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ENVIRONMENTAL CONCERNS AND POSSIBLE STRATEGIES TO REDUCE THE POTENTIAL RISKS OF PLANT MOLECULAR FARMING - REVIEW

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Abstract

The production of useful recombinant proteins was perceived since the existence of genetic modification technologies, in so doing, transgenic plants have been materialized. Transgenes flow remains as one of the global concern in the area of plant molecular farming that deteriorates the environment. This review aimed to evaluate the potential risks of plant molecular farming on the environment and their possible controlling strategies by assessing secondary data. Various sources of literatures have stated that the possible environmental risks of molecular farming rely on genetically engineered organisms are including but not limited to creating new or more vigours insect pests and pathogens; exacerbating the effects of existing pests via hybridization with related transgenic individuals; harm to non target species such as soil organisms, non- pest insects, birds and other animals; disruption of biotic communities including agroecosystems; irreversible loss or changes in species diversity or genetic diversity within species. These potential risks of molecular farming could harm the well-being of humans, animals and the environment at large. A number of different interdependent options such physical and biological approaches have been put in place to reduce food/feed chains contamination or environmental pollution due to PMF. Some the most important gene flow barriers are production of proteins by cell suspension culture, chloroplast transformation, cytoplasmic male-sterility, sexually incompatible crops, seed terminator, tissue specific expression technology, labs filters, and greenhouse/glasshouse and isolation distances. Some sources indicated that, the overall acceptability of molecular farming applications seemed less appreciable by the society. It is suggested that the potential environmental risks from the plant molecular farming can be reasonably minimized by controlling of the gene flow from the transgenic to conventional plants.

Keywords: Plant molecular faming, GM crops, Environmental concerns, Environmental risk reducing strategies

Összefoglalás

A hasznos rekombináns fehérjék előállítása a genetikai módosítási technológiák kifejlesztése és a transzgénikus növények megjelenése óta folyik. A transzgének áramlása azonban globális problémát jelent a növényi molekuláris gazdálkodásban, mivel rombolhatja a környezetet. Jelen tanulmány célja a növényi molekuláris gazdálkodás potenciális környezeti kockázatainak értékelése másodlagos adatok elemzésével, valamint javaslatot tesz a lehetséges szabályozási stratégiákra is. Irodalmi források szerint a genetikailag módosított szervezeteket használó molekuláris gazdálkodás környezeti kockázatai – nem kizárólag – új vagy erősebb kártétellel rendelkező rovarkártevők és kórokozók megjelenését; a meglévő kártevők kártételeinek súlyosbodását; a nem célzott fajok károsodását; a biotikus közösségek, köztük az agroökoszisztémák megzavarását; a fajok sokféleségének vagy a fajokon belüli genetikai sokféleségnek visszafordíthatatlan változását is előidézhetik. A molekuláris gazdálkodás ezen lehetséges kockázatai veszélyt jelenthetnek az emberek, az állatok és a környezet egészére nézve egyaránt. Fizikai és biológiai elhatárolási megközelítéseket vezettek be az élelmiszer/takarmányláncokban a növényi molekuláris gazdálkodás miatt megjelenő szennyeződések és a környezetszennyezés csökkentése miatt. A fehérjék termelése sejtszuszpenziós tenyésztéssel, kloroplaszt transzformációval, a citoplazmatikus hímsterilitás, a nemi szempontból inkompatibilis növényfajok használata, a terminátor és a szövetspecifikus expressziós technológiák alkalmazása, a laboratóriumi szűrők, és az izolációs távolságok megtartása jelentik a génáramlás megakadályozásának legfontosabb eszközeit. A tanulmány rámutat arra, hogy a molekuláris gazdálkodás alkalmazásainak általános társadalmi elfogadhatósága kevésbé érezhető. Lehetséges megoldás, hogy a molekuláris gazdálkodás környezetre gyakorolt negatív hatásait főként a génáramlás – GM növényből a nem génmódosított növényekbe történő mozgásának – ellenőrzésével lehetne minimalizálni.

Kulcsszavak: növényi “molekuláris gazdálkodás”, GM növények, környezeti aggályok, környezeti kockázatokat csökkentő stratégiák

Introduction

Since gathering ear till today plants have been a potential source of medicinal drugs. In line with (Grifo et al., 1997) report nearly 57% of the well-studied drugs had exhibited a minimum of a single main active ingredient initially purified from plant source. Winslow & Kroll (1998) had reported also that about one fourth of medicines usage was sourced from plant origin. Plant molecular farming uses either whole organisms, various plant parts or cultured cells as bioreactor, and that encompasses genetic modification of agricultural products for the production of commercially valuable and pharmaceutical oriented proteins and chemicals on large scale and at low costs (as reviewed by Tarinejab & Rahimi, 2015). It is also believed that this plant based technology can potentially solve the current demand for the biomedicine (Ahmad, 2014). In this regard, scholars could select suitable host plants to be used in the plant molecular farming biotechnological program. As a result, numerous host plants' selection criteria had been studied eventually the most informative and profitable ones are identified such as total biomass yield, ease of transport, storage attributes, and value of recombinant proteins, life cycle, required area, maintenance costs, labor availability, edibility, and cost of the final product (Fischer et al., 2004; Schillberg et al., 2005). To this end, some of the lists of crop that have been manipulating in molecular farming are tobacco, canola, potato, safflower, alfalfa, lettuce, soybean, rice and maize. Among these crops rice and maize has been recognized as the most suitable and the later had exhibited the highest biomass yield from the domain of food crops with soften transformation and maximum final product production (Ramessar et al., 2008). Generally plant based pharmaceuticals are found to be safer, storable, less costly and produced in bulk (Ahmad, 2014). Despite its importance and remarkable insights there exist two main classes of risks of molecular farming. One affects human beings and other harms the environment, and other organisms; it was evident that grain crops found to be the most suitable for this technology, but it is full of controversy the grain transformation using agrobacterium in the production of pharmaceutical proteins; the immune system can be incapacitated the medicines produced in plants and rather be the initiator for allergic reactions (Hout, 2003). Similarly, (Tarinejab & Rahimi, 2015) had reported that the use of transgenes could impose a higher degree of replication and transmission of genes, toxic recombinant proteins deposition in the ecosystem which in turn led to food web contamination and increased costs of remedies. This review paper briefly assessed some progresses of molecular farming with a central focus on environmental associated risks and the complementary strategies to reduce the possible negative impacts of the molecular farming in the ecosystem and human health aspects.

