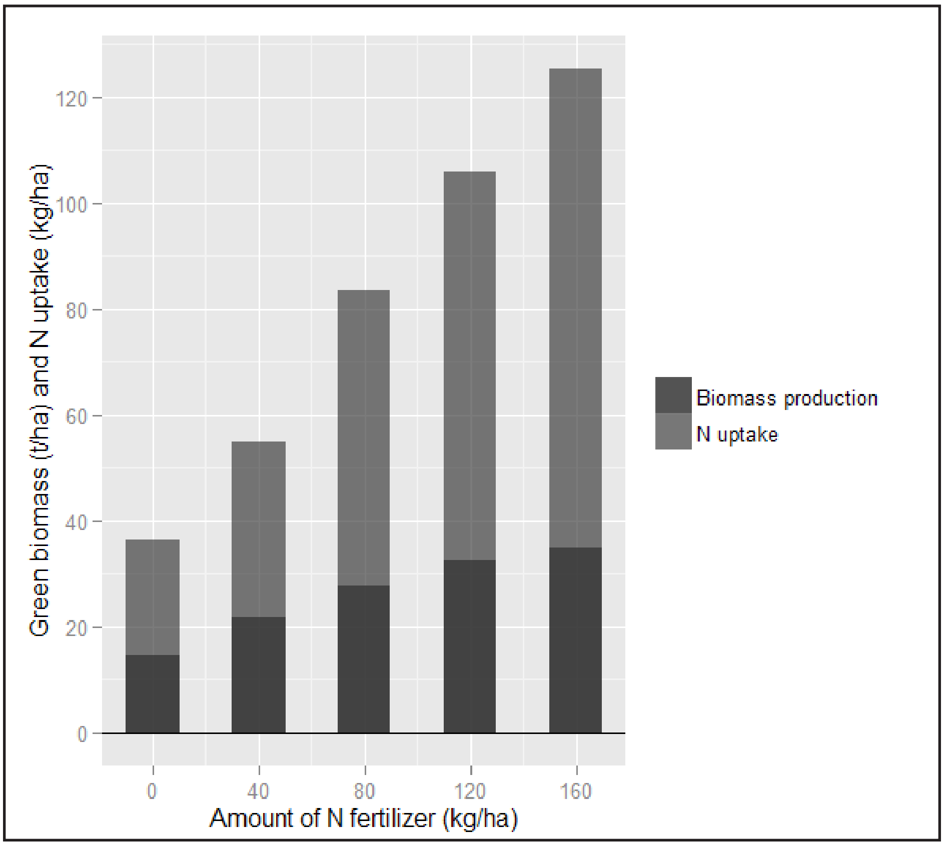

Figure 2. Mean green biomass and nitrogen uptake of oil radish catch crop applying increasing nitrogen fertilization to the winter barley (main crop) based on data of 1997-2002

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Table 4. Nitrogen balance of winter barley production without and with catch crop oil radish

Elements of N balance		N fertilization rates (kg ha^{-1})				
		0	40	80	120	160
Inputs	Winter wheat straw manure (3-5% N) (N kg/ha)	9.2	12.3	21.2	25.9	26.9
	C/N compensation (1 kg N/100 kg DM) (N kg/ha)	28.4	35.7	45.3	53.0	56.7
	Winter barley N rates (N kg/ha)	0.0	40.0	80.0	120.0	160.0
Total input		**37.6**	**88.0**	**146.5**	**198.9**	**243.6**
Output without catch crop	Winter barley uptake (N built in grain and straw) (N kg/ha)	-45.5	-62.5	-89.0	-104.4	-116.9
Output with catch crop	Winter barley uptake (N built in grain and straw) (N kg/ha)	-45.5	-62.5	-89.0	-104.4	-116.9
	Catch crop uptake (N kg/ha)	-36.3	-54.8	-84.0	-105.9	-125.3
Balance without catch crop (N kg/ha)		**-7.9**	**25.5**	**57.5**	**94.5**	**126.7**
Balance with catch crop (N kg/ha)		**-44.2**	**-29.3**	**-26.5**	**-11.4**	**1.4**

fraction must be maintained below the limit of 50 kg ha^{-1} in fall. The amount of N taken up by the catch crops might be different. According to Józsa (1985) about 70-100 kg ha^{-1} N were taken up by the different catch crops during the green manuring. The yield of stubble sowing of mustard and rapes were about 20 t ha^{-1} with the amount of 55-65 kg N ha^{-1}. Sowing these crops at the beginning of August, it corresponds to 100 kg N ha^{-1} (Kahnt, 1986). Our results are in accordance with it.

N balance (winter barley-oilseed radish)

The effect of green manure on retaining soil nitrogen was calculated in a N balance model (Table 4.). The input side of the balance consisted of the N fertilizer rates of WB, the N content of preceding crop's straw and the quantity of added N fertilizer for decomposition (C/N) of straw manure. On the other hand the output included the amount of N uptake in grain and straw (balance without catch crop), further to it the balance with catch crop included the N uptake of catch crop biomass as well.

Figure 3. shows that except the zero plots (N0) the balance without catch crop is positive in each variant and it is increasing parallel with the N doses of the preceding crop. It means that in case of the highest N treatment there is 126 kg ha^{-1} N surplus in the soil on average of the years. In case of N1-N4 the N use efficiency without catch crop is decreasing continuously with the increasing N fertilization; 71%-61%-52%-48%. Consequently: the higher the fertilizer rates of the previous crop, the lower the N efficiency of the N fertilization is, and higher the amount of N residue in the soil.

Taking into account the amount of N removed by the green biomass of oil radish the balance with catch crop can be calculated. The balance demonstrates that the catch crop eliminated the surplus N fertilizer from the soil completely. It is presumably incorporated into the green manure in organic form. Subsequently, after ploughing down oil radish and transformation of N in the soil, N would be available to the afterward plants. In this way it is possible to retain the N residue against leaching (Torstensson, Aronsson, 2000), which promotes environmentally sound agriculture (Askegaard et al., 2005). Based on these results it can be stated that the N surplus in the soil can be mitigated successfully by stubble

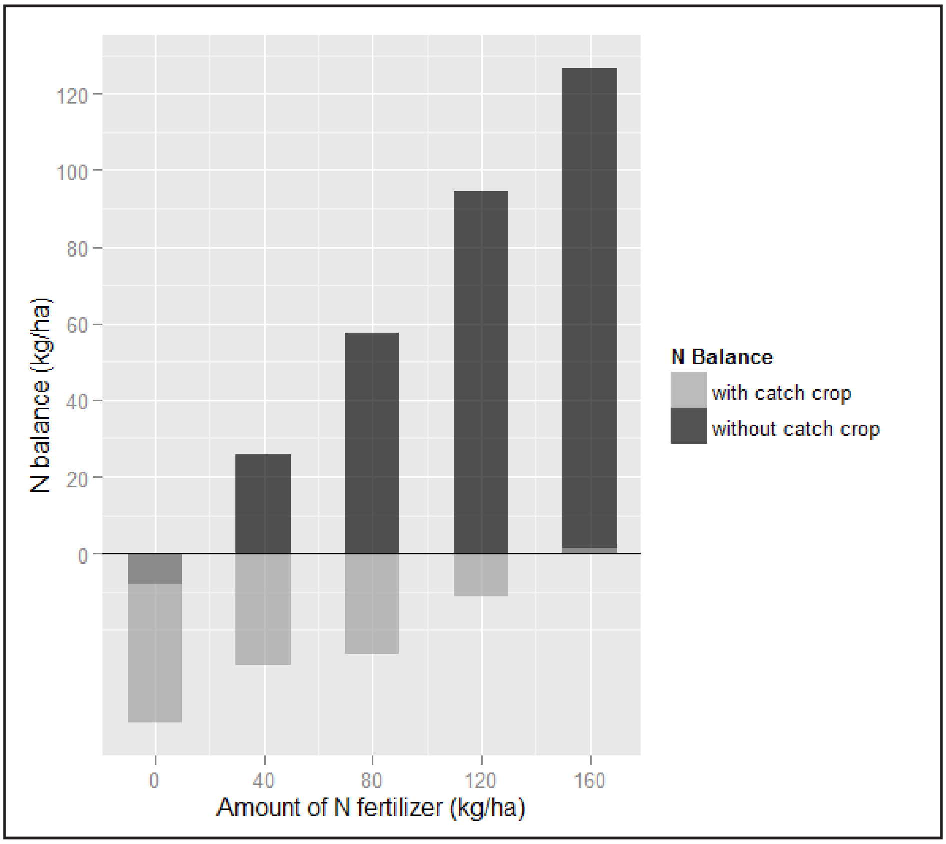

Figure 3. Nitrogen balance with and without oilseed radish catch crop applying increasing nitrogen fertilization to the winter barley (main crop) based on mean data of 1997-2002.

sown oil radish which takes up the residue N from the soil. Large quantity of N is left in the soil after the previous crop if the N fertilization was excessive resulting positive N balance.

Conclusions

Based on our study large quantity of N is left in the soil after the previous crop if excessive N fertilization is applied which results positive N balance. N fertilization rate of preceding crop higher than 120 kg ha^{-1} did not improve significantly the green biomass of oil radish in our study. Under the climatic conditions of the studied years oil radish reached its potential production when 120 kg ha^{-1} N fertilization was given to the previous crop. Mean N removal by catch crop oil radish was increasing with increasing fertilization rates during the studied six years. Maximum N uptake was 120 kg ha^{-1} based on the average of the years, but in the rainy 1998 year the N removal reached the 200 kg ha^{-1}. Significant differences were mostly observed between variants where difference in N fertilization rates was 80 kg ha^{-1}. N fertilization rates are highly correlated with green biomass (0.876-0.999) and catch crop's N uptake (0.929-0.991) and are also correlated with N content of dry material with varying correlation strength by years (0.637-0.956). N content of oil radish was between 0.92 and 5.47 % depending on the N fertilization rate of the preceding crop and climatic conditions. When catch crop is sown between winter barley and a spring row crop, such as maize, excess N in the soil will not be lost by leaching or released into the atmosphere but will be used by the catch crop. In our study the amount of N taken up by the catch crop increased with increasing N fertilization rates of the previous crop.

The N balance calculations proved that catch crop application in the crop sequence could avoid the risk of N leaching even if the level of N input was high, because N turnover of the crop rotation remains balanced by the green biomass' N uptake.

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Our study verifies that the N surplus in the soil can be mitigated successfully by stubble sown oil radish which takes up the residue N from the soil. In this way excess N - which would cause environmental pollution due to leaching or erosion - can be fixed in the catch crop, which amount can be beneficial for the crop following the catch crop. In this way, further to environmental considerations, also the cost of fertilization can be reduced due to decreasing N loss by the catch crop. The only investment needed for catch crop production is the cost of the seed, which is one order magnitude less than that of N fertilizer per ha. Considering it and the favourable effects of catch crop e.g. on soil structure, weed and erosion control, we can assume that integrating catch crop in the crop rotation will decrease the costs of plant production and increase net primary production in a long run.

For the better understanding of the catch crop's influence on plant production's N balance, analysis of meteorological data, nitrate leaching, nitrogen content and yield of row crop which follows the oil radish catch crop could be analysed as well in the future.

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COLOUR IDENTIFICATION OF HONEY AND METHODICAL DEVELOPMENT OF ITS INSTRUMENTAL MEASURING

Rubina T. SZABÓ[1] – Miklós MÉZES[1] – Tamás SZALAI[1] – Edit ZAJÁCZ[2] – Mária WEBER[1]

[1] Faculty of Agricultural and Environmental Sciences, Szent István University, Páter K. u. 1. 2100 Gödöllő. Hungary; E-mail: szabo.rubina@mkk.szie.hu
[2] Research Centre for Farm Animal Gene Conservation, Isaszegi u. 200, 2100 Gödöllő, Hungary

Abstract: Colour is one of the most important sensory traits of honey for the consumers. Honeys originating from different plant species are different in colour, but there could be variability within them as well, if originating from different geographical locations. Colour of the honey usually is determined by subjective methods. We determined colour categories by Lovibond method, and compared the results with reflectance spectrometry by Minolta Chromameter for broadening the possibilities of determining the colour of the honey and getting an objective image. First purpose of the study was to find whether the results by Lovibond method are in concordance with those got by reflectance spectrometry. The second purpose was to decide whether white or black backgrounds get the more accurate result when using the Minolta equipment. The results revealed that Minolta Chromameter is suitable to determine honey colour and the reflectance spectrometry data is comparable with the Lovibond method. Additionally white background is advised to be used by this instrument The L* ($r=-0.884$; $p< 0.001$) showed close significant correlation with the Lovibond categories.

Keywords: honey, subjectivity, colour, Lovibond, Minolta Chromameter.

Introduction

Honey is a natural material produced by honeybees (*Apis mellifera*). It is an over-saturated sugar solution, containing a high percentage of sugar (e.g. arabinose, fructose, galactose, glucose) and not more than 20% water (Körmendy and Rácz, 2009; Nyawali *et al.,* 2015). Honey is a natural products in which nothing is added or taken away from it (Wilczynska, 2014). Next to different carbohydrates there are minerals, amino acids, pigments, organic acids, enzymes, vitamins, aromatic and colour materials are also present in honey (Szalay, 2002; Bentoncelj *et al.,* 2007; Czipa *et al.*, 2015; Dominguez & Centurión, 2015). The colour of the honey is determined by its ingredients (e.g. mineral content), and by the type of polyphenols (Can *et al.,* 2015; Czipa *et al.*, 2015).Among the main colour materials, flavonoids are the most important (e.g.: 6-flavonol, 4-flavonol, pinocembrin, pinobanksin, galangine, luteoline) (Szalay, 2002; Turkmen *et al.*, 2005; Gheldof and Engeseth, 2002; Gheldof *et al.*, 2002). Colour spectrum of the honey can spread from colourless (light) to amber yellow or even to black (Mateo Castro *et al.,* 1992).

Colour is one of the most important feature in consumers' decisions and main attribute in food products, therefore affects the price of honey in the world market (Gonzales *et al.,* 1999; Quintas *et al.,* 2007, Dominguez & Centurión, 2015; De Silva *et al.,* 2016). The lightness of honey plays appreciable role in the preference of the consumers. In many countries, the price of the honey is related to its colour. The general acceptance of the honey's colour is very widely but generally the lightly coloured honeys have a better price (González - Miret *et al.,* 2007; De Silva *et al.,* 2016). There is a close correlation between colour and mineral content, pollen content, plant origin, geographical origin and also between colour and physical traits of the honey, such as electrical conductivity (Tuberoso *et al.,* 2004; Habib *et al.*, 2014; Czipa *et al.*, 2015; De Silva *et al.,* 2016). The colour of honey also depends on its ash content, the temperature and the storage time (Gupta *et al.*, 1992; González - Miret *et al.,* 2007; De Silva *et al.,* 2016). Different types of honey get darker with diverse speed and to different proportion which depends on acidity, sodium- and fructose content. There are natural changes in colour

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during crystallisation: the honey typically gets lighter. Furthermore, processing and handling of the honey, and circumstances and duration of the storage also can have measurable effect on its colour, making it darker. Caramelisation reaction can change the colour of honey. The HMF (hydroxymethylfurfural) content alone is not able to explain colout changes through the caramelisation reaction. (Quintas *et al.,* 2007). Amino acid and mineral content is broader in darker honeys, and they also have more tyrosine and tryptophan content, while lighter honeys not (Negueruela and Perez-Arquillue, 2000; Gonzales et al., 1999; Turkmen *et al.,* 2006). There is a close correlation between the colour and the antioxidant capacity of the honey. According to some researchers, darker honeys are having higher antioxidant content (Frankel *et al.,* 1998; Beretta *et al.,* 2005; Saxena *et al.,* 2010). In conclusion, a lot of facts suggest that colour being an important issue in case of honey, however, there is no officially standardised method available for its measurement(González -Miret *et al.,* 2007).

Organoleptic analysis of food from the consumer's viewpoint is rather common, as with applying that direct, immediate information can be gathered from the costumers (Stolzenbach *et al.,* 2011). The colour change kinetics is important for industrial process design and control so we have to decide which tools would be the best for measure the colour of honeys (Quintas *et al.,* 2007). Defining the colour is not an easy task and in a way a sensory perception and a subjective interpretation at the same time. Environmental circumstances have different impacts on the perception of a certain colour (Konika-Minolta, 1998), nevertheless, there are instruments available to measure the colour of the honey. The idea of classification leads to the development of several honey colour scale, e.g. Pfund or Lovibond scale (Quintas *et al.,* 2007). Pfund colour measuring is the well-known visual comparing instrument in case of honey, which results are given in mm (Koerner, 2005). The Pfund colorimeter is a simple instrument which has a reference unit (Pfund scale) (Dominguez & Centurión, 2015). Traditionally Lovibond 2000+ equipment is also used for the visual analysis of the honey. During this process it is compare six glasses of different shades of yellow and the given honey sample. These methods do not distinguish small colour differences and depend on person observing (Dominguez & Centurión, 2015). While the methods listed above can be affected by the environmental conditions, reflectance spectrometry (Minolta Chromameter) operates always with the same light conditions and illumination, so the circumstances of the measurement are constant. The most popular colour distance is based on the CIELab method, where L* (lightness), a* (degree of greenness/redness) and b* (degree of blueness/yellowness) values which is applied widespread for measuring colour of subjects and food products (Negueruela and Perez-Arquillue, 2000; Konica-Minolta, 1998, Wilczynska, 2014). This colour system is practical because any colour can be defines by a mixture of red, blue and green colours (Quintas *et al.,* 2007).

The purpose of the present study was to compare objectively the results gathered by Lovibond and Minolta equipments about the colour as consumers perceive subjectively, independently of the ingredients and other physico-chemical properties of honeys. Our aim was also compare white and black backgrounds so better background could be chosen for Minolta equipment.

Materials and methods

Samples

A total of 21 honey samples of different plant origin was analysed collected from producers, honey traders and shops (Table 1.). Majority of the samples originated from Hungary, and some of them from other countries. Have to stress, that it is always essential to work with

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Table 1. Origin of honey by plants

Plant	Number of honey samples
Mixed wildflower	5
Acacia	4
Linden	3
Common milkweed	1
Lavender	1
Orange	1
Forest wildflower	1
Raspberry	1
Sycamore maple	1
Chestnut	1
Sunflower	1
Wild privet	1

Table 2. The categories of Lovibond

Values	Categories
8	Water white
17	Extra white
34	White
48	Extra light amber
83	Light amber
114	Dark amber

Table 3. Connection of the visual perception and the ΔE^*_{ab} values according to the equation proposed by Lukács, 1982

Domain	Perceptible difference
$\Delta E^*_{ab} \leq 0,5$	non-perceptible
$0,5 < \Delta E^*_{ab} \leq 1,5$	barely perceptible
$1,5 < \Delta E^*_{ab} \leq 3$	perceptible
$3 < \Delta E^*_{ab} \leq 6$	visible
$6 < \Delta E^*_{ab}$	huge

fluid and clear honey samples, as the light scattering of crystallised honey is different. Due to that already crystallised honeys have to be melted in a water bath at a maximum of 40 ^{o}C, then cool back to room temperature. Visible physical contaminations have to be removed by filtration, sample have to be homogenised by a mixer. When filling out honey, air bubbles have to be avoided, so it is necessary to let the cuvette rest, or a real-sonic cleaner is advised for eliminating bubbles: these is how we have done in every case.

Measuring colour and its backgrounds

First we analysed the colour of samples by Lovibond instrument, by its colour disk samples can be classified into colour categories. Three independent persons made the analysis parallel, next to natural light. The categories of Lovibond are shown in the Table 2.

Then we measured colour by the Minolta Chromameter® CR 410 type instrument as well. The same honey samples was measured as by Lovibond. Minolta Chromameter is built from anti-reflexion glass; we placed honey samples on its cuvette suitable for measuring the colour of liquids and powders. The resulting L* value refers to the lightness of the sample (0=black; 99=white), a* value refers to the redness of the sample (in +60 direction red, in -60 direction green) and the b* value gives the yellowness of the sample (in +60 direction yellow, in -60 direction blue) (Wilczynska, 2014). ΔE^*_{ab} value is necessary to be used for evaluating the colour of honey samples from the consumer's point of view based on the values measured in the L*a*b* colour system, according to the following formula (Lukács, 1982):

$$\Delta E^*_{ab}=\sqrt{(\Delta L^*)^2+(\Delta a^*)^{2+}(\Delta b^*)^2}.$$

Differences among results can be compared and also evaluated in regard to the visual perception based on it (Table 3).

As background for the Minolta equipment white (L*= 63.15; a*=2.21; b*=2.44) and black (L*=38.04; a*=1.06; b*=-2.44) colours were used. White colour means the sum of colours, while black the lack of colours (Negueruela and Perez-Arquillue, 2000). Statistical correlation between results of Lovibond and Minolta Chorameter were analysed by Pearson's test using R 3.2. 0 software.

Results and discussion

Three of four acacia honeys belonged to the brightest categories (water and extra white) and had the highest L* only in case of the

Table 4. Results of Lovibond

Honey	Examiner 1	Examiner 2	Examiner 3	Final result
Acacia (3)	17	17	17	17
Acacia (1)	8	8	8	8
Acacia (2)	8	8	8	8
Acacia (4)	83	83	48	83
Orange	83	83	83	83
Lavender	114	114	114	114
Raspberry	83	83	83	83
Sunflower	48	83	48	48
Forest wildflower	34	48	34	34
Chestnut	48	83	48	48
Sycamore maple	83	83	83	83
Common milkweed	34	34	17	34
Wild privet	34	34	34	34
Mixed wildflower (1)	48	48	48	48
Mixed wildflower (3)	83	83	83	83
Mixed wildflower (4)	114	114	114	114
Mixed wildflower (5)	114	114	114	114
Mixed wildflower (2)	48	34	48	48
Linden (1)	48	48	48	48
Linden (2)	48	48	48	48
Linden (3)	83	83	83	83

white background. This tendency is similar to previously reported data (Wilczynska, 2014).

Correlations between the categories of the Lovibond instrument and the results got by using Minolta Cromameter showed the following results. Table 4 and 5 show the results separately. In case of using white background, the L* values showed close significant negative correlation with the Lovibond categories (r=-0.884; p<0.001). It means that honeys with higher a* value (in +60direction red, in -60 direction green), falls into higher Lovibond category. In case of b* values and Lovibond categories, no significant correlation was found (r=-0.188; p=0.427).

If the correlation analysis was made with using black background, there was a significant negative correlation between L* values and categories formed by Lovibond (r=-0.616; p< 0.01). In case of black background the correlation coefficient is weaker than the coefficient of the white background. The a* values showed close negative correlation with the Lovibond categories (r=0.816; p< 0.001) but the b* values not (r=-0.079; p=0.741). The results of a* and b* values of black background show similar tendency with white background values. Comparing the two different background it can be conclude that negative correlation between L* value and Lovibond categories is stronger in case of the white background. Similarly, stronger but positive correlation was found between a* value and Lovibond colour categories when white background was used. In case of the b* values there were no correlations with either background, giving a reason for further studies.

The ΔE^*_{ab} value gives the visible difference between two samples. By dint of it, honeys which belong to the same category of Lovibond can be confronted. If honeys have the least difference (non- or barely perceptible) in a same Lovibond category, the two honey colour measuring methods are related. Generally,

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Table 5. Results of Minolta Chromameter

Honey	White background			Black background		
	L*	a*	b*	L*	a*	b*
Linden (1)	14.16	3.54	-7.45	35.81	0.3	7.31
Common milkweed	60.17	2.44	27.78	35.76	0.87	4.3
Lavender	36.22	17.62	9.77	30.3	4.43	-0.84
Orange	48.12	14.45	29.04	33.08	3.99	3.64
Forest wildflower	57.35	2.35	33.39	35.23	0.75	5.07
Mixed wildflower (1)	56.57	0.16	42.05	35.74	0.14	7.8
Raspberry	45.4	15.33	24.84	32.05	4.05	1.97
Sycamore maple	44.18	16.17	23.17	31.82	4.13	1.63
Chestnut	51.34	10.29	32.92	33.37	2.78	3.75
Mixed wildflower (3)	49.98	6.77	31.57	33.97	2.47	5.17
Mixed wildflower (5)	31.12	10.69	0.74	29.25	2.96	-2.62
Acacia (3)	64.37	-0.97	22.7	36.54	-0.01	3.14
Sunflower	52.06	0.81	33.67	47.74	-1.02	26.18
Mixed wildflower (4)	37.4	8.03	9.55	36.87	6.48	8.62
Wild privet	61.77	-1.31	37.2	35.81	-0.26	5.71
Acacia (1)	66.77	-1.26	15.03	36.7	0.04	1.29
Acacia (2)	65.47	-0.38	20.28	36.52	0.23	2.41
Mixed wildflower (2)	55.36	3.87	36.42	34.99	1.25	5.82
Linden (2)	55.6	5.08	33.61	34.42	1.47	4.4
Linden (3)	43.64	11.9	21.31	31.76	3.23	1.26
Acacia (4)	49.53	13.89	30.59	32.95	3.73	3.22

the Lovibond categories and the ΔE*values are connected. If the two backgrounds were compared, small or big variances were detected, mainly in case of raspberry, sycamore maple and chestnut honeys. In case of white background, between raspberry honey and sycamore maple honey the difference of visual perception was ‘perceptible’ (E*=2,23), between raspberry honey and orange honey was ‘visible’ (E*=5,08), between raspberry honey and linden honey (3) was ‘visible’ (E*=5,23), between raspberry honey and acacia (4) honey was ‘huge’ (E*=7,22), between raspberry honey and mixed wildflower honey (3) was ‘huge’ (E*=11,81).

If the black background was used, the difference of visual perception between raspberry honey and sycamore maple honey was ‘non-perceptible’ (E*=0,42), between raspberry honey and linden honey (3) was ‘barely perceptible’ (E*=1,12), between raspberry honey and acacia (4) honey was ‘perceptible’ (E*=1,57), between raspberry honey and mixed wildflower honey was ‘visible’ (4,05), between raspberry honey andorange honey was ‘perceptible’ (E*=1,96).

In case of white background, between sycamore maple honey and linden honey (3) the difference of visual perception was ‘visible’ (E*=4,69), between sycamore maple honey and acacia (4) honey was ‘huge’ (E*=9,43), between sycamore maple honey and mixed wildflower honey (3) was ‘visible’ (E*=13,88), between sycamore maple honey and orange honey was ‘huge’ (E*=7,28).

If the black background was used, the difference of visual perception between sycamore maple honey and linden honey (3) was ‘barely-perceptible’ (E*=0,97), between sycamore maple honey and mixed wildflowerhoney (3) was ‘visible’ (E*=4,46), between sycamore maple honey and acacia (4) honey was 'perceptible' (E*=1,99), between sycamore maple honey and orange honey was 'perceptible' (E*=2,38). In case of white background, between chestnut honey and linden honey (2) the difference of visual perception was 'visible' (E*=6,77), between chestnut honey and mixed wildflower honey (2) was 'huge' (E*=8,34), betweenchestnut honey and linden honey (1) was 'huge' (E*=55.30), between chestnut honey and sunflower honey was 'huge' (E*=9,54), between chestnut honey and mixed wildflower honey (1) was 'huge' (E*=14,61).

If the black background was used, the difference of visual perception between chestnut honey and linden honey (2) was 'perceptible' (E*=1,8), between chestnut honey and mixed wildflower honey (2) was 'visible' (E*=3,04), between chestnut honey and linden honey (1) was 'visible' (E*=4,98), between chestnut honey and sunflower honey was 'huge' (E*=26,91), between chestnut honey and mixed wildflower honey (1) was 'visible' (E*=5,38).

Conclusions

Many type of honey can be found in the market which differs in package, prize, colour or origin. Hence estimate the preference of the costumers would be important specifically for honey color. By means of this, the beekeepers would target produce, especially in migratory beekeeping (Czipa *et al.,* 2012; Gyau *et al.,* 2014).

The colour of honey must be objectively measured to classify the product for the processing industry and the quality control.

Lovibond results showed some subjective error, if more than one people is involved in making the measures. All honey originating from different plants can be ordered to one of the Lovibond categories but minor differences between colours cannot be enlightened by using it. However, its great advantage is, that the equipment itself is simple and portable, so measures can be made even in field condition, and also, its use does not require previous training.

Minolta Chromameter is suitable for measuring honey colour, as values resulted by using it are in concordance with colour categories developed by Lovibond. According to our result, by using it very detailed pieces of information can be gained about the colour of the honey. Due to the stability of circumstances always can get accurate and objective results. Further advantage of the equipment, that it is portable, and can also be used for determining colour of other substances. With Minolta Chromameter human errors can be reduced (Dominguez & Centurión, 2015).

The results of Lovibond and Minolta are comparable and correlate in case of white and black backgrounds. The use of the Minolta instrument was resolved but there was not enough result about different background effect. Based on the L*, a*, b* (L*=63.15, a*=2.21, b*=2.44) applying a white background is advised for correct colour measurement of honey if the L*, a*, b* parameters are separately highlighted. However if we want to measure the difference of visual perception, the use of black background (L*=38.04; a*=1.06; b*=-2.44) is the better choice. This result is similar to Negueruela and Perez-Arquillue, 2000.

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THE IMPACT OF N AND P SUPPLY ON THE PERFORMANCE OF YIELD COMPONENTS OF WINTER BARLEY (*HORDEUM VULGARE* L.)

Szilvia SURÁNYI – Zoltán IZSÁKI

Faculty of Economic, Agricultural and Health Sciences, Szent István University, Tessedik Campus, H-5540, Szabadság utca 1-3. Szarvas, Hungary. E-mail: suranyi.szilvia@gk.szie.hu

Abstract: Winter barley is one of the most important fodder plants due to its nutritional value, but the sown area size is rather small among the cereal crops. Barley production has a profound impact on the success of animal husbandry in Hungary. Relevant foreign and Hungarian publications on fertilisation and investigating contexts between yield components of winter barley can only be found in few recent periodicals. The aim of this investigation was to gain data about the effect of nitrogen and phosphorus supplies on the plant height, length of ear and grain yield per ear as well as the yield of winter barley. The long-term fertilisation experiment was set up in 1989 on a chernozem meadow soil, calcareous in the deeper layers, with four levels of N, P and K supplies respectively. The present paper discusses the N and P fertilisation results obtained in the 2011 year of the experiment. We measured the highest values - height (78.88 cm), length of ear (7.25 cm) and grain yield per ear (18.9 pieces per ear) - in the case of AL-P2O5 level of 194 mg kg-1and N 240 kg N ha -1supplies. Maximum grain yields were recorded at AL-P2O5 level of 194 mg kg -1 as well, but in the case of N, the 160 kg N ha -1 supply was proved to be adequate.