Table 1. Acceptability of plant molecular farming (PMF) applications as revealed by a case study in Canada, Alberta, University of Calgary in 2005.

Applications	**Fully acceptable**	**More acceptable**	**Less acceptable**	**Unacceptable**
Interleukin in tobacco	8	25	13	2
Edible vaccines (Norwalk in potatoes)	10	25	11	2
Gastric lipase in corn for cystic fibrosis	6	26	15	1
Trypsin grown in corn for industrial uses	1	14	21	11
Bioplastics grown from corn	6	21	14	6
Overall impression of PMF	3	29	10	6

(adapted from Einsiedel and Meldock, 2005)

Potential risks of plant molecular farming in the environment

Both the potential benefits of plant molecular faming and it's possible influence reaches to human biengs, animals and the extended environment too; and the target of assessment for human, animals and environmental safety issues become a priority due to their exposure to the plant molecular faming products (Breyer et al., 2009). A case study shown that the overall acceptability of plant molecular farming application was found very low impressive to the end users (Table 1). It is evident that the active ingredients of the pharam plants could enter to the water bodies and even eaten by animals which eventually affect these entities, and this could result desensitization of the vaccine so that it would stop its functionality as well (internet1).

Such types of environmental concern issues due to plant molecular faming had manifested in the year 2002 in United States of America and recorded as first public incident. In the same year, transgenic maize was grown in the field of soybean to harvest trypsin the pharmaceutical active ingredient followed by soya production, however, 13,500 tons of soybean produce was damped because it was found contaminated by the prior plantation. According to Fernandez et al. (2014) together with different regulatory agencies reached into consensus as any regulatory review should encompass environmental concerns such as weedy nature of the crop, out-crossing ability of the transgenic crops with their wild relatives or cultivated crop species and influence on non-target living things.

One of the most threating burning issues of plant based pharmaceuticals are poising of the food chain. So far, studies had revealed that crossing of conventional genetic materials with transgenic pollen sources be it by using the same harvesting equipment, process without precise decontamination, growing crops adjust to transgenic crops or ignoring of the soil from proper decontamination ahead of non-transgenic cropping practiced (Rigano and Walmsley, 2005). To this end, the ultimate seed bank could be even distorted as a resultant of contaminated non-genetically engineered crops and weeds (Mallory-Smith & Sanchez Olguin, 2011). In a similar fashion and even more intense herbicide resistance genes could transfer from crops to weeds and posed difficulty to control these weeds (Gressel, 2015). According to (Breyer et al., 2012) ingestion of the recombinant proteins and/or the transgenic plant itself could cause a potential skin or eye problem and allergy primarily in children. The problems associated with plant molecular farming are not imagery, rather it could be demonstrated by these two examples- the case of ProdiGene and StarLink concerns (Murphy, 2007). Originally, ProdiGene is a vaccine used to prevent bacteria-induced diarrhea in pigs produced from a transgenic corn, though it was non-toxic to human but it was strictly advised not to be a part of the human food chain

(Hileman, 2003). Similarly, millions of tons of non-transgenic corn was contaminated by the StarLink transgenic across the United States. The cost of recollection and dumping of the contaminated corn by Aventis was estimated $ 500 million (Murphy, 2007). It is not likely to be true, however, a gene could follow from transgenic crop to noxious weed then after this weed to another non-genetically engineered crops. Along this line, these contaminated weeds could harbor that transgene permitting expansion to non-engineered crops. The most important environmental concerns about the use of GM crops for various purposes are: increased use of toxic pesticides, unforeseen consequences (Pleiotropy) and genetic contamination (internet2). Moreover, the possible risks of genetically engineered organisms to the environment including but not limited to creating new or more vigours insect pests and pathogens; exacebating the effects of existing pests via hybridization with related transgenic individuals; harm to non target species such as soil organisms, non- pest insects, birds and other animals; disruption of biotic communities including agroecosystems; irreversible loss or changes in species diversity or genetic diversity within species (as reviewed by Snow et al. 2005). As a worse case scenario, mutation and extinction of species may become a dominant event and cause abnormalities within the large biological entities (Godheja, 2013). However, they are some still argue that, as tittle is known about the drawbacks of plant molecular farming to the environment, and human health since the technology is relatively new, and most of the research works are strictly laboratory based with a few filed trials (Hout, 2003). These interrelated plant molecular farming concerns need due attention starting from their production technology selection up to proper usage to make them user and eco-friendly so as to ensure sustainability.

Strategies to minimize the potential risks of plant molecular farming

So far, three dominant entries of transgenes into the ecosystem have been identified. These ways of spread are volunteer plants (Michael et al., 2010), pre and during harvesting shattering of seeds and cross-pollination with the adjust crops (Gressel, 2015). The tradeoff of plant molecular farming hits the environment, human welfare and at large the economy, this calls the development of mitigation measures and implementing of strategic controlling means to the spread of transgenes (Clark & Maselko, 2020). These potential risks of blending and pollution of GM crops utilized in plant molecular faming associated with agriculturally vital crops could be minimized by using non-food/forage crops of PMF. In this respect, various strategies such as production of recombinant proteins by cell suspension culture in bioreactors, restrict physical agronomic confinement, post-harvest field monitoring and sanitation, use of late maturing or early maturing cultivars at the different time period to ensure harvesting before or after other crops intended for food /feed and processing are among the frequently used ones (Obembe et al., 2011; Spok et al., 2008). Moreover, contaminating gene flow can be blocked by implementing various facilities like greenhouses, glasshouses, hydroponics; and biological advancements such as chloroplast transformation, cytoplasmic male-sterile transgenic plants, creating of sexually incompatible crops, seed terminator, parthenocarpy and tissue specific expression technology (Valkova, 2013; Salehi, 2012). To harvest the maximum benefit of plant molecular farming without or with minimum environmental drawbacks, it is highly recommended synthesizing scientific and regulatory risks assessment, and management strategies and standards too (Jouzani & Tohidfar, 2013).

Regulatory frameworks

There exists regulatory frameworks and guidance to plant molecular farming, and here the case of the United States and the European Union is briefed as below. The Coordinated Framework for Regulation of Biotechnology has come to existence for the first time in 1986. The agency called Animal and Plant Health Inspection Service has been responsible for regulating the plant molecular farming (PMF) production process while the Food and Drug Administration (FDA) targets the end products safety and pharmacological aspects. For example the use and cultivation of GM crops outside the delineated and predetermined growing sites need an authorization (internet 3) In the European Union GMO regulatory frameworks have been formulated. The Directive 2001/18/EC has been in account for regulating the boundless activities either for experimental or commercial conscious release of GM crops (EC, 2001). This Directive 2009/41EC has also allowed the limited use of GM micro-organisms considering their likely harmful outcome for human health and the environment with due emphasis to their accident preventive and control of wastes (EC, 2009). Recently some amendment was made by the Directive 2015/42 EU, and it stated that member states could cultivate GM crops by employing suitable measures to get rid of possible cross-border contamination into neighboring member states where cultivation of GM crops is prohibited (EU, 2015). From these directives and regulatory frameworks one can understand that GM based plant molecular farming technologies remain as one of the potential concern to the ecosystem.

Physical and biological transgene flow mitigation approaches

Gene flow is a natural process in which plant populations exchange genes due to the crossing of gametes at varying frequencies (Cerdeira & Duke, 2006). This happens within the closely related and rarely between species. Following this path, some persuading confirmations of transgene flow has been realized for example in cotton, maize and soybean (Baltazar et al., 2015; Dong et al. , 2015; Londo et al., 2011). Nearly all transgenes have been gotten away into their partner and wild relatives. In spite of the fact that gene flow changes between species, crops and environmental zones/environments but intraspecific gene flow (> 10%) is not an exceptional in adjoining populations. While in outcrossing species, 1% gene flow at thousand meters' confinement is not unordinary, and size is indeed higher than the mutation rate (Rizwan et al., 2019). Therefore, this global concern needs sound mitigation approaches besides to regulatory frameworks and appropriate production of molecular farming, there are a number of different interdependent alternatives grouped as a physical and biological gene flow mitigation approaches that can reduce food/feed chains contamination or environmental pollution due to PMF.

Table 2. Some selected compatible strategies for minimizing the potential risks of PMF to the environment.

Types of approaches	Specific Cases	Purpose-Examples	References
Physical containment	Plastic tunnels and greenhouse	Production of biopharmaceutical alfalfa for therapeutic proteins	(Zayon & Flinn, 2003)
	Delineated land	To eliminate the risk of gene flow to non-farming plants and wild relatives	(Howard & Hood , 2007)
	Isolation distance	Minimum gene contamination-via gene flow	(Linder et al., 1998)
	Non-transgenic trap plants	Reduced gene contamination-due gene flow	
Biological blockage	Plastid transformation	The production of vaccine antigens and pharmaceutical	(Daniell, 2006)

Greenhouse/glasshouse meshes, filters in the laboratories and isolation distances in the field serve as physical barriers. It seems less likely to record non-success story of physical containment in the lab or greenhouse, however, which is not the case in the field. In line with (Fox, 2003) report traces of transgenes from previously cultivated ProdiGene harboring maize were found on small magnitude of maize leaf trash adhering to the following crop. Biologically, uncontrolled hybridization can be reduced to the possible minimum tolerable rate by mismatching the relative flowering times of GM and non-GM crops. In this way, gene flow would be prevented whenever the anthers pollinate pistils before flowers open (Gruber & Husken, 2013). Some of the most powerful physical and biological strategies to reduce the potential risks of plant molecular farming to the environment are listed below (Table 2).