Keywords: nitrogen, phosphorus, winter barley, supply

Introduction

Barley is a member of the *Gramineae* family. It is a self pollinating diploid species with 14 chromosomes. The wild ancestor of domesticated barley, *Hordeum vulgare* ssp *spontaneum* is originated from the Fertile Crescent and believed to be one of the earliest domesticated grain crops. Outside this region, the wild barley is less common and is usually found in disturbed habitats (Zohary and Hopf 2000). However, in a study of genome-wide diversity markers Dai et al (2012) found Tibet to be an additional centre of domestication of cultivated barley.

Barley is the third most important cereal of ours from the point of view of both human consumption and of animal nutrition in Hungary. Barley is eaten by all domestic animals. The most ratio is used in the swine feeding - within it in swine fattening (Schmidt, 2004). Its protein content respectively and more essential amino acids ratio (f.i. lysine, threonine, tryptophan) are higher than in maize, but it does not reach the wheat characteristic value. Its fibre content is higher than that of both referred cereals thus it has excellent dietetic effect for swine. Content of NSP (no starch polysaccharide) is considerable, which is with unbeneficial effect digestion of poultry, thus poultry should be fed with it to a lesser extent (Schmidt, 2003; Mézes and Hausenblasz, 2009). Critical point of the barley production is the N- fertilization, which basically determines the yield and quality, furthermore some economic value measurement factors. Berhanu et al. (2013) found, that N supply was in close correlation with the productivity of winter barley. Kádár (2000) determined that abundant NP- supply can increase expressly speed of N- uptake of winter barley in the early phase of development respectively the plant is able to supply its N-need on the coming into ear. Dunai et al (2014) evaluated the impact of NPK fertilisation in single and combined applications with organic manure. It was stated, that crops can directly produce higher yields and while the more moist conditions increase the microbial activities, the efficiency of fertilizers can be better. These soil physical results enable crops to obtain more yields with using mineral and organic fertilizer in combined applications. Yielding ability of barley highly depends on the performance of yield components. Spikelets are arranged in triplets that alternate

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along the rachis. In wild barley and other species of *Hordeum*, only the central spikelet is fertile, while the other two are reduced. This condition is retained in certain cultivars known as two-row barleys. A pair of mutations (one dominant, the other recessive) result in fertile lateral spikelets to produce six-row barleys (Zohary and Hopf 2000). Recent genetic studies have revealed that a mutation in one gene, *vrs1*, is responsible for the transition from two-row to six-row barley (Komatsuda et al 2006).

N and P impact on barley yield and yield components has been recently discussed in a short communication by the authors (Surányi and Izsáki 2016).

In this work we looked for an answer on how the N-, P- and K- supply of the soil affects the height, length of spike individual productivity of winter barley and what impact it has on the yield.

Materials and methods

Long-term mineral fertilisation experiments were set up at the experimental station of the Szent István University, Faculty of Economic, Agricultural and Health Sciences, Szarvas, in 1989. The soil of the experimental area had the following parameters: chernozem meadow soil, calcareous in the deeper layers, 85–100 cm humus layer, $pH_{(KCl)}$ 5.0–5.2, humus content 2.8–3.2%, upper limit of plasticity according to Arany (K_A) 50 and clay content 32%. The fertilisation was treatment of 4-4 nitrogen (N)-, phosphorus (P)- and potassium (K)- levels carried out in all possible combinations of four levels each of N, P and K, giving a total of 64 treatments, set up in a split–split plot design with three replications. The following fertiliser rates were applied: N: N_0 = 0, N_1 = 80, N_2 = 160 and N_3 = 240 kg N ha^{-1} year^{-1}; P (P_2O_5): P_0 = 0, P_1 = 100 kg ha^{-1} year^{-1}, P_2 = 500 kg ha^{-1} 1989, 1993 and 2001, and P_3 = 1000 kg ha^{-1} year^{-1} in 1989, 1993 and 2001; K (K_2O): K_0 = 0, K_1 = 300 kg ha^{-1} year^{-1} between 1989 and 1992 and 100 kg ha^{-1} year^{-1} from 1993, K_2 = 600 kg ha^{-1} in 1989 and 2001, 1000 kg ha^{-1} in 1993, and K_3 = 1200 kg ha^{-1} in 1989 and 2001 and 1500 kg ha^{-1} in 1993. The high rates of P and K replenishment fertilisation were used to create clearly distinct supply levels in the soil in order to investigate plant responses to nutrient status. The plot size of the sub-subplots was 4×5 = 20 m². For the plant investigations and the yield components analysis two times one meter row samples were taken from all treatments of the K_1 plots.

Results and discussion

Relationship between the N, P-supply and the height of winter barley

Increase of the N- doses (Figure 1.) – in mean P- treatments – rises stem length of winter barley (45.76; 63.47; 71.38; 75.76 cm), but we have to envisage in case of the excessive N-supply risk of lodging respectively its effect of environmental risk. The expanding P- supply does not show so pronounced effect as in case of N- supply (64.41; 63.77; 63.84; 64.36 cm). The adequate P- supply enhance growth and maturity of plant and at the same time increases resistance against lodging. Compared to P_0N_0 the P- supply increase resulted decrease in the length of straw. The lowest length of straw was observed at P_2N_0- supply level, while the highest values in case of maximum N- supply level (240 kg ha^{-1} N year^{-1}) on the same P-level (P_2, AL- 194 mg kg^{-1}). The increase of N-supply is in close relationship (r=0,95) with length of straw of winter barley (Figure 2.), but the P-supply did not affect significantly the investigated parameter.

Relationship between the N- and P-supply and length of spike

The increasing dose of N- supply (0; 80; 160; 240 kg ha^{-1} N year^{-1}) – on the average of P-supply – increased length of spike of winter barley (5.23; 6.22; 6.65; 7.04 cm) (Figure 3.), but different P- doses acted on reducing of investigated parameter (6.41; 6.21; 6.20; 6.31 cm). We measured the shortest length of spike in case of P_3N_0 – supply (4.73 cm), the longest

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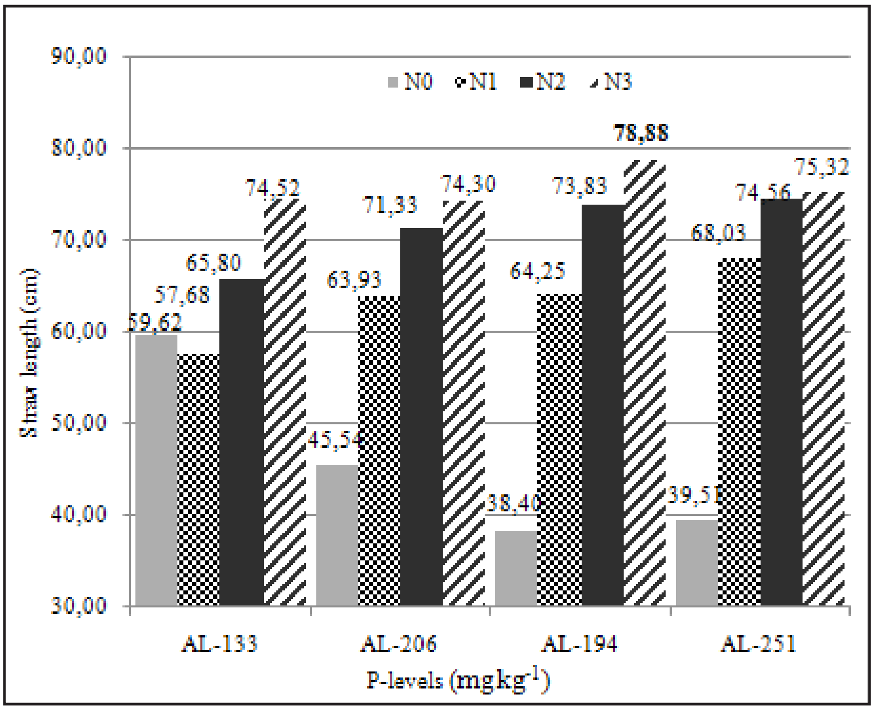

Figure 1. Effect of N- and P- supply on stem length of winter barley (cm)

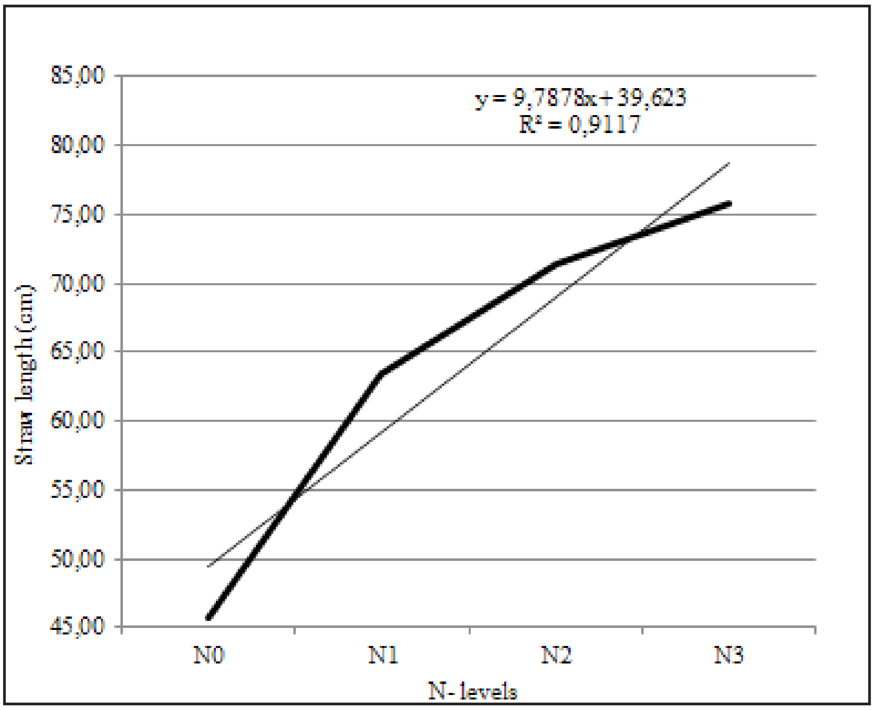

Figure 2. Correlation between N-supply and stem length of winter barley (cm)

in case of P_2N_3- supply (7.25 cm). Between N- supply and length of spike winter barley there is a very close correlation (r= 0.97) (Figure 4.), but P- supply did not result in significant changes.

Relationship between N-, P-supply and the grain number per spike

In case of this examined parameter, the increasing N-supply also rose the grain

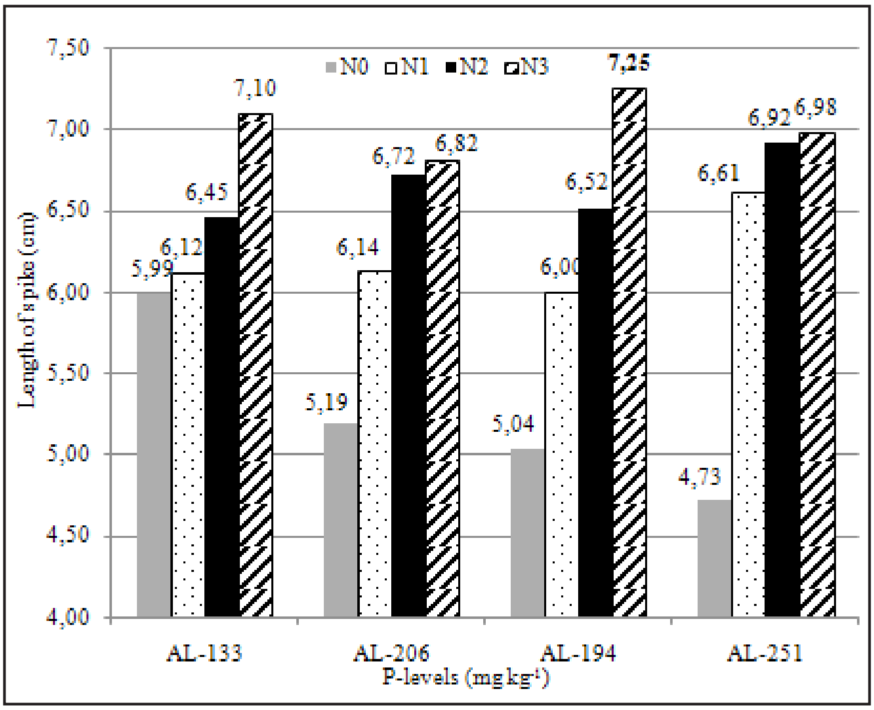

Figure 3. Effect of N- and P- supply on length of spike (cm)

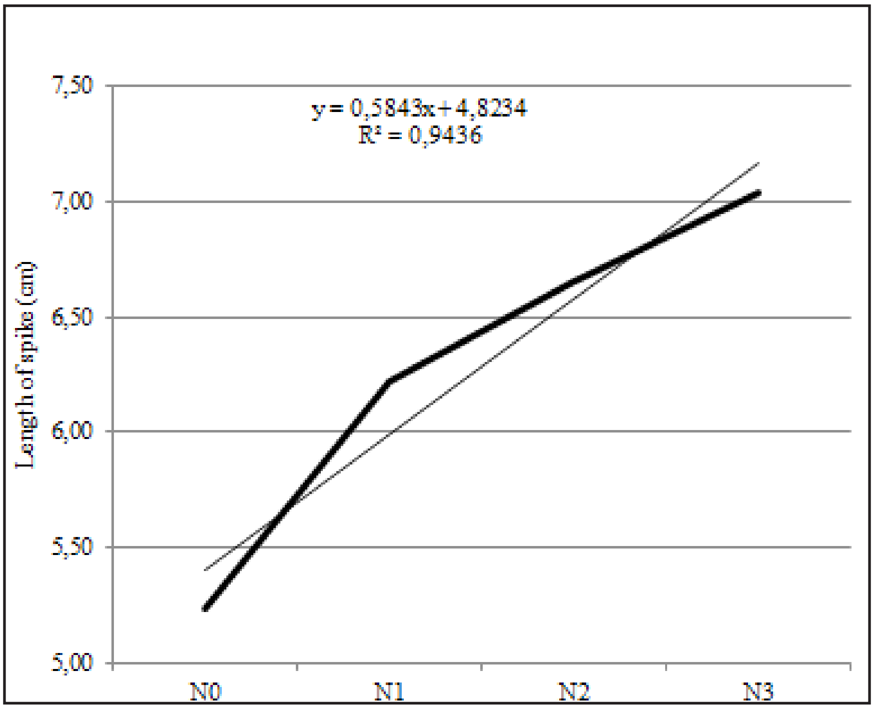

Figure 4. Correlation between the N-supply and length of spike (cm)

number per spike (Figure 5.) – on the average of P- supplies (13.64; 16.43; 17.46; 18.19 grain number per spike) and we found close correlation (r=0,95) between grain number per spike and N-supply, too (Figure 6.). The P-fertilization – without N- treatment – acted on the decrease of the grain number per spike. We got the smallest grain number in case of P_3N_0 – supply, the most in case of a N_{240} kg ha^{-1} year^{-1}, by P_2-level (18.9 grain number per spike).

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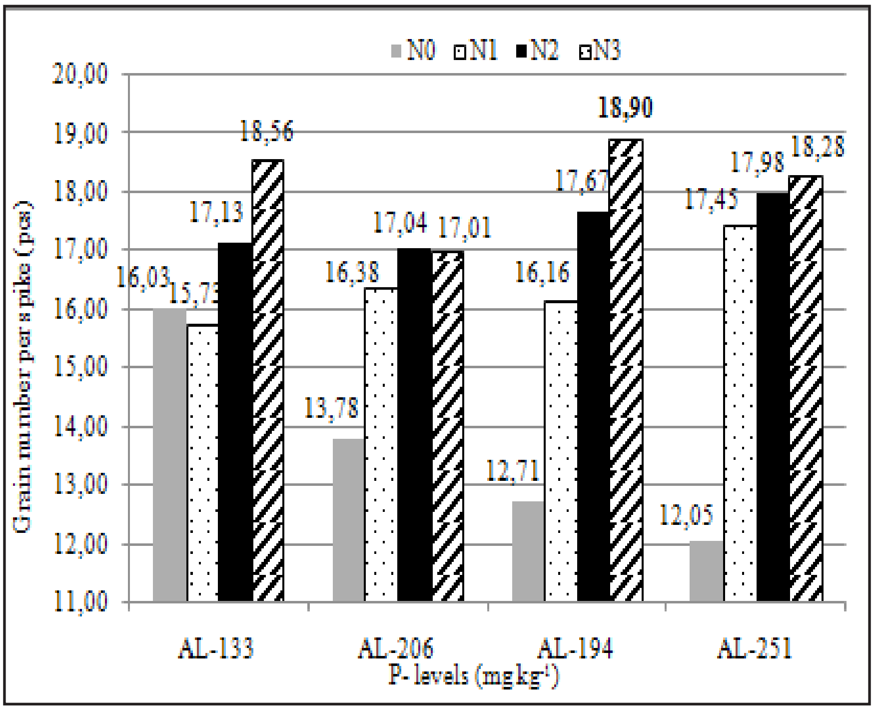

Figure 5. Effect of N- and P– supply on grain number per spike (pcs)

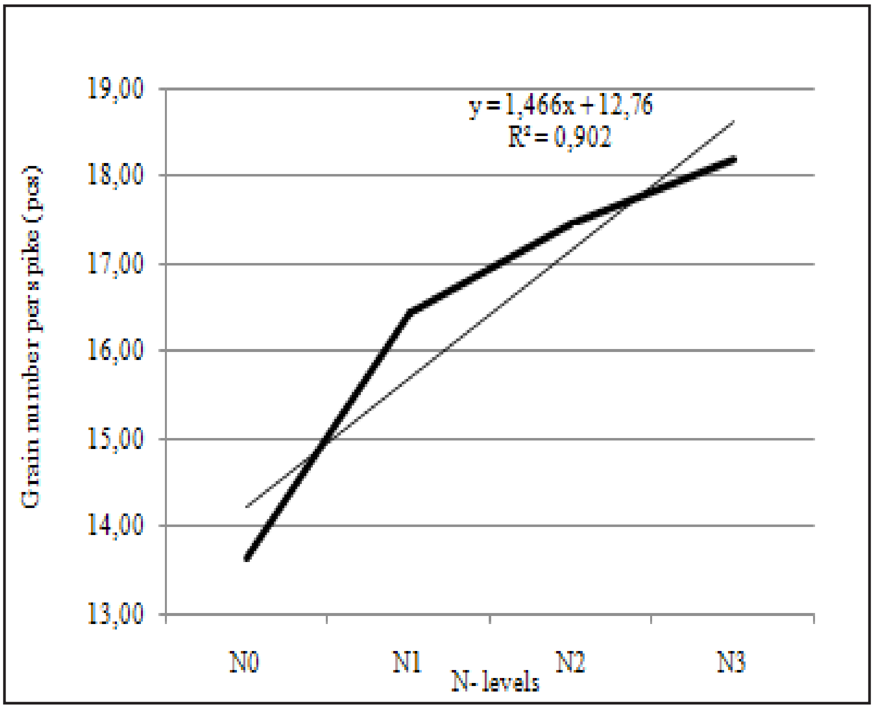

Figure 6. Correlation between the N- supply and grain number per spike (pcs)

Relationship between N-, P- supply and the yield of winter barley

In case of yield quantity, N- supply as well as P- supply affected on yield. Increasing the N- and P- supply (P_2N_2) after a level caused the decrease of yield (Figure 7.). The highest yield was obtained in case of P_2N_2. We found very close correlation in case of N- supply (Figure 8.) and P-supply, too.

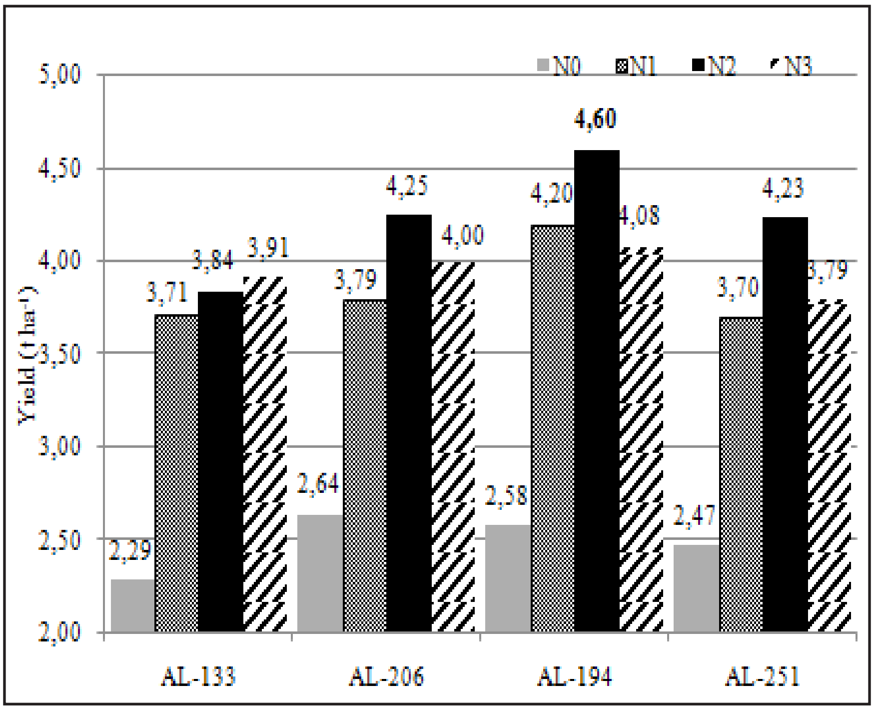

Figure 7. Effect of N- and P- supply on yield of winter barley (t ha^{-1})

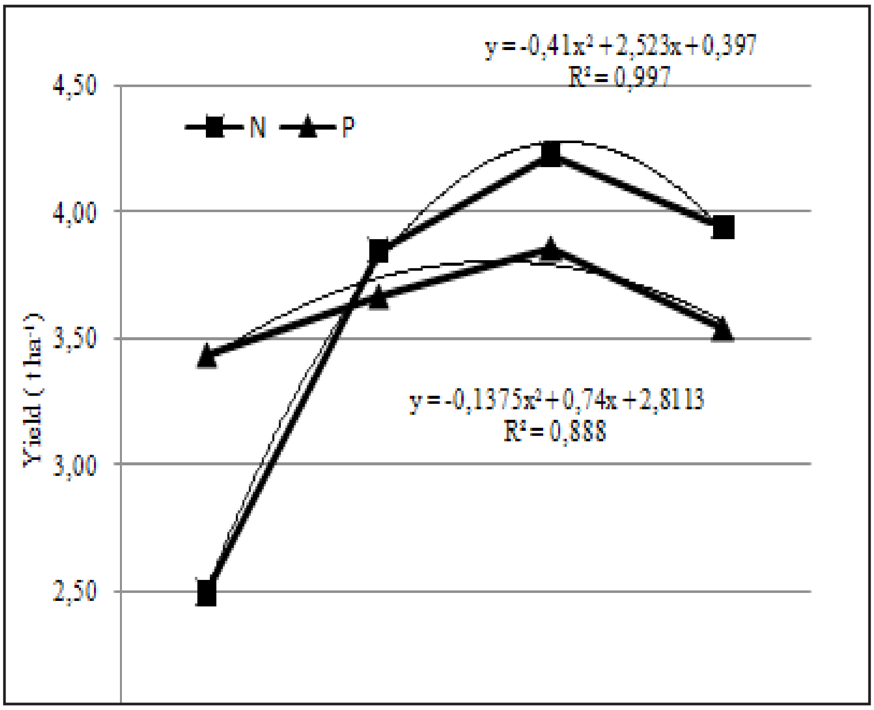

Figure 8. Correlation between the N- and P- supply and grain (t ha^{-1})

Conclusions

We measured the most extended height of plant, length of spike and the grain number per spike in case of the maximum N- supply (N_{240} kg ha^{-1} $year^{-1}$), but the maximum yield was resulted by N_2- supply ((N_{160} kg ha^{-1} $year^{-1}$). We received the longest of stem at P_2N_3- level, but the excessive N- supply raised predisposition on lodging, which may lead to yield decrease. The P- supply applied did not show so much effect

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on the height, length of spike and on the grain number per spike of winter barley as we found in case of N- supply. Yield of winter barley was increased at 160 kg N ha^{-1} $year^{-1}$. A bigger N-supply caused decrease of yield figures. The P-supply resulted in the maximum yield on 194 mg kg^{-1} AL- P_2O_5- level. The excessive P-level (251 mg kg^{-1}) caused decrease of yield.

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THE EFFECT OF MULCHING ON THE ABUNDANCE AND DIVERSITY OF GROUND BEETLE ASSEMBLAGES IN TWO HUNGARIAN POTATO FIELDS

Péter DUDÁS[1] – Csongor GEDEON[2] – László MENYHÁRT[3] – Gergely AMBRUS[1] – Ferenc TÓTH[1]

[1]Institute of Plant Protection, Faculty of Agricultural and Environmental Sciences, Szent István University, Hungary, H-2100 Gödöllő, Páter Károly u. 1. E-mail: dudpet@gmail.com

[2] Department of Agrochemistry and Plant Nutrition, Institute for Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research, Hungarian Academy of Sciences, Hungary, H-1022 Budapest, Herman Ottó út 15. E-mail: csongorg@gmail.com

[3] Department of Economic Methodology, University of Pannonia Georgikon Faculty, H-8360 Keszthely, Deák Ferenc u.16. E-mail: menyhart-l@georgikon.hu

Abstract: The benefits of mulching in potato (*Solanum tuberosum* L.) production are manifold, such as it provides shelters for natural enemies of pests. The aim of our study was to detect the effect of two organic mulch types on the abundance, diversity and species composition of carabid beetles. Our potato plots were located in two sites with similar habitats including similar soil characteristics. Each plot was treated with hay and leaf litter mulch (hay, leaf, control) with 4 replications at each site. It resulted in 24 samples in each study site annually. Carabid beetles were collected by using pitfall traps in between 2011-2013. We found that due to mulching a larger and more diverse carabid population occurred on potato plots. Both mulching types increased the total number of carabids captured having a 17% higher abundance on hay mulched plots and 14% higher abundance on leaf litter mulched plots. According to the results 28 % of the collected species was found only on mulched plots, whereas only 13 % of the captured species were found only on unmulched areas. For some of the species, especially for *Brachinus crepitans* (L.), the number of individuals was significantly higher on mulched plots. The two different mulching materials had very similar effects on assemblages of carabid species. The species composition of ground beetles was considerably affected by the two locations, and to a lesser extent by the different time periods. While the dominant species of Budaörs was *B. crepitans*, the most abbundant carabid beetle at Hidegkút belonged to genus *Harpalus*. Neither hay nor leaf litter were able to constantly increase the biodiversity of the carabid assemblages on potato plots, because the effect of the two years overwrote the between-treatment effects on carabid diversity. The positive effect of organic mulching on carabid diversity however, was found significant in both years of 2011 and 2012.

Keywords: potato, leaf litter mulch, hay mulch, pitfall trap, Carabidae

Introduction

Since the appearance of the potato beetle in most region of Hungary, potatoe can only be grown successfully if the defense against is regular (Sáringer, 1998). Several of the larger *Carabus* species are effective predators of the Colorado potato beetle (Scherney, 1959) and wireworms (Dunger, 1983). Sorokin (1976) described 14 carabid beetle species as natural enemies of the Colorado potato beetle in Eastern Europe (Heimpel and Hough-Goldstein, 1992). A survey of North American potato fields found that *Lebia grandis* (Hentz) and *Poecilus chalcites* (Say), both of the family Carabidae, were natural enemies of the Colorado beetle (*L. decemlineata*). An adult *L. grandis*, being one of the most important predators of the Colorado beetle (Hemenway and Whitcomb, 1967), can consume up to 47 eggs of the beetle a day (Groden, 1989). One of the most frequent carabid species in Hungary, *Poecilus cupreus* (*L.*) feeds on the egg and larvae of the Colorado beetle. The *Broscus cephalotes* (L.) is efficient predator of *L. decemlineata* too (Merkl and Vig, 2011). A North-American paper supported that the population size of the Colorado beetle (*L. decemlineata* Say) was lower on potato mulched with straw (Stoner et al., 1996; Brust, 1994) and claimed that the number of pest was lower because the straw applied on the soil surface provided the predators with

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hiding places, and the habitat suffered less disturbance. According to Dvořák et al. (2012) the number of Colorado beetle larvae was reduced in parcels covered by chopped grass and these parcels provided bigger potato tubers on average. The higher tuber yield in chopped grass mulch might have been due to the higher nitrogen availability in the soil (Dvořák et al. 2013). Straw mulch was found to be beneficial to the microflora of the soil (Flessa et al., 2002). The surplus of organic material distributed over the surface of the soil increases its organic matter content and has a beneficial effect on the macrofauna as well (Pauli et al., 2011). A study (Kromp, 1999) supported that the species composition and the number of individuals of carabid beetles were lower on conventional potato plots than on organic ones; it also suggested that geographical location may have a significant effect on the densities of carabid species. Another study on the carabid fauna in France found that the thickness of the leaf litter influences the density of *Abax ater,* Villers, as it may be harder for a carabid predator to catch the prey under a thick layer of leaf litter. This study indicated that the thicker the layer, the lower the number of carabid beetles was (Guillemain et al., 1997). That fenomenon may appear in mulched fields as well, since the width of leaf litter is increased. Tuovinen et al. (2006) tested whether covering of the soil surface itself has an effect on carabids and they found that organic mulch materials were more favourable for carabid beetles than the conventional plastic sheets on strawberry plots.

Our aim was to investigate the effect of two different organic mulching materials (leaf litter and hay) on the carabid assemblages. We conducted our experiments within similar conditions with two different mulch types at two locations. Then we investigated whether species composition would differ between sites and mulch types and studied the mulch preference of carabid species.

Materials and methods

For our research, we settled for a mountainous area with brown forest soil (Várallyay & Szűcs 1978). Quality of soils is showen in Table 1. 12 soil samples were collected from the upper 8 cm of the topsoil at each experimental plot. Our experimental plots were located in a suburban area with continuous mixed forests in the outskirts of Budaörs (47°47'25.6" N, 18°95'90.3" E) (Pest County, Central Hungary) and Hidegkút (47°00'20.6" N, 17°.83'05.9" E) (Veszprém County, West Hungary) with a total area of 168 m^2 per location, including tramlines. There were 12 plots, each were 3 × 4 m, with 4 repetitions and 3 treatments (hay mulch, leaf litter mulch and control).

There were 2 pitfall traps in each plot, a total of 24 traps per location. We grew the potato in organic farming system. We used Barber pitfall traps to sample the carabid beetles. All traps were emptied fortnightly between June and September of 2011-2013. On mulched plots we removed all mulch from the direct surroundings of the traps to level their rims with the soil surface. Animals were killed with acetic acid (5%). Carabids were identified to species level by using the guides "Carabidae of the Czech and Slovak Republics" (Hůrka, 1996) and "Die Käfer Mitteleuropas Band 2 Adelphaga 1 Carabidae (Laufkäfer)" (Müller-Motzfield, 2004). Effects of the mulching treatments and locations on the abundances of the captured Carabids were tested by using two-way ANOVA tests, the pairwise comparisons were performed with LSD test.