Conclusion and future prospects

Historically, plants have been a potential source of medicinal drugs. Plant molecular farming uses either whole organisms, various plant parts or cultured cells as bioreactors, and produce pharmaceuticals at large scale and low costs. Despite the current technological developments and the potential merits of plant molecular farming for the betterment of mankind there are also uncertainties associated with it. As a matter of fact, genetically engineered crops based plant molecular farming is found to be capable of contaminating the environment, non-GM plants, wild relatives and even weeds this eventually led to food/feed chain contamination. It is also evident that the active ingredients of molecular farming products could enter to the water bodies, and even be eaten by animals which in turn affect these entities, and this could result desensitization of the vaccine so that it would stop its functionality. The impact of plant molecular farming in the environment, biodiversity richness, human health, and the economy could be kept down via controlling the gene flow of the transgenes. In doing so, it is suggested that regulatory frameworks shaped the appropriate production and utilization of molecular farming applications. To this end, there are also a number of different interdependent options assembled as a physical and biological approaches that can reduce food/feed chain contamination and environmental pollution due to PMF. Specifically, gene flow can be prevented by various physical and biological barriers. On one hand, plastic tunnels, greenhouses, delineated land, isolation distances and non-transgenic trap plants serve as physical barriers against gene flow. On the other hand, plastid transformation, cytoplasmic-male sterility, seed terminator technology, transient expression, cell-suspension culture, and creating sexually incompatibility crops and some others have been practicing to reduce the potential risks of plant molecular farming to the environment at large. The sector still remains challenging and suspicious. It is therefore attention is needed in implementing all possible advancements in the field enabling reduction of gene flow into the agricultural production systems, and the environment at large. Equally, unbiased risk assessment to evaluate the merits and demerits of new traits to the environment will remain instrumental to the efficient application of plant molecular farming.

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internet 3 http://usbiotechreg.nbii.gov

A DNA BASED METHOD TO DETERMINE THE ORIGIN OF HONEY PRODUCED BY *APIS CERANA*

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Abstract

The value of a product is derived from various components, such as its functionality, availability on the market, origin, raw material, exclusivity; in case of food products not least taste and health effects. In this approach honey has an outstanding position because of its reputation in the food and health industry. The honey of the Asian honey bee is unique and of great value, and because of this it is often prone to adulteration. Asian honey is restricted to those countries where the Asian honey bee is endemic. Beekeeping with this species of bee in these areas is part of cultural tradition. The idea of this work is to design a fast and accurate detection method for adulteration, in the interest of preserving the value of the honey produced by the Asian honey bee (*Apis cerana*). The purpose of the study was to point out the genetic differences between two close honey bee species (*A. cerana* and *A. mellifera*) and to use mitochondrial loci to develop a molecular method to confirm the entomological origin of honey deriving from a Cerana apiary. The markers designed besides being suitable per se can be adopted as a part of a genetic analysis panel.

Keywords: *Apis cerana*, ethnic honey, molecular marker, honey authenticity, adulteration

Összefoglalás

Egy termék értékének megállapításában számos tényező részt vesz. Ilyen paraméterek az adott produktum piaci felhasználhatósága, elérhetősége, eredete, alapanyagának minősége, exkluzivitása. További kritériumok merülnek fel élelmiszeripari termékek esetében, mint például az ízvilág és az egyre népszerűbb egészségre gyakorolt hatás. Ebből a megközelítésből a méz kiemelkedő helyet foglal el, mivel elismerten jelentős termék az élelmiszeripar és az egészségügy szempontjából egyaránt. Az ázsiai méh (*Apis cerana*) méze egyedülálló és különlegesen értékes, és emiatt gyakran van kitéve hamisításnak. Az ázsiai méh által termelt méz korlátozottan elérhető, leginkább csak azokban az országokban, ahol ez faj endemikus. Az ázsiai méh első sorban Kínában, Szibériában, Indiában és Japánban terjedt el. Ezeken a területeken a méhészkedés hozzátartozik a kulturális hagyományokhoz. Ezen munka középpontjában a keleti méh által termelt méz értékmegóvása áll, egy olyan módszer fejlesztése által, ami gyors és pontos módja a hamisítás kimutatásának. A tanulmány célja két közeli rokon méhfaj (*Apis cerana* és *Apis mellifera*) genetikai különbségeinek kimutatása és mitokondriális lókuszok segítségével olyan marker fejlesztése, aminek segítségével kimutatható a méz keleti

méhtől való származása. Mindamellett, hogy önmagukban is használhatóak a tervezett primer párok, beilleszthetőek már meglévő genetikai vizsgálati panelekbe is.

Kulcsszavak: *Apis cerana*, helyi méz, molekuláris marker, méz eredetiség, hamisítás

Introduction

Currently, food fraud is an increasing and spreading problem, causing economic loss, and nutritional drawbacks, and because of this, it may lead to health consequences. In a narrower interpretation, Economically Motivated Adulteration (EMA), is intentional fraud for a financial gain (Everstine et al., 2013). The most popular articles of food exposed for adulteration are olive oil, milk and honey (Moore et al., 2012). According to the Codex Alimentarius FAO (2001), honey is a sweet viscous fluid obtained from the nectar of flowers or from secretions of living parts or excretions of plant-sucking insects on the living parts of plants, and produced in the honey sac of bees. Being a valuable, high-quality food with many health benefits, honey is a product that is often subjected to adulteration, through mixing with cheaper honey, or sugars, or even mislabeling the product. Despite being unethical this can also be harmful to human health. The adding of different foreign matters, colorants, aroma may lead to hyperglycemia, causing in many cases type II diabetes, obesity, hypertension (Ajibola et al., 2013). Moreover the added alduterants of unknown origin can have harmful effects on organs (Shapiro et al., 2008; Soares et al., 2017) and may even cause death by increasing visceral fat and total body fat (Samat et al., 2017).