Table 1. Basic soil characteristics of potato plots at Hidegkút and Budaörs

Place	Soil sample depth (cm)	pH (H_2O)	$CaCO_3$%	Soil salinity (EC 2,5 mS/cm)	Saturation Percentage
Budaörs	0-8	7.63-7.68	24.41	0.303	58
Hidegkút	0-8	7.52-7.78	23.4	0.286	54

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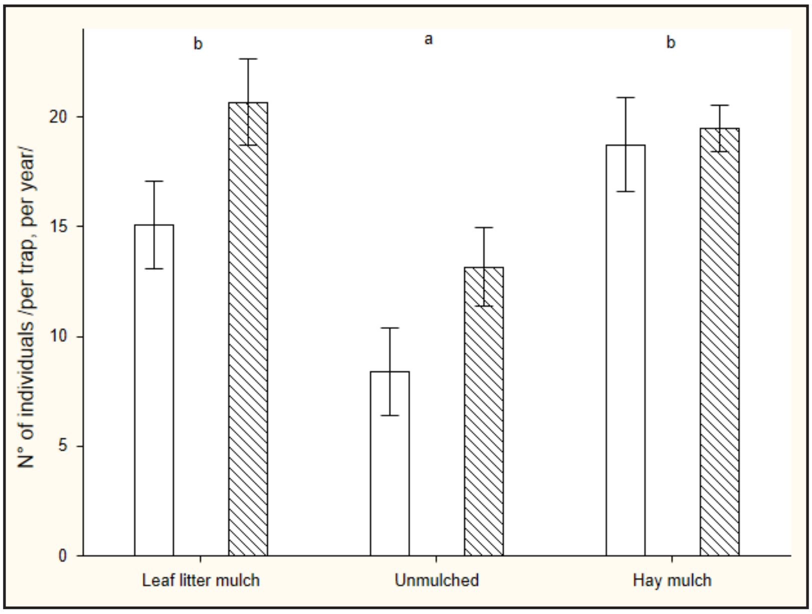

Figure 1. Average capture of carabid beetles of mulched and unmulched potato plots at Hidegkút and Budaörs from three years, 2011, 2012 and 2013 open bars: Budaörs, shaded bars: Hidegkút (bars denotes mean and whiskers standard error

Biodiversity was analyzed by using Shannon-index. Shannon index was calculated for each plot per site, per year and per treatment. Linear model was created to estimate Shannon index with three explanatory factor variables: site, year and treatment. Since the effect of the site was not significant, it was removed from the final model. The final model estimates the Shannon index as the function of the year and the treatment. Modell diagnostics justified the homogeneity of variance and the normality of the residuals.

Results and discussion

Application of organic mulches significantly increased the number of carabid individuals in potato plots (F(66,2)=11,72, p<0.001). During the whole sampling period the overall number of individuals collected on unmulched plots was 533, but the figure for leaf mulch was 439 higher, and for hay mulch, 641 higher. The averaged values of captured Carabids by treatment are shown in Figure 1. The results of the two-way ANOVA are presented in Table 2. Locations and mulching treatment affected the captured number of individuals significantly (p<0.001). We found a slightly but singnificantly higher abundance of carabid beetles at Hidegkút site (F(66,1)=5,94 (p=0,017). Mulching increased the number of individuals significantly (F(66,2)=11,72; p<0,001), with 66% at hay mulching and 77% at leaf litter mulching. According to LSD test there was no significant difference between the two types of mulching treatments, but number of individuals captured in either mulching was significantly higher compared to the captures of unmulched sites. Overall we found 46 species during the three years (Table 3). 13 species were solely captured on mulched plots, whereas 6 species occurred only on control (unmulched) plots. We found no difference in

Table 2. Results of the final 2-ways ANOVA model (dependent variable: number of individuals)

Effect	SS	Degr. of freedom	MS	F	p
Sites	245.7	1	245.7	5.94	0.018
Treatments	969.4	2	484.7	11.72	<0.001
Sites x treatments	80.1	2	40.1	0.97	0.385
Error	2729.4	66	41.4		

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Table 3. Carabid beetles of mulched and unmulched potato plots at Hidegkút and Budaörs in 2011-2013 (L: leaf litter mulch; U: unmulched; H: hay mulch)

Species	**L**	**U**	**H**	**Grand total**
Abax parallelepipedus (Piller et Mitterpacher 1783)	4	0	2	6
Amara similata (Gyllenhal 1810)	1	0	0	1
Calathus erratus (Sahlberg 1827)	1	0	1	2
Callistus lunatus (F. 1775)	18	0	27	45
Carabus scabriusculus Olivier 1795	0	0	1	1
Cicindela germanica L. 1758	0	0	1	1
Harpalus pumilus Sturm 1818	2	0	2	4
Ophonus laticollis Mannerheim 1825	0	0	1	1
Ophonus rupicola (Sturm 1818)	1	0	0	1
Poecilus cupreus (L. 1758)	2	0	6	8
Syntomus pallipes (Dejean 1825)	4	0	4	8
Trechus quadristriatus (Schrank 1781)	2	0	1	3
Zabrus tenebrioides (Goeze 1777)	1	0	3	4
Acupalpus meridianus (L. 1761)	1	1	3	5
Amara aenea (De Geer 1774)	6	6	8	20
Amara equestris (Duftschmid 1812)	4	3	3	10
Anchomenus dorsalis (Pontoppidan 1763)	6	8	80	94
Brachinus crepitans (L. 1758)	67	22	187	276
Brachinus explodens Duftschmid 1812	0	1	15	16
Calathus fuscipes (Goeze 1777)	22	14	35	71
Carabus coriaceus L. 1758	23	13	20	56
Harpalus affinis (Schrank 1781)	2	1	6	9
Harpalus albanicus Reitter 1900	2	3	1	6
Harpalus calceatus (Duftschmid 1812)	15	5	16	36
Harpalus caspius (Steven 1806)	59	41	51	151
Harpalus dimidiatus (Rossi 1790)	64	25	54	143
*Harpalus distinguend*us (Duftschmid 1812)	85	43	64	192
Harpalus griseus (Panzer 1797)	55	29	39	123
Harpalus rubripes (Duftschmid 1812)	3	6	0	9
Harpalus rufipes (De Geer 1774)	344	187	408	939
Harpalus serripes (Quensel 1806)	8	9	11	28
Harpalus smaragdinus (Duftschmid 1812)	3	1	1	5
Harpalus tardus (Panzer 1797)	76	49	61	186
Licinus cassideus (F. 1792)	1	2	5	8
Microlestes maurus (Sturm 1827)	30	15	9	54
Ophonus azureus (F. 1775)	28	19	25	72
Ophonus cribricollis (Dejean 1829)	11	10	16	37
Ophonus melletii (Heer 1837)	1	2	0	3
Ophonus signaticornis (Duftschmid 1812)	20	9	4	33
Pterostichus melas (Creutzer 1799)	0	1	3	4
Calathus ambiguus (Paykull 1790)	0	1	0	1
Cicindela campestris L. 1758	0	1	0	1
Harpalus atratus Latreille 1804	0	1	0	1
Ophonus diffinis (Dejean 1829)	0	1	0	1
Ophonus rufibarbis (F. 1792)	0	1	0	1
Parophonus dejeani Csiki 1932	0	3	0	3
Total number of individuals	972	533	1174	2679
Number of species	35	33	36	46

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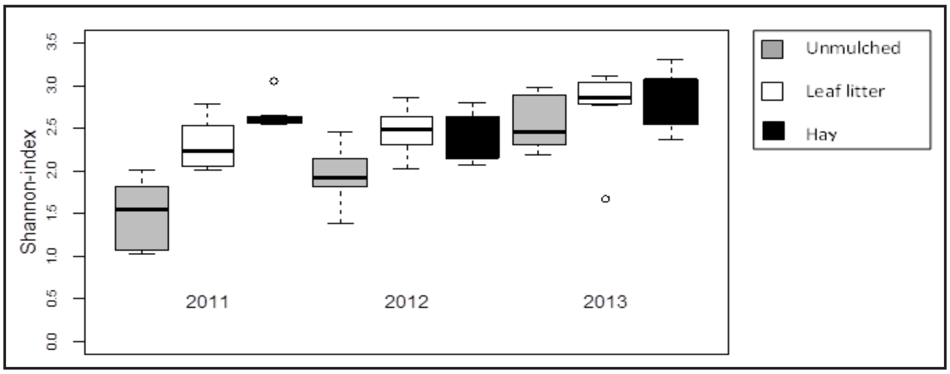

Figure 2. The biodiversity of captured carabid beetles of mulched and unmulched potato plots at Hidegkút and Budaörs from three years, 2011, 2012 and 2013

the number of species between the two types of mulching, however, the number of species on either mulching type was significantly higher than that on the unmulched, control area. The value of R^2 was 0.52. The results of the model are presented in Table 4. The biodiversity in 2012 did not differ significantly from 2011 but it was significantly higher in 2013. Both the leaf litter and the hay mulch significantly increased Shannon diversity (Figure 2). The most abundant carabid species of the study areas, in decreasing frequency, were *H. rufipes*, *Harpalus tardus* (Panzer), *Harpalus distinguendus* (Duftschmid), *Harpalus dimidiatus* (Rossi) at Hidegkút; and *Brachinus crepitans* (L.), *H. rufipes*, *H. distinguendus, Ophonus azureus* (F.) at Budaörs. The carabid fauna of the two locations overlapped considerably.The number of species was higher in Hidegkút, as there were only 37 species collected, while at Budaörs, the total number of species was 29. The combined number of individuals was 1636 for Hidegkút and 1043 for Budaörs. Carabid species that were captured in only one of the locations were found in small quantities, except for *Harpalus griseus* (Panzer), which was found in Hidegkút only, and was the fifth most frequent species of that location. Unmulched plots however, displayed lower diversity figures, and in every year, the mulched plots were the more diverse ones (Figure 2). There was no significant difference in species diversity between the two sites. Both locations (Hidegkút and Budaörs) had similar environmental conditions, which suggested similarity in species diversity.

For three consecutive years we studied the density of carabid assemblages of potato fields both without mulch and with organic mulch of two different structural types. The species composition of the two locations matches the typical carabid fauna of agroecosystems: the range is dominated by *Harpalus* species, while members of less frequent genera (*Ophonus, Brachinus*) prefer dry habitats (Saska and Honek, 2004; Kocourek, 2013).

Hidegkút and Budaörs are ruderal, agricultural open-habitats and as a result we found similar, mostly thermofilic species on both sites (Müller-Motzfield, 2004). The use of organic mulch, such as hay or leaf litter, had a positive effect on the carabid assemblages of potato, and this difference was significant. Mulching

Table 4. The averaged values of captured Carabids by treatment

	Estimate	Standard error	t	p
(Intercept)	1.77	0.09	18.83	<2×
year (2012)	0.14	0.10	1.34	0.186
year (2013)	0.56095	0.10	5.45	7.75×
treatment (leaf litter)	0.51	0.10	4.97	4.99×
treatment (hay)	0.62	0.10	6.04	7.52×

in general was found to increase the diversity of carabid species. We assume that organic mulch (hay and leaf litter, or similar materials) imitates natural habitat structures and has the potential to attract carabid species that do not occur on unmulched areas into arable plots. The species richness of mulched plots was significantly higher than that of unmulched ones. Carmona and Landis (1999) examined the influence of soil cover (mulching) and of the plot margin on the activity density of carabid beetles. They found a significant difference between the number of individuals of mulched and unmulched plots, although this finding was most prominent in the period between June and August. Shearin et al. (2008) marked and released *H. rufipes* individuals to compare the number of recaptured individuals on mulched and unmulched plots. They captured twice as many beetles on mulched plots than on unmulched ones. Enhancing the organic content of soil surface has an undoubtedly positive influence on the carabid assemblage of the habitat. Several studies of the movement patterns of carabid beetles have shown that carabids generally move randomly about in a favorable habitat but switch to a more straightforward course in an unfavorable habitat (Rijnsdorp, 1980; Wallin and Ekbom, 1988). The highest number of individuals captured belonged to species *H. rufipes*. This beetle is one of the most important carabids of agricultural areas and is found in great abundance from July to September on arable lands. *Poecilus cupreus* (L.) however, although described as a species of great abundance between May and June on arable lands (Juen et al., 2003), was scarcely captured in our experiment, with only 8 individuals caught on our plots. The activity peak of *H. rufipes* was observed between July and September. Similarly to our findings, the number of *Abax parallelepipedus* (Piller et Mitterpacher), *Amara aenea* (De Geer), *Calathus fuscipes* (Goeze), *Callistus lunatus* (F.), and *Carabus coriaceus* (L.) individuals captured by Traugott (1999) was also significantly lower than that of the most abundant species. This Austrian study found *H. rufipes* the most abundant species too, but *P. cupreus* was captured in notably larger abundance than in our experiment. Our study confirmed the presence of carabid species that are usually found along the edges of forests (Roume et al., 2011), namely *Anchomenus dorsalis* (Pontoppidan), *Trechus quadristriatus* (Schrank). The number of these species was relatively low, as they are not among the most important species of arable lands and are assumed to have been attracted into our experimental potato plots by of the presence of the organic mulching material. As a matter of fact, the appearance of both species was expected, because our experimental plots are surrounded by forested areas. The relatively high frequency capture of *B. crepitans* left us unsurprised, because this species is known for being frequent on disturbed areas such as mine tailings (Sár and Dudás, 2002), and on limestone terraces of abandoned quarries (Novotna and Šťastna, 2012). The occurrence of *B. crepitans* however, was especially high in one of the locations and we assume that the differences between the two habitats account for that. *H. distinguendus* (Tóthmérész et al., 2011), on the other hand, which prefers open habitats, was found in the largest number on plots covered with leaf litter mulch but was hardly found on unmulched plots. *H. tardus* (Small et al., 2006), which prefers open habitats and was also one of the most frequent species, was found in the largest number on mulched plots. There were other, frequent species such as *H. griseus* (Magura et al., 2008).

This species prefers open habitats generally. We found it in the largest number on plots covered with leaf litter mulch but it was hardly found on unmulched plots. One of the most frequent species of the Budaörs location, *B. crepitans* (Roume et al., 2011), which prefers open habitats, was found in large numbers on plots mulched with hay and was scarcely found on unmulched plots.

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Our results suggested that the two types of mulching materials did not cause a significant difference between the species diversity of carabid beetles (Figure 2). The reason of the similarity in diversity of carabids might be the small size of the plots or that the quality of cover is less important for ground beetles than the presence or absence of a shelter (and it seems that mulch as a soil cover that imitates the natural layer of decaying plant material is accepted as shelter). One must also note that the abundancy of potential prey also plays an important factor in the abundance and diversity of carabid beetles of an area (Guillemain et al., 1997). According to our study, year had no effect on the diversity of carabid beetles (Figure 2).

Conclusions

Our results suggested that our study should be extended to more locations and larger study plots and more types of mulch. In addition we should conduct further experiments to find out more about the micro-habitat preferences of the dominant species (*B. crepitans*, *Harpalus* spp.) of Carabid assemblages.

The impact of organic mulch types on the diversity of carabid beetles was significant in our plots. The number of individuals of species that were found only on mulched (covered) soil was low, leading us to the assumption that soil cover helps these somewhat rare species to spread and change their position between habitats. We found that the differences between the number of individuals of mulched and unmulched plots are explained by the presence or non-presence of the most frequent species of the area; whereas there are rare species behind the differences between the diversity figures. Mulching has a similar effect on both frequent and rare species, that is, to maximize their safety and survival on mulched areas both frequent and rare species prefer covered surfaces to open areas. The change that took place during the course of the study in land use (from grassland to potato production) increased the diversity of carabid beetles.

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CAGE CULTIVATION OF BESTER IN EAST KAZAKHSTAN

Damir ZHARKENOV[1] – József PEKLI[2] – Olga KIRICHENKO[3] – Katalin ZSUGA[4] – Toleuhkan SADYKULOV[5]

[1]Kazakh Fishery Research Institute, Almaty, Kazakhstan
[2] Szent István University, H–2100 Gödöllő, Páter Károly utca 1. E-mail: jozsef.pekli@gmail.com
[3]Altai branch, Kazakh Fishery Research Institute, Ust-Kamenogorsk, Kazakhstan
[4]AGRINT International Agriculture Trading and Consulting Ltd., H–2100 Gödöllő
[5]Kazakh National Agrarian University, Almaty, Kazakhstan

Abstract: The results of cage breeding of sturgeon hybrids in the third fish farming zone's climatic conditions are given. It is also shown the achieved fish breeding and biological features, the dynamics of the growth rate, the viability of the object and the possibility of breeding stocking material of hybrid sturgeon in providing certain technological conditions.

Keywords: sturgeon hybrids, cage breeding of juveniles, temperature regime, growth rate, stages of cultivation

Introduction

In the 21th century the water and food lack and safety are the main fundamental factors of life on Earth. The largest water consuming in the world is agriculture, food production. Nowadays one of the main economic and social problems is to lessen lack of protein of the population. The fishing, developing fish breeding can contribute to this considerably by setting up modern, water-saving recirculation systems (RAS, Recirculation Aquacultural Systems), and cage cultivation (Zsuga et al. 2015). Aquaculture development is an actual in ensuring food safeness of fish products supplying any state. For this aim the Republic of Kazakhstan has a huge fund of small fishery water bodies scattered practically throughout the country (Pekli et al. 2014).

Organization of fish breeding and fishing operations in small water bodies is associated with certain difficulties: the ambiguity of climatic and hydrological conditions. At the same time, climatic and hydrological differences allow to diversify the profile of fish farms significantly, taking into account the scientific advices. Fishery is an economic sector that fits the natural capabilities of the Republics of Central Asia. Development of the aquaculture of fresh water can provide significant measures to provide quality food for the peoples and opens up new possibilities on the area of exporting goods (Pekli – Zsuga 2015).

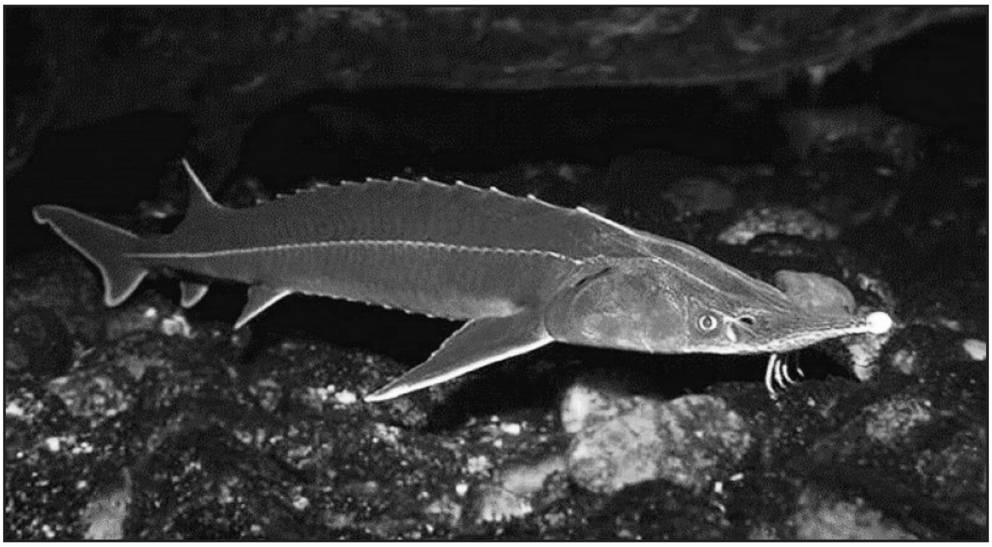

Photo 1. Bester hybrid (*Huso huso x Acipenser ruthenus*)

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Sturgeon fish occupy a special place in the world of aquaculture. Bester is a hybrid species, the result of different variants of beluga (Huso huso) female and sterlet (Acipenser ruthenus) male crossing (Photo1). It is suitable for aquaculture because it shows better growth rates than its male and female parents (Burtsev, 1997). The results of genetic identification showed that bester hybrids are more similar to beluga – 0.68, in comparison with sterlet – 0.45 (Yarmohammadi et al. 2012).

Bester produces high quality caviar at a younger maturity age as compared to pure beluga. The bester has been cultured not only in Russia but also in other countries, such as Germany, Hungary, France and Japan (Omoto et al., 2005). It has excellent fish-breeding characteristics, and food value. High dietary qualities of bester production continuously provide a great demand on it. Bester has great potential for intensive aquaculture technology due to its quality caviar and meat production. Yet, in the field of bester sturgeon nutrition many authors consider that further investigation is desirable (Dediu et al. 2011).

The relevance of commercial breeding of sturgeons is due to their reduction in natural reservoirs. The implementation and working out of sturgeon's commercial breeding technologies in different types of fish farms are very important and actual task. The proven cultivation technology of individual sturgeon species will allow showing the ability of producing valuable commodity products in adapted small water bodies. This technology will be transferred for implementation to other fish farms of the eastern region of the republic in order to increase the efficiency of their production. The purpose of this research is to study the adaptation and implementation of innovative technologies of growing sturgeon hybrid (Bester) in cages in water bodies *("pond in pond")* with a small area in the third fish farming zone climatic conditions.

Material and Methods

The work includes research papers of 2013 under the program *019 „Hosting measures for the dissemination and implementation of innovative practices"*, aimed at studying the possibility of implementing innovative experience of cage fish breeding in farms of Eastern Kazakhstan.

All-breeding works carried out in stages at two experimental bases - in laboratory conditions in the aquarium fish tank („mini-RAS") and in fish farming capacity in conditions of the farm „Silver bream", located on the Tainty reservoir.

Tainty reservoir is located 85 km in the south-west of the city Ust–Kamenogorsk and formed by locking up the Tainty and Bestau rivers, the covered area 61 hectares. Minimum level falls to the winter months. The average depth is 5 m. The ground of the coastal bottom is rocky, the open part is gumbo. Overgrowing of the reservoir is low, 2 – 3 %. From rigid surface vegetation found cattails, sedges, from the underwater – pondweed.

Bester larva grown in the farm conditions has been under constant surveillance. Valuations have been carried out every 10 days. The hydrochemical and ichtyological monitoring of the habitat and gibrion (Bester larvae) in water in fish tanks has been systematically held in laboratory conditions. The amount of the collected material is shown in Table

Table 1. The amount of collected material

Name of works	The amount of material
Hydrochemical samples	92
Water temperature measurements	192
Measurement of the oxygen regime	192
Sanitary inspection of sturgeon's fry (specimens)	406
Grows and fatness of fishes (specimens)	406

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1.For analysis of water quality the following components were measured: dissolved oxygen, pH, calcium, magnesium, potassium, sodium, bicarbonate, chloride, sulphate, total hardness, ammonia, nitrite, nitrate, sulphate.

The matching of analyzes results with fishery MPC conducted by conventional „Generalized list of MAC ." (Izmaylov 1990).

In the process of ichthyology researches were assessed:

- the general health status of the fry;
- size and weights features of fry
- presence of morphopathological abnormalities.

While carrying out fishery works on the topic were used own innovative engineering on sturgeon breeding, prepared by experts of LLP "KazNIIRH."

Results and discussion

Phase I.

Three-day Bester larvae were delivered on 25th of May for growing process. At this time the water temperature in the reservoir was only 7.4 ^{0}C in the daytime. For this reason the larvae were placed at first in the laboratory for rearing in an aquarium mini-installation, until the temperature was at least 12 ^{0}C in the reservoir.There is very important to insure both the suitable water quality and solving of the accidental problems for the satisfactory development of larvae. In the process of sturgeon larvae growing in laboratory were made daily observations of the gas mode, physicochemical properties and content of biogenic ions.

For sturgeon cultivation in a closed cycle is necessary to ensure the normal oxygen conditions. In the hatchery tank for water saturation with oxygen was used two submersible compressors. In addition, the water passing through one of the compressors and the external filter, passed through additional flutes, saturating the water with oxygen and creating the necessary water flow. Due to this the content of dissolved oxygen in the hatchery tank were stable and were at a high level during the whole period of rearing. The value of dissolved oxygen was in the range 7.8 – 8.6 mg/dm^3 (85 – 95.2% saturation).

Sturgeon tolerates fairly wide variations of pH, but most optimal values lead at a pH range of 6.5 - 8.5. The pH value was in the lab hatchery aquarium averaged 7.8. In some instances 8.17 maximum values were observed in the morning that seems to have been due to the accumulation of waste products in the fish aquarium. In this case the changing of the water in an amount of 30 % of the total reduced the pH to 7.5 – 7.6.

Photo 2. General view of the cage line in Tainty reservoir

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Table 2. The composition of water and water quality standards for sturgeon growing

Indicators	**Measurement unit**	**Tainty reservoir**	**$MPC_{aquaculture}$**
pH	-	7.5	7.2 - 9.0
Oxygen	mg/dm^3	9.0	6 - 8
Permanganate oxidation	mgO_2/dm^3	2.6	до 10
Ammonium nitrogen	mg/dm^3	0.0	0.5
Nitrite	mg/dm^3	0.0	0.08
Nitrate	mg/dm^3	0.48	40.0
Phosphate	mg/dm^3	0.11	-
Chloride	mg/dm^3	1.74	50
Sulphate	mg/dm^3	13.9	50
Total hardness	$Mg\text{-}EQ/dm^3$	1.3	60 - 80
Total mineralization	mg/dm^3	143.5	400 - 900

The concentration of ammonium ions in the tank during the larvae hatching period varied in a fairly wide range - from 0.0 to 1.95 mg/dm^3. To improve the hydrochemical regime was made water changing (about 30 % of the total). Increasing water temperature to 19.5 ^{0}C made a possible to reduce the concentration of ammonium ions to zero values. Along with this, in one of plates of the outer filter was loaded zeolite as an adsorbent that allowed partially lowering the concentration of ammonium ions and significantly reducing the turbidity of water.

High-protein feed residues deposited on the soil surface of the aquarium were a source of nitrite in the water. On some days, the contents of nitrite in the water in the fish tank aquarium reached a value of 0.63 mg/dm^3. Partial water changing reduced the content of nitrite to the optimal values. The concentration of nitrate in the process of growing in the fish tank does not exceed the normative values and changed in the range of 0.0 – 11.4 mg/dm3.

Feeding of larvae began on the third day with yolk after placing them in the conditions of the aquarium. After some days they were fed with starter feed imported with grit sizes up to 1 mm, with addition of live food (Oligochaetes). At the rate of 10 % by weight of the larvae feeding was at intervals 5 times a day, the last was at 6.00 p.m. Later the ration was changed towards increasing the share of dry starter feed, gradually bringing its value in the daily diet to 25-30 % or more.

Photo 3. The cage with bester larvae

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Thus, one of the required conditions of feeding in the initial stage is a strict management of standards in the calculation of daily rations, which depends on the temperature, the growth indicators and the mandatory inclusion in the diet of live food.

Bester larvae grown in laboratory conditions in general showed a high survival on a first phase, composed exactly 30 days from the date of putting them into the system, as indicated by the value of their survival 48 %. The maximum deviation of the larvae was observed on 9th-10th day after hatching 40 %, which is coincided with the transition to active feeding larvae. At this time due to technical problems, larva was in critical water exchange and water purification more than a day that affected its survival. Later departure stabilized (12 – 16 hours cultivation) at a very low level 2-3 % per day.

An analysis of fish breeding and biological features of the growing fry according to the first month cultivation results showed the following results: the average growth of the bester larvae from the starting was 97.7 % by weight and 71% by length of the fish, reaching an average of 7.25 cm in length and 2.64 g weight of their body. These results are higher than the corresponding features from the experience of Korean (average weight was 1.0 grams on 43rd day of cultivation) and Russian (the average weight was 0.9 – 1.0 g on day 30th growing day) fish farms (Burtsev et al. 2007, Filippov et al. 2004).

After 30 days in late June bester larvae were transferred from the “mini-RAS” and placed in a cage line of Tainty reservoir (Photo 2.). There were also the larvae grown in suited base condition. Since young bester, grown under different conditions, and vary greatly in their size and weight characteristics, was performed calibration in order to align its growth. All the plant material was sorted and put on into 3 cages:

- cage number 1– “large” fry, weight 3 grams or more
- cage number 2 – “medium” fry, weight at least 2 grams
- cage number 3 – “small” fry, weight under 2 grams.

Phase II.

The hydrochemical compositions were measured before the transplant of young bester in cages of water of Tainty reservoir. The composition of the water and the value of the water quality standards for sturgeon growing are given in Table 2.

Optimization of the temperature regime, providing favourable conditions for growing of sturgeon, is the basis of growing technology in the closed cycle of water

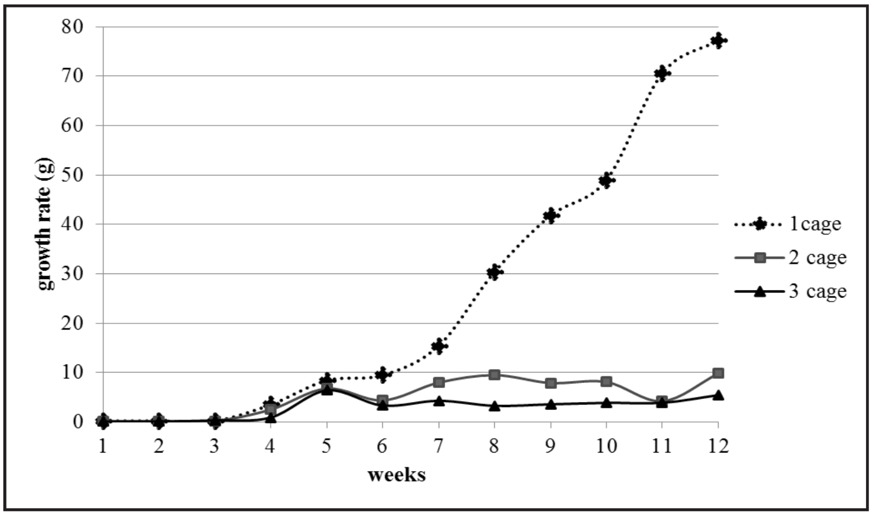

Figure 1. The dynamic of growth of juvenile Bester in a cage line (July – October).

supply. Changes in temperature influence to an oxygen consumption, a rate of development and growth, as well as on an intensity of consumption and digestion. During the fish works implementation in the reservoir operational control over the temperature regime was carried out daily.

Since early July, the second phase of growing fry bester started in a cage line of Tainty reservoir (Photo 3.).

For the favourable development of sturgeon temperature plays an important role. During the growing period water temperature (July-August) was significantly below the optimal temperatures for juveniles rearing. So, the average temperature in the month of July totalled 17.4 ^{0}C, while the standard is in accordance with the aquaculture norms is 23 – 26 ^{0}C, in August average temperature was even lower and amounted 16.2 ^{0}C.