There are several methods for adulteration, which mainly focus on the chemical composition of the honey. Consisting mainly of carbohydrates and water (Bogdanov, 2009), the most obvious way of EMA is the mixing of different sugars in the honey, this way increasing the volume, but lowering the quality. The sugar profile of honey can be analyzed with Gas

Chromatography (GC) and Liquid Chromatography (LC); this way a doubtful origin can be detected (Ruiz-Matute et al., 2010). Another technique for evaluating honey adulteration is Near Infrared Transflectance Spectroscopy, showing beet invert and corn syrup residues in honey (Zhu et al., 2010). The Fourier transform infrared (FTIR) spectroscopy and attenuated total reflection (FTIR-ATR) approach is an effective method for detecting the botanical origin of the honey, making the classification possible based on melissopalynological data (Svečnjak et al., 2015). Another important characteristic of honey is its protein profile. The level of protein in honey highly depends on the type of flora and hence it is variable. Honey of different plant origin can be tracked by their pollen protein content, using it as a chemical marker. Silver staining SDS-PAGE is an analytical method for identifying and isolating protein molecules from pollens (Won et al., 2008).

A current diagnostic method used in honey analysis for food control is DNA based techniques. PCR based methods use exponential amplification of target-specific DNA and a signal can be detected only if the target DNA is present. In the case of honey analysis, this applies to the genetic material of the honey bee and floral DNA as well (Laube et al., 2010). HRM (High-Resolution Melting) is adequate in the case of honey samples because it allows genotyping and serotyping of pathogenic microorganisms and detection of food allergens (Druml and Cichna-Markl, 2014).

For identifying the origin of the honey not only its botanical origin has to be examined, since entomological authentication can provide valuable information about the product (Kek et al., 2017; Soares et al., 2017; Utzeri et al., 2018). Recently, a real-time PCR method using the tRNAleu-cox2 intergenic region was designed by Soares et al. (2018) to distinguish between species *A. cerana* and *A. mellifera*.

The Asian honey bee produces a significant amount of honey too Hisashi (2010). In comparison to the European honey bees the collected amount is lesser, but its characteristics justify its importance on the honey market (Partap and Verma, 1998; Verma, 1990). According to the study of Won et al. (2009) the price of the honey produced by the indigenous honey bees in Asia can be up to five times higher than the one produced by *A. mellifera.* However, from the beginning of the 20th century, the western honey bee started to spread on the continent, thanks to beekeepers who imported the species in the hope of a greater profit (Partap and Verma, 1998). Because of this, the western honey bee poses a threat to the endemic species, causing the decline of *A. cerana* colonies, potentially leading to extinction in its native environment (He et al., 2013; Partap and Verma, 1998; Theisen-Jones and Bienefeld, 2016). Although the European honey bee shows a more effective foraging behavior, the Asian honey bee has beneficial traits that make them valuable for beekeepers in the East. The imported species *A. mellifera* integrated itself instantaneously in the new environment, especially the Himalayan region, but fortunately beekeepers in Nepal and Bhutan still prefer to opt for the endemic species, *A. cerana* being suitable for beekeeping in the mountains (Partap and Verma, 1998).

Moreover, due to its natural *Varroa* resistance, *A. cerana* requires no treatment with acaricides, meaning that the honey will not be exposed to miticide related chemical residues. *A. cerana* honey is sometimes considered to be of superior quality compared to that of *A. mellifera*, especially in parts of China and India, with increasing demand (Abrol, 2013; Hu, 2015 and Puttaraju, 2015, personal communication). *A. cerana* colonies can also be reproduced and scaled up with very little additional input: strong colonies can be divided easily (Abrol, 2013). Because of the higher quality and price, honey produced by Eastern honey bees is potentially exposed to EMA on a global scale. Our aim was to preserve the value of Cerana honey, by developing a fast and cost-effective molecular, PCR-based detection method, which can be a useful tool against food fraud, causing serious economic damage.

Material and method

Sample collection

Honey from two distant points of the distribution area of the Asian honey bee was used, collected in Japan (apiary at Tamagawa University, Japan) and India (organic apiary Uttarkhand, India). Japanese honey and honey bee were collected in a private apiary while the certified organic honey produced by the Himalayan subspecies of *A. cerana* derived from an apiary in India. For negative control honey of western honey bee was used (Hungary, private apiary). We used honey bee individuals of each species as the positive control.

DNA extraction

Extraction of DNA was preceded by dissection of the honey bees. *A. mellifera* and *A. cerana* bee individuals were stored frozen at -70°C right after sampling respectively preserved in RNA later. For DNA extraction, we used muscle tissue from hind legs bees using QIAGEN DNeasy Tissue Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's protocol. Honey samples needed preprocessing before extracting total DNA. Therefore 5 grams of honey has been measured from each sample. Tubes containing the honey were filled with 45 ml distilled water and put in 40°C water bath for 30 minutes with constant shaking. Subsequently, we centrifuged the tubes at 4,000 rpm for 30 minutes. Supernatant was discarded and the pellet was used for the DNA extraction, using the kit above, according to the manufacturer's instructions. Yield and purity of the extracts were determined by spectrophotometry (260/280 nm ratio and absorbance spectrum) using NanoDrop™ 2000/2000c Spectrophotometer (Thermo Scientific™, Waltham, MA, USA).

Primer design and PCR amplification

To detect the genetic differences between *A. cerana* and other Apis species primers were designed to target a part of the *cytb* region of two honey bee species. The sequences used were downloaded from GenBank and are shown in an additional table (*Table 1.*).

Table 1. Accession numbers of the A. mellifera and A. cerana honey bee sequences used for primer design.

Apis mellifera	*Apis cerana*
EF184057.1	FJ229480.1
EF184058.1	FJ229478.1
GU979492.1	FJ229476.1
EF184041.1	FJ229475.1
EF184043.1	FJ229473.1
EF184042.1	FJ229471.1
JQ778297.1	EF467437.1
JQ778298.1	EF180095.1
JQ778299.1	F180094.1
JQ778301.1	F180093.1
JQ778300.1	EF180092.1
JQ778302.1	EF180091.1
JQ778303.1	EF180090.1
EF184046.1	
EF184047.1	
GU979494.1	

The *cytb* sequences were aligned and compared using BioEditv. software, version 7.0.4. (Hall, 1999) to detect species-level differences. Primers were designed using the Primer3web version 4.1.0. primer designer website. Two *Apis cerana* species-specific primer pairs (Acer_short forward 5’-TGAGGTGCAACAGTAATTACAAATTTAC-3’, Acer_short reverse 5’-ACTTCACTTTATTTTACCTTTAGTAATT-3’; Acer_long forward 5’-TGAGGTGCAACAGTAATTACAAATTTAC-3’, Acer_long reverse 5’-

ATAATTAATTTTCAATATCCTTATTATTT-3’) were meant to result in different fragment lengths. The primer pair indicated as ’short’ resulted in a 143 bp amplicon, whilst the ’long’ one generated a 300 bp product.

Reactions were performed in Rotor-Gene Q 5Plex HRM (Qiagen, Germany) appliance, using the following cycling profile: initial denaturation at 95 ºC for 3 min; 40 cycles at 95 ºC for 30 s, 55ºC for 60 s, 72 ºC for 60 s and 72ºC for 10 min for final extension. The PCR-mixture (Thermo Scientific™, Waltham, MA, USA) contained5 µl 10X DreamTaq Green Buffer, 1 µl of forward and reverse primers, 5 µl dNTP Mix, 1 µl DreamTaq DNA Polymerase, 36 µl nuclease-free water, 2 µl DNA template in a total volume of 50 µl.

The amplified fragments were visualized in a 1% agarose gel containing Ethidium bromide 1x (Merck, Germany) for staining and carried out in TBE 1x (Duchefa, Netherlands) Agarose Electrophoresis Buffer. The fragments were chemically purified using the QIAquick GelExtraction Kit (Qiagen, Germany) according to the protocol of the manufacturer and 10 µl was loaded into the wells of the gel per each sample. The results were confirmed by Sanger sequencing on 3130 Genetic Analyzer (Thermo Fisher Scientific, USA) using the same primers listed above.

Results

DNA extraction

The honey samples were of different floral origin, thus containing large amount of plant DNA. Consequently, we had to extract a greater amount of DNA, to ensure it includes residues from the honey bee. The quantitation of the extracts resulted in yield between 11 and 128 ng/µl with a purity ranging from 1.9 to 2.2, which was proved to be adequate.

Primer design and PCR amplification

Using the primer pairs designed, the expected mitochondrial region of *cytb* gene was successfully amplified in the *A. cerana* bee and honey sample as well. The primer pair Acer_short resulted in an amplicon of the size of 143 bp, while Acer_long primers exhibit a band on the gel of the size of 300 bp. This fragment seemed unreliable for detection, as the fragment is barely visible on the gel electrophoresis image, thus we did not use it in further reactions. In case of the *A. mellifera* bee and honey samples, which were used as negative controls, no amplification was detected (*Fig. 1*).

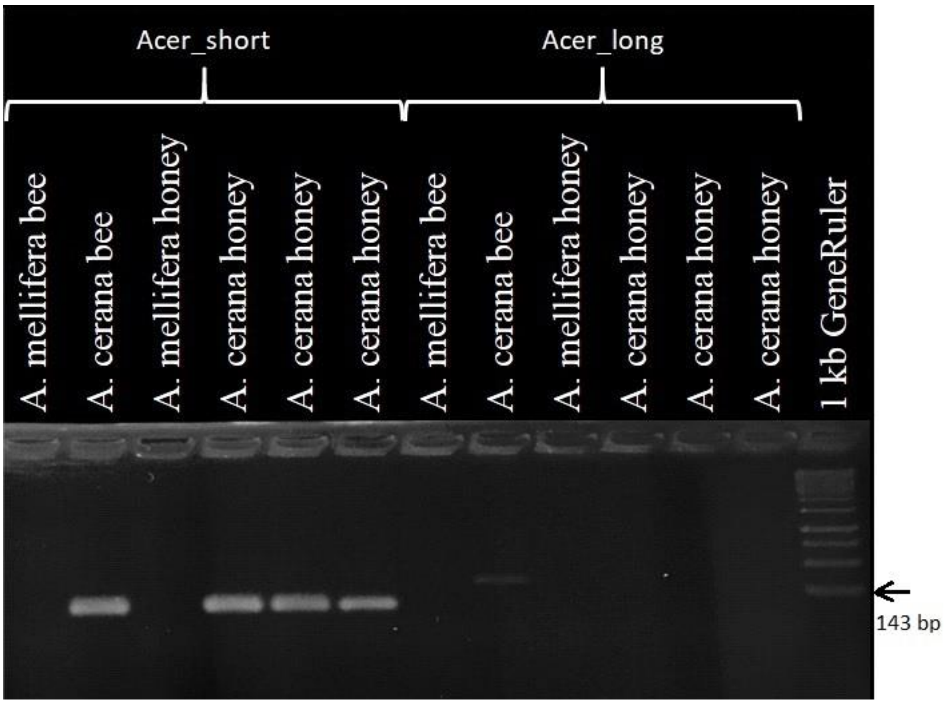

Figure 1 The gelelectrophoresis image of the fragment samplified with the primer pairs designed.