In the process of young Bester rearing the oxygen regime monitoring was daily carried out, water sampling to determine an active reaction of environment and concentrations of ions were carried out every 10 days. Oxygen regime of the reservoir was optimal for rearing juveniles throughout the fish works carrying out – the concentration of dissolved oxygen was in the range of 7.9 to 9.2 mg /dm3, the average saturation of water with oxygen was 87.8 %.

By the results of the cage fish culture experiments in Hungary, the best weight gain of Bester was at 18 – 23°C temperatures and an oxygen content of water higher than 6 mg/dm3. There was a dramatic decrease in the appetite of fish, however, if DO content decreased below this value (Müller – Váradi).

Characteristic pH value of Tainty reservoir water is 8.2 to 8.3. In the period from July to August, the pH of water in the test cage line was in the range of 7.9 – 8.65 and the minimum values were recorded during the period of rainfall. In this period were took place an increasing of the hydrological level of the reservoir and the degree of water flowage, and as a consequence, the decrease of pH value.

Ammonium nitrogen excreted by fishes in water as the end product of metabolism. Ammonium ions (NH4+) in large quantities are toxic. The concentration of ammonium ions in the period of fry rearing in cages averaged 0.23 mg/dm^3. The growth of the ammonium ions content in water was recorded on 19 July and was caused by the processes of decay of uneaten feed and waste products of juveniles actively flew in the case of water heating. To reduce the content of ammonium ions were conducted an additional cleaning of the cages, a removal of algae in the area of the cage lines, after which there was a decrease of ammonium ions concentration.

Nitrite is toxic for fish, since it breaks the binding of oxygen by haemoglobin. During the work, the content of nitrite was at an optimal level (0 – 0.02 mg/dm3) and also does not exceed the recommended aquaculture norms.

The nitrate formed from nitrite in a process of nitrification, and it is much less toxic than nitrite. The content of nitrate in the process of cage line cultivation did not exceed normative values and varied from 0 – to 2.99 mg/dm3.

The phosphate content during the growing period in cages amounted 0.0 – 0.34 mg/dm^3 (there is no limit value by normative).

Comparing recorded values of the main hydrochemical indices of water with the regulatory requirements of the allowable values for aquaculture water gives grounds to verify that fluctuations in the values of above mentioned indicators in most cases did not exceed the permissible norms and ensured a normal growth of sturgeon.

In order to ensure greater survival of planting stock and the efficiency of the growth process, the gotten larvae before putting in the cage lines of fish farm were grown in adapted „mini-RAS" aquarium with the required water exchange and water purification. All

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Table 3. Fish breeding and biological indicators in a cage Bester line

Indicators	Phase I.	Phase II.	Phase III.
Growing period (days)	30	60	47
Planted on the cultivation (pcs)	500	226	197
The initial mass (gram)	0.09	2.30	20.31
The final mass (gram)	2.64	20.31	30.83
Average daily gain (milligram)	88	300	263
Fulton condition factor	0.70	0.42	0.46
Survival rate (%)	48	92	87 (28)

received Bester larvae was divided in two control groups: one was put in a laboratory's aquarium, the second was delivered to Tainty reservoir. Rearing was carried out in the reserve tank, installed in a trailer. Medium size of Bester larvae figures on the total body length, measured up to the end of the caudal fin rays averaged 1.5 cm while the size features of the planting stocks varied from 1.3 to 1.7 cm.

Analysis of the size and weight indicators juveniles in the second growing period shows the highest growth in both length and weight, the "large" fry from cage number 1, where the average weight gain for the reporting period reached 700 mg, the "medium" group 90 mg, and the" small "fry 50 mg.

Some variation of the average values of weight in the group of "medium" and "small" fry is due to the heterogeneity of its size and composition of the different growth rates of individuals. Aligning growth is achieved by periodic calibration. The gradual decline in nutritional status of young bester in the process of growing is quite natural and is due to the acceleration of the growth of individual species and the intensification of metabolism in the body, most of all when entering the body of nutrients begins spent on protein growth.

In addition, a prolonged period of adaptation to the changed conditions of juvenile protection had a role in the decline in nutritional status of fish, in this case, after the transportation it from the laboratory to the cage line reservoir.

Thus, it should be stated that as a result of transplantation, and the second calibration stage of cultivation, the growth rate increased significantly only in the young group of "large" fish. In the "medium" and "small" group heterogeneity growth trend persists that apparently depends on genetic potential of an individual which was obtained from the original parental forms (Figure 1). The grown in a natural pond fry bester showed good viability in the second phase, as indicated by the value of survival rate of the planted (92%) – in Table 3).

Phase III.

The third phase of growing fry bester lasted 47 days; from early September to mid–October. The hatchery process associated with a significant backlog growth in size and weight of fish from the category "medium" and "small" specimens. Attempt to stimulate it through the use of immune modulator and probiotics.

Analysis of fish breeding (Photo 4.) and biological indicators on the results of fry rearing in the third phase showed the following results: the mean growth of fry bester, by all size groups, from the initial value was about 52 % by weight and about 4 % by the length of the fish. It is noted the apparent excess of weight gain over the linear growth of fish, which in principle is a natural process. The general fall in the rate of growth due to the decline in water temperature, which slows down the metabolic processes in the body, including the digestive system and, ultimately, affects the largest growth rates.

Using krezatcin and probiotics had some effect – the growth processes of young "medium"

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Photo 4. Valuation of young bester in cage cultivation

and “small” groups increased: for example, if the weight gain in the category of “large” fish in the same period amounted to slightly more than 10 %, then the “small 40 %, and the “average” 135 %. The survival rate of young fish at the end of the third stage was 87 %. In late October, the fry Bester was placed for the winter lowered in special cages to a depth of 3 – 5 meters.

Conclusions

Adapted and improved technology will allow sturgeon breeding farms on the example of the possibility of obtaining valuable commodity products adapted to small ponds. Here are some recommendations developed during the mining process.

Artificial breeding of valuable fish becomes more effective when the larvae, before boarding a cage fish farm line were grown to full-system RAS providing optimal exchange of water and water treatment.

It is necessary to undertake a systematic monitoring of hydrochemical water for the most important indicators – water temperature, oxygen content, an active reaction (pH) and ions, nutrients (ammonium, nitrite, nitrate).

The system RAS provide a full cycle of water treatment; to reduce the toxic effects of metabolic products is expedient as filter substances in the biofilter used natural adsorbents – zeolites, expanded clay. Water in pools for rearing trays or fed directly from the reservoir, which will be installed cages, preferably adjusted to the optimum temperature 18 – 23 ^{o}C with oxygenation of 6 – 8 mg /L, pH: 6.5 – 7.5 with average rigidity.

The use of soils in the basins is impractical because it is difficult to clean from the products of metabolism, feed residues.

For sturgeon typical uneven growth, manifested in the end result in a large spread by weight of fish, so the fish sorting should be performed periodically during the growing process, separating them by size groups: small, medium and large.

During breeding larvae and young fish, it is necessary to enter a live feedstuffs in the diet, depending on the water temperature, growth parameters (average linkage) fish and their quantity; daily diets calculated taking into account the use of live food. If you are unable to sort and place separately juveniles, it is imperative to organize a few tables of food in different places, in order to reduce competition during feeding. In case of a different quality of growth processes of the young, in order to stimulate growth in the contingent of “small” fish enter into the diet dressing vitamins, probiotics and produce handling juvenile

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immune modulator. To minimize the stress load on the weighing of fish, produce electronic scales in the tank with water, adding ascorbic acid feed rate of 10 grams per 1 kg of feed. Thus, experience shows that the availability of the sufficient necessary equipment and suitably trained fish farmers, allows rearing sturgeon larvae from stage a three-day weather conditions in Eastern Kazakhstan (third aquaculture zone) and economically feasible.

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EFFECT OF SHORT-TERM AFLATOXIN EXPOSURE IN COMBINATION WITH MEDICINAL HERB MIXTURE ON LIPID PEROXIDATION AND GLUTATHIONE REDOX SYSTEM IN LAYING HENS

Mangesh NAKADE[1] – Krisztián BALOGH[1] – Erika ZÁNDOKI[2] – Mátyás CSERHÁTI[3] – Miklós MÉZES[1] – Márta ERDÉLYI[1]

[1] Szent István University, Faculty of Agricultural and Environmental Sciences, Department of Nutrition, H-2103 Gödöllő, Páter Károly u. 1., Hungary; E-mail: Mezes.Miklos@mkk.szie.hu

[2] Kaposvár University, MTA-KE Mycotoxins in the Food Chain Research Group, H-7400 Kaposvár, Guba Sándor u. 40., Hungary

[3] Szent István University, Faculty of Agricultural and Environmental Sciences, Department of Environmental Safety and Ecotoxicology, H-2103 Gödöllő, Páter Károly u. 1., Hungary

Abstract: Aflatoxins are well known hepatotoxic mycotoxins, which mainly contaminate the cereal grains. Those induce lipid peroxidation and impair the antioxidant, including glutathione redox system in long-term studies. The purpose of present study was to investigate the short-term (36-hour) effect of feeding aflatoxin B1 (AFB1) contaminated diet alone or in combination with a medicinal herb mixture on lipid peroxidation (conjugated dienes and trienes, and malondialdehyde), and on parameters of the glutathione system (reduced glutathione and glutathione peroxidase) in blood plasma, red blood cell haemolysate, liver and kidney homogenate of 49-week old Bovans Goldline laying hens. The results revealed that AFB1 (125 m /kg feed) did not have effect on feed intake, body and liver weight, but increased malondialdehyde content was observed in blood plasma and red blood cell haemolysate as effect of feeding AFB1 and medicinal herb mixture at 36th hour of the trial. However, the same diet resulted in lower malondialdehyde content in liver, but not in kidney. Reduced glutathione concentrations showed variance among treatments; thus due to inclusion of medicinal herb mixture in the diet lower values were measured in red blood cell haemolysate. Glutathione peroxidase activity was significantly lower in all treated groups as compared to the control at 36th hour of the trial in blood plasma, but not in other tissues. The results are contradictory with previous findings, probably due to the short-term exposure, and/or to medicinal herb mixture supplementation as it could moderately modify the effect of AFB1.

Keywords: Aflatoxin B1, malondialdehyde, glutathione, glutathione peroxidase, medicinal herb

Introduction

Cereal grains and their by-products are important ingredients in poultry diet. Cereals are the main components of complete feeds that may be contaminated with mycotoxins, secondary metabolites of moulds (Binder et al., 2007). Feed spoilage by moulds may result in heating, reduced palatability and loss of nutritive value (Christensen, 1974). Among various mycotoxins, aflatoxins (AF) are often encountered in feed ingredients even at high concentrations in many parts of the world under different environmental conditions (Jelinek et al., 1989). Among avian species, goose is the most sensitive to the prooxidant effect of mycotoxins, followed by duck and chicken, while the most sensitive indicators are liver, blood plasma, and red blood cells (Mézes et al, 1999).

However, mycotoxin contamination levels in animal feedstuffs are usually not high enough to cause an overt disease, but may result in economical loss due to changes in growth, production and immunosuppression (Richard, 2007). In poultry species, the economic losses associated with aflatoxin exposure include poor feed conversion and growth, increased mortality, decreased egg production, leg problems, and carcass condemnations (Smith and Hamilton, 1970; Hamilton, 1971; Huff et al., 1992; Yunus et al. 2011). Aflatoxins are toxic metabolites of certain strains of *Aspergillus flavus* and *Aspergillus parasiticus* moulds (Manafi et al., 2011), and aflatoxin B1 (AFB1) is the most toxic among aflatoxins, which causes a wide variety of adverse effects, such as decrease of feed intake, reduced immune response, impaired production

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traits, and liver damage depending on the concentration, duration of exposure, animal species, its age, health and nutritional status during the exposure (Diaz 2005). Aflatoxin B1 residues arise food safety problems, because those are present in eggs and flesh of laying hens fed aflatoxin B1 contaminated diet (Herzallah, 2013).

Most of the mycotoxins, e.g. aflatoxin B1, T-2 toxin and ochratoxin A (Balogh et al., 2007; Pál et al., 2009), provoke oxygen free radical formation. The body is protected against reactive oxygen metabolites by the biological antioxidant defence system, which includes antioxidant enzymes and low molecular weight antioxidants (Surai, 2002). It has been suggested that the cellular first line of antioxidant defence is based on the activity of enzymes, such as superoxide dismutases, glutathione peroxidases (GPx) and catalase. Also, the rate of lipid peroxidation is reported to be increased in different tissues as a result of feeding mycotoxin-contaminated diet containing AFB1 or T-2 toxin (Surai et al., 2002) and ochratoxin A (Balogh et al., 2007), which reduces the efficiency of the biological antioxidant defence system.

Phytobiotics, such as essential oils of herbs are considered to be important natural antioxidants both in animal and human nutrition. Compared to synthetic antioxidants, their antioxidant capacity is measurable only at relatively high doses and is mainly proven in the Fe^{2+}/ascorbate system (Bozin et al., 2006). Kim et al. (2009) reported *in vivo* antioxidant effects where dietary supplementation with garlic husk resulted in significantly lower thiobarbituric acid reactive substances (TBARS) values in chicken. In this study a mixture of eight medicinal herb extract was used. Two of them (rosemary [*Rosmarinus officinalis*] and oregano [*Origanum vulgare*]) have antioxidant bioactive component(s), and a third one (Mary thistle [*Silybum marianum*]) is hepatoprotective (Mirzaei-Aghsaghali, 2012).

Our hypothesis, and thus, the purpose of the study was to investigate the short-term effect of AFB1 on lipid peroxidation and some glutathione redox parameters in laying hen and evaluate the efficacy of a herbal mixture (Herbamix™) for counteracting AFB1 in experimentally contaminated layer diet.

Materials and methods

Animals and experimental design

Total of 60 Bovans Goldline laying hens, being at 90% daily egg production at 49 weeks of age were divided randomly to four experimental groups (two replicates each): a control (aflatoxin content < 1.0 μ/kg) and three treated groups fed with aflatoxin (total aflatoxin content was 170.3 μ /kg; AFB2: 39.0 μ/kg; AFG1: 2.0 μ/ kg; AFG2: 4.3 μ/ /kg), herbal mixture, and aflatoxin + herbal mixture, respectively. For experimental contamination of the feed aflatoxin was produced in ground corn which was artificially infected with an aflatoxin producing *Aspergillus flavus* strain (ZT80) isolated by Dobolyi et al. (2011). Appropriate amount of the fungal culture was mixed with the complete feed, according to its total aflatoxin content. Herbal mixture, namely Herbamix Basic Premix™ (Herbamix Trade Ltd., Budapest) was added to the complete feed in powder form at the dose of 600 mg/kg. Hens were kept in deep litter condition. The feeding trial lasted for 36 hours, after 12 hours of feed deprivation. Because of the short-term exposure the toxin dose was much higher than the regulatory limit of 20 μ /kg for aflatoxin B1,, in complete feed (Commission Regulation 574/2011).

Aflatoxin content of the loaded complete feeds was analysed with AFLAPREP HPLC method after immunoaffinity clean-up (Food Analytica Ltd, Gyula). Five animals from each group were taken randomly at 12, 24 and 36 hours after start the experiment. Body weight and liver weight was measured, and blood and tissue (liver and kidney) samples were taken at extermination.

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Ethical issues

The experiment was carried out according to the Hungarian Animal Protection Act, in compliance with the EU rules. The experimental protocol was authorised by the Food Chain Safety and Animal Health Directorate of the Pest County Agricultural Office, under permission number XIV-1-001/1880-5/2011.