Discussion

The amount of the honey bee's DNA in honey depends mostly on beekeeping and honey extraction technology. Technology applied for western honey bee uses honey extractors leaving the honeycomb undestroyed, thus being recyclable. In case of the eastern honey bee, honeycomb is removed from the hive and honey can be extracted by crushing and straining (Reuber, 2015). This implies that honey of *A. cerana* may contain an increased amount of DNA coming from larvae and pupae tissue.

Soares et al. (2018) used the $tRNA^{leu}$-cox2 intergenic region to differentiate between the two honey bee species concerned. A recent study focused on the entomological differences between Apis species as well, using qualitative end-point PCR using EPIC markers coupled with specific fragment restriction (Moškrič et al., 2020) In addition to this, our group had focused on *cytb* mitochondrial gene. A completion to the previous study a confirmatory application was designed to a possible further profiling panel. Meta-barcoding of mixed pollen samples has revealed that multi-locus approach is more reliable than single-locus analyses (Sickel et al., 2015). Our primers resulting the short amplicon proved to be accurate, it amplified the target region, and we did not detect any non-specific fragments in honey produced by *A. mellifera* honey bees. The long amplicon cannot be used as a confirmatory marker, we can assume, that DNA in honey is mostly fragmented and the chance of longer fragments deriving from honey bees remaining in the samples is low, DNA of bee origin representing the lesser portion of the nucleic acid content of the honey DNA. Despite resulting in a fragment in case of the bee samples, long amplicon primers yielded in variable amount and inconsequently in honey.

Conclusion

The development of a simple method for the detection of *Apis cerana* honey in mixed honey samples was successful. This novel DNA marker may be used in a multiplex PCR system with previous primers designed for the same reason but targeting other loci. It allows confirming the entomological origin of the honey to protect this unique product. PCR amplification being exponential even for samples of small and degraded DNA content as DNA based methods can be highly sensitive.

In practice the method provides quick and reliable information about Asian honey bee derived honey exposed to fraudulent procedures, this way it may prevent beekeepers from economic damage.

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GREENHOUSE GAS EMISSIONS IN THE COUNTRIES OF THE VISEGRAD GROUP: AN ANALYSIS OF SUSTAINABLE AGRICULTURE AND ENVIRONMENTAL MANAGEMENT

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Abstract

Our study analyses the greenhouse gas emission intensity of the main agricultural products in the Visegrad Four. The time interval under consideration is 2000-2017. According to the statistical calculation, the GHG emission intensity of the production of the Visegrad Group's agricultural products was similar for most of the products that we examined. In the case of lamb production and egg production, we found significant differences between the countries emission data. All countries except the Czech Republic have reduced their emissions in respect of the production of cereal products, with the same variability. We have observed a steady decline in pork production emissions in all member states except Slovakia. All countries have reduced GHG emissions intensity in terms of emissions related to the production of cow's milk.

Differences between countries can be due to the differences in the market conditions and the different structures of agricultural sectors of the four member states.

Keywords: Visegrad Four, agri-environment, emissions intensity

Összefoglalás

Tanulmányunkban számba vettük a főbb mezőgazdasági termékek üvegházhatású gáz kibocsátási intenzitását a Visegrádi Négyek tekintetében. A vizsgált időintervallum 2000-2017 közötti időszak. Statisztikai számítások elvégzését követően elmondható, hogy az országok mezőgazdasági termékeik előállításának ÜHG kibocsátás intenzitása legtöbb vizsgált termék esetében hasonlóképpen alakult. Bárányhús előállítás, valamint a tojás termelés esetében tapasztaltunk jelentősebb eltéréséket az országok kibocsátási adatai között. Gabona termékek előállítása tekintetében azonos hullámvonalak leírása mellett - Csehország kivételével- mindegyik ország csökkentette kibocsátását. Sertéshús előállításának kibocsátása tekintetében folyamatos csökkenést figyelhettünk meg - Szlovákia kivételével- minden tagország esetében. Tehéntej előállításával kapcsolatos kibocsátások tekintetében pedig kivétel nélkül mindegyik ország csökkentette ÜHG kibocsátás intenzitásának mértékét. Az országok közötti különbségekért a piaci viszonyok eltérő alakulása és a mezőgazdaság eltérő felépülése is felelős.

Kulcsszavak: Visegrádi Négyek, agrár-környezet, kibocsátás intenzitás

Introduction

Agriculture is a major emitter of gases responsible for climate change. It is important to examine the evolution of emissions in details whereas agriculture is a major cause and a victim of climate change. We examined the extent of the emissions of the gases responsible for climate change in the framework of previous studies. We placed special emphasis on the analysis of emissions from agriculture, we dealt with the examination of the proportion of GHG emissions by agriculture per produced product. We tried to explore the correlations between macroeconomic indicators and environmental indicators in light of climate change. Being an area defined by environmental policy, we also paid attention to the policy indicator throughout the preparation of the study. The research seeks answers to the following questions: Which product is produced in the most GHG- intensive sector? How did the emissions values develop in the case of the members of the Visegrad Group? What differences and similarities can be observed?

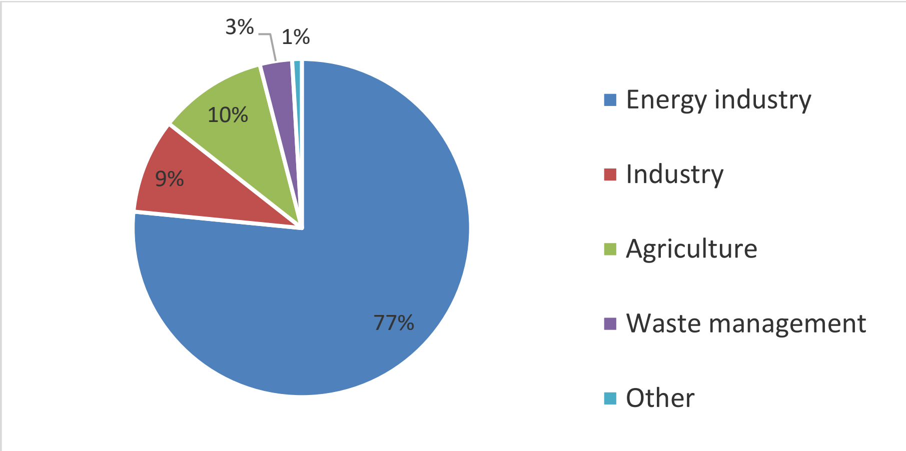

Figure 1 GHG emissions of V4s by sectors in 2018 (Source: EUROSTAT,2020)

Agriculture accounts for ~ 10% of GHG emissions (Figure 1). Agriculture GHG emissions in all member states showed a steady slow increase from 2000 to 2018, while emissions from the economy, on the whole, decreased over this period. Based on this, the role of agriculture in reducing emissions cannot be neglected.

Agriculture accounts for a 10% share of all GHG emissions. Of the greenhouse gases, the primary sector is responsible for a significant, about 80-90% of ammonia emissions. The largest emitters are livestock farming and manure management (Pogány, 2011). In addition, the activities of these sectors are responsible for a significant part of N_2O emissions. More than that, a large part of methane emissions is due to the production of livestock sectors. By correct management and efficient disposal of emission by-products, GHG emissions can be reduced (Sárváry, 2011; Szaktudás Kiadó Ház Zrt, 2008; Hongdou et al., 2018).

An assessment of the environmental impacts of livestock production was carried out by Williams et al., in 2006. In the course of the analysis products with a detrimental effect on the environment of the entire population of rotating farm animals were taken into account. Inputs and outputs were defined for units of animal products. In their study, they listed more important environmental burden values than GHG production at CO_2 equivalent (Balogh, 2021).

In addition to livestock production, the role of crop production is also paramount, as plants also absorb and emit CO_2 during their lifetime. As well as soil being one of the largest carbon reservoirs, its proper use is essential for emission reductions (Sárváry, 2011).

The climatic effect of crop production is two-way. Due to photosynthesis, it allows the sequestration of CO_2, however, CO_2 emissions from plant respiration also occur. Furthermore, fertilizers used by crop production increase the NO_x content of the atmosphere. In addition, it indirectly contributes to air dust pollution through the use of crop-growing machines (Taylor & Entwistle, 2015) CO_2 emissions from crop production are mainly due to emissions from agricultural machinery. Nitrogen fertilizers, animal and green manure are responsible for NO_x emissions (Szabó, 2010; Foley et al., 2011).

Increasing the sustainability of agriculture and combating climate change are some of the main objectives of the CAP 2020 reform. The European Green Deal is all intended to promote safer and more climate-friendly food production through the Farm to Fork Strategy and the Biodiversity Strategy. The CAP will develop its support systems for the post-2020 period, with a focus on these. The European Commission proposes that member states allocate at least 40%

of the funding to help to green during the organization of funding for the next 2021-2030 period (European Commission, 2020).

Materials and methods

The data is accessed from FAOSTAT's database (FAOSTAT METADATA, 2021). It was carried out an examination of the GHG emission intensity of the goods included in the agri-environmental indicators. This indicator shows greenhouse gas emissions per unit of product. Data were available for a variety of agricultural products. The time interval examined was 2000-2017. The indicator is calculated and published based on FAOSTAT's data. It is defined as the quotient of production and output data. In addition to taking into account external and internal factors, it is important to note that the indicator only includes data that are produced within farms. The objective of environmental indicators is to facilitate national and regional agri-environmental trend analysis and to provide member states with reference information. In our study, we tested the products we considered most important. Our data were organized in a Microsoft Excel database manager and evaluated using statistical calculations (CV, percentage change determination, average). Furthermore, we compared the intensity of the countries' CO_2 equivalent calculated GHG emissions.

Results

We primarily examined GHG intensity emissions from crop production. The highest values for the development of emissions per kilogram of cereals were found for Poland at 0,3265 kg CO_2 per kg of cereals in 2006. While Hungary observed the lowest value for the minimum value of 0.1316 kg CO_2 eq/kg of cereals in 2005. Based on the analysis of the country averages, we calculated the highest average CO_2 intensity for Poland with 0.2598 kg CO_2 eq/kg of cereals, followed by the Czech Republic with 0.2354 kg CO_2 eg/kg of cereals. Slovakia had an average of 0.2059 kg CO_2 eq/ kg of cereals in the time period studied, while Hungary had only 0.16695 kg CO_2 eq/kg of cereals.

The Czech Republic had the highest variability with a CV of 15.36%, followed closely by Hungary with 14.56%. Slovakia is the third with 13.04% and Poland is the fourth with 9.88%. The differences between countries are not considered significant, but the data are very volatile. In addition to variability, the Czech