Biochemical analyses

Level of conjugated dienes (CD) and conjugated trienes (CT) in liver, as markers of initial phase of lipid peroxidation, was measured by the absorbance of samples at 232 nm and 268 nm after extraction in 2,2,4-trimethylpentane (AOAC, 1984). Malondialdehyde (MDA) content, as marker of terminal phase of lipid peroxidation, was measured with the method of Placer et al. (1966) in blood plasma and according to Mihara et al. (1980) in liver and kidney homogenates. Reduced glutathione (GSH) content was measured as non-protein sulfhydryl groups with Ellmann's reagent (Sedlak and Lindsay, 1968), and glutathione peroxidase (GPx) activity according to Lawrence and Burk (1978) in blood plasma, in red blood cell haemolysates, and in the 10,000 g supernatant fraction of tissue homogenates. GSH content and GPx activity were calculated to protein content which was determined with biuret reaction (Weichselbaum, 1946) in blood plasma and red blood cell haemolysate, while Folin-phenol reagent (Lowry et al., 1951) was applied for the 10,000 g supernatant fraction of tissue homogenates.

Statistical analyses

Statistical analysis of data (one-way analysis of variance with Tukey's post-hoc test, calculation of means and standard deviations) was performed with GraphPad Prism 5 for Windows (GraphPad Software, San Diego, CA, USA).

Results

There was no morbidity or mortality in the experimental groups during the trial.

Feed intake (Table 1), liver weight and relative liver weight (Table 2) did not change significantly during the experimental period between the treatment groups.

Initial phase of lipid peroxidation, characterised with levels of conjugated dienes and trienes, was not affected by feeding aflatoxin contaminated diets (data not shown), but its terminal phase, described with malondialdehyde concentration, showed increased intensity as a result of combined treatment with medicinal herb mixture and AFB1 containing diet (Tables 3-6). Significantly higher malondialdehyde concentration was found at 36th hour of treatment in blood plasma and red blood cell haemolysate samples of the group treated with the combination of AFB1 and medicinal herb mixture than the Control (Tables 3 and 4). However, variable changes were found in the liver (Table 5). There was significant difference between single Herbamix and combined Herbamix+AFB1 treatments, as the combination showed remarkably lower value. AFB1 alone did not cause significant

Table 1. Individual and combined effect of aflatoxin B1 and medicinal herb mixture (Herbamix™) on calculated individual feed intake (g in each period/hen) of laying hens in different periods of exposure

Groups/Timeframe	0-12 h	12-24 h	24-36 h
Control	146.50	4.06	144.50
Herbamix	150.00	0.00	147.50
Aflatoxin B1	150.00	0.00	146.50
Aflatoxin B1 + Herbamix	146.25	1.56	140.50

Table 2. Individual and combined effect of aflatoxin B1 and medicinal herb mixture (Herbamix™) on body weight, liver weight and relative liver weight (mean±SD; n=5)

	Control	**Herbamix**	**Aflatoxin B1**	**Aflatoxin B1 + Herbamix**
	Body weight (g)			
12th hour	1986± 116.4	1940± 149.5	1941± 155.2	1859± 100.8
24th hour	1819± 158.1	1782± 276.0	1924± 92.8	1817± 63.5
36th hour	2023± 178.5	2047± 228.5	1945± 183.0	1997± 128.0
	Liver weight (g)			
12th hour	41.91± 6.59	39.85± 2.47	41.54± 3.58	42.88± 3.45
24th hour	34.22± 4.39	34.97± 8.08	41.57± 8.54	37.41± 2.86
36th hour	53.56± 2.98	48.69± 9.45	48.52± 4.49	48.80± 4.97
	Relative liver weight (g/100 g body weight)			
12th hour	2.10± 0.24	2.06± 0.20	2.14± 0.10	2.31± 0.25
24th hour	1.89± 0.25	1.95± 0.20	2.16± 0.44	2.06± 0.17
36th hour	2.66± 0.27	2.37± 0.29	2.50± 0.14	2.44± 0.17

Table 3. Individual and combined effect of aflatoxin B1 and medicinal herb mixture (Herbamix™) on some parameters of glutathione redox system and lipid peroxidation processes of blood plasma (mean±SD; n=5)

	Control	**Herbamix**	**Aflatoxin B1**	**Aflatoxin B1 + Herbamix**
	MDA (μmol/L)			
12th hour	15.40**a**± 0.79	15.93**ab**± 1.51	18.48**bc**± 1.90	19.13**c**± 1.21
24th hour	19.53± 1.74	21.06± 2.78	21.14± 0.67	18.09± 0.84
36th hour	17.72**a**± 1.32	22.28**ab**± 5.04	25.24**ab**± 2.35	28.71**b**± 3.60
	GSH (μmol/g protein content)			
12th hour	3.43± 0.71	3.51± 0.73	3.71± 0.77	4.14± 0.93
24th hour	4.84± 1.07	3.95± 0.81	5.34± 2.09	4.89± 2.33
36th hour	2.83± 1.05	2.76± 0.74	2.42± 1.15	3.22± 1.81
	GPx (U/g protein content)			
12th hour	4.60± 0.65	4.72± 1.55	5.07± 1.38	4.97± 1.71
24th hour	7.49± 1.79	5.66± 1.02	6.98± 1.88	6.93± 1.94
36th hour	6.93**b**± 1.94	4.17**a**± 0.98	3.82**a**± 0.28	4.04**a**± 0.98

[a,b] Means designated with different letters within the same rows mean significant difference ($p<0.05$)

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Table 4. Individual and combined effect of aflatoxin B1 and medicinal herb mixture (Herbamix™) on some parameters of glutathione redox system and lipid peroxidation processes of red blood cell haemolysates (mean±SD; n=5)

	Control	Herbamix	Aflatoxin B1	Aflatoxin B1 + Herbamix
	MDA (μmol/L)			
12th hour	29.84b± 1.07	30.47b± 2.66	29.73b± 1.59	26.13a± 1.49
24th hour	38.57± 11.02	35.48± 7.03	37.32± 8.46	50.35± 14.13
36th hour	44.88a± 0.84	52.92ab± 5.58	45.32ab± 5.55	54.00b± 6.91
	GSH (μmol/g protein content)			
12th hour	8.92± 0.82	7.23± 0.67	7.88± 1.34	7.90± 0.87
24th hour	6.91± 3.46	6.18± 2.05	5.16± 1.59	4.36± 2.41
36th hour	5.79b± 0.42	4.11a± 1.04	4.65ab± 0.40	4.87ab± 0.99
	GPx (U/g protein content)			
12th hour	6.09± 1.01	6.25± 1.89	6.08± 0.79	6.05± 0.15
24th hour	5.55± 1.97	4.80± 1.19	4.04± 0.99	3.50± 1.03
36th hour	4.62± 0.56	4.14± 0.87	4.30± 0.46	4.24± 0.98

[a,b] Means designated with different letters within the same rows mean significant difference ($p<0.05$)

difference as compared to other experimental groups. In case of kidney, there were no significant changes in malondialdehyde concentration (Table 6).

Reduced glutathione concentration showed significant variance across the groups only in red blood cell haemolysate (Table 4) where medicinal herb mixture treatment resulted significantly lower level as compared to untreated Control, but in blood plasma (Table 3), liver (Table 5) and kidney (Table 6) no significant changes were found during the 36-hours period of experiment. Glutathione peroxidase activity was significantly lower in all treated groups as compared to the Control at 36th hour of experiment in blood plasma (Table 3), but there were no measurable changes in the other tissues during the experimental period (Tables 4-6).

Discussion

The result of present study showed that short-term aflatoxin B1 exposure did not have effect on feed intake and liver weight, which is probably due to short period of investigation, because long-term experiments showed marked feed refusal effect of AFB1 and liver weight was also increased (Aly Salwa and Anwer, 2009). However, AFB1, even after short-term exposure, induced lipid peroxidation, which was confirmed by the significant increase of the termination phase parameter (MDA) in blood plasma and red blood cell haemolysate, but not in liver or kidney. MDA concentration in blood plasma and red blood cell haemolysates revealed significant differences between the treatment groups at hors 12 and 36 but not at hour 24. This results probably due to the lack of feed intake in the period between hours 112 and 24, because of dark period. Additionally MDA concentration in liver and kidney was numerically lower in aflatoxin B1 contaminated feed fed group as compared to the control, which is probably duet to the impairment of lipid metabolism as effect of AFB1 (Siloto et al., 2013), while

Table 5. Individual and combined effect of aflatoxin B1 and medicinal herb mixture (Herbamix™) on some parameters of glutathione redox system and lipid peroxidation of liver homogenates (mean±SD; n=5)

	Control	**Herbamix**	**Aflatoxin B1**	**Aflatoxin B1 + Herbamix**
	MDA (µmol/g wet weight)			
12th hour	8.28± 1.12	8.37± 2.41	10.59± 1.34	8.33± 1.68
24th hour	8.01± 2.66	7.21± 4.24	7.32± 2.96	6.76± 1.54
36th hour	13.61**ab**± 1.74	14.28**b**± 2.39	11.68**ab**± 1.59	10.70**a**± 1.65
	GSH (µmol/g protein content)			
12th hour	4.66± 1.20	4.64± 0.76	5.25± 1.87	6.06± 2.22
24th hour	3.78± 0.68	3.83± 0.93	3.17± 0.53	4.00± 0.71
36th hour	6.84± 0.68	7.79± 0.83	7.28± 0.41	6.61± 1.72
	GPx (U/g protein content)			
12th hour	4.22± 0.91	4.57± 0.83	5.13± 1.80	5.74± 2.20
24th hour	3.41± 0.88	3.75± 0.86	3.28± 0.49	4.01± 0.56
36th hour	7.01± 0.70	7.30± 0.50	7.04± 0.55	6.61± 1.77

[a,b] Means designated with different letters within the same rows mean significant difference ($p<0.05$)

Table 6. Individual and combined effect of aflatoxin B1 and medicinal herb mixture (Herbamix™) on some parameters of glutathione redox system and lipid peroxidation of kidney homogenates (mean±SD; n=5)

	Control	**Herbamix**	**Aflatoxin B1**	**Aflatoxin B1 + Herbamix**
	MDA (µmol/g wet weight)			
12th hour	7.50± 1.58	9.84± 2.76	10.10± 1.07	10.29± 2.73
24th hour	12.44± 2.82	15.98± 3.46	19.36± 7.25	12.97± 2.56
36th hour	15.40± 2.86	11.57± 2.39	13.26± 2.50	15.28± 4.01
	GSH (µmol/g protein content)			
12th hour	5.29± 0.42	5.19± 0.66	5.79± 0.62	5.24± 0.31
24th hour	6.08± 1.15	4.58± 1.53	5.81± 0.89	6.11± 0.40
36th hour	6.70± 0.31	7.08± 0.86	6.83± 0.50	7.55± 1.84
	GPx (U/g protein content)			
12th hour	5.17± 0.44	5.62± 0.60	6.39± 1.13	6.06± 0.43
24th hour	4.79± 0.78	3.96± 0.94	4.81± 0.83	4.94± 0.34
36th hour	4.67± 0.68	5.14± 0.41	5.49± 0.59	5.55± 0.53

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MDA concentration partly depend on the actual lipid content of the sample (Dworschák et al., 1988) The results in case of liver are contradictory to previous findings which are showed significant MDA formation in liver, but after long-term aflatoxin B1 exposure or application of higher doses (Surai, 2002). Aflatoxin B1 in combination with medicinal herb mixture revealed lower value of MDA, thus lower rate of lipid peroxidation in liver and kidney, which alludes to reduced oxygen free radical formation when aflatoxin was combined with the herbs.

Among the parameters of the glutathione redox system, GSH content changed only in red blood cell haemolysate, as medicinal herb mixture resulted in significantly lower value than in the other groups. The exact cause of this difference is not known yet, but it probably caused by the changes of the intensity of glutathione synthesis in the red blood cells. It is an important finding, because erythrocyte glutathione plays an important role in mitigating the damaging effects of reactive oxygen species (ROS) present in the circulation (Mak et al., 1994) which causes continuous oxidation of haemoglobin within the cytosol of the erythrocyte (Hsieh and Jaffe, 1975). GSH reacts with ROS and degrades hydrogen peroxide and lipid peroxides by glutathione peroxidase or modifies toxic xenobiotics by glutathione *S*-transferase (Pace et al., 2003). The activity of glutathione peroxidase did not vary significantly in red blood cell haemolysate, liver and kidney, but it was reduced in all treated groups in blood plasma. This result is different than previous finding where AFB1 exposure resulted in significant drop in GPx activity in liver, however that was a long-term trial (Shi et al., 2012).

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OBITUARY

JÚLIUS ŠÚTOR (1935-2016) PROMINENT HYDROLOGIST PASSED AWAY

Márton JOLÁNKAI

Szent István University, 2100 Gödöllő, Páter Károly utca 1. E-mail: jolankai.marton@mkk.szie.hu

Július Šútor (1935 – 2016)

Dihydrogen-oxide. A most essential substance of life. Colourless, transparent, tasteless, scentless compound of oxygen and hydrogen in liquid state, convertible by heat into steam and by cold into ice. Our vocabulary has countless words concerning the form, appearance and the like of this material; ice, snow, hail, hoar, rime, moisture, dew, vapour, steam – and sea, ocean, lake, pond, river, creek, brook, stream, fluid, drop and droplet – and also such phenomena as cloud, fog, rain, shower, tide, flood or on the contrary, words that may indicate the lack of that, from wilting to drought. Any of these words has a certain meaning providing us with information about this substance. The history of mankind is also a history of struggles to control and regulate water. Hydrology is the scientific study of the movement, distribution, and quality of water on Earth including the hydrologic cycles, water resources and environmental watershed sustainability.

A man whose life was dedicated to explore, experiment and explain the nature of water passed away. Július Šútor was not a hydrologist only, but a scientist building strong relations within the international scientific community for a better understanding.

Július Šútor was born on the 6th October 1935 in Vel'ké Uherce, Czechoslovakia. He was educated at the secondary school of Prievidza where he completed his final exam in 1954. He continued his studies at the Faculty of Mathematics and Physics of the Komensky University, Bratislava. He graduated in 1959. Soon afterwards he started his scientific career at the Institute of Hydrology of the Slovak Academy of Sciences, his only workplace in his life. He started as a young research fellow, served at all levels of the scientific hierarchy. Between 1992 and 2004 until his retirement, he was the director of the institute. Actually he never ceased his research work. Until the last days of his life he was a key person in the field of hydrology, water management, soil science and land use.

His scientific activity was remarkable. His research has yielded internationally renowned results especially in the field of hydrology, water management and hydromechanics. One of his major works was the complex survey describing the hydrological conditions of Slovakia. He has developed a wide range of methodological innovations as well. His methods in relation with the determination of subsoil water properties and kinetics are in use up to now in many countries.

He defended his PhD (RNDr) dissertation in 1966, and he was given the DrSc title of the Slovak Academy of Sciences in 1982. He was the president of the Association of Hydrologists of Slovakia. He was an active member of the Alps-Adria Scientific Cooperation. His publication and dissemination activity was also remarkable. He was editorial board member of several scientific journals: Hydrology and Hydromechanics, International Agrophysics, and Acta Hydrologica Slovaca.

Things that belong to each other may merge – says an old proverb. Simply, if we, neighbours do not cooperate, do not help ourselves on a mutual basis no one in the world will do a favour to us. Common problems can only be solved by common efforts. We can learn a lot from highly educated wise scientists of the great and wealthy nations in the world. However they can never accomplish the work of ours. The biggest result of Július Šútor was that he could build and maintain bridges between scientists of various countries even in peculiar periods of history.

In the name of the scientific community, we would like to express our thanks to his work and results, his friendly personality, and at the same time we would like to say farewell. Professor Šútor, rest in peace!

Július Šútor and György Várallyay at the AASW Špičák, 2010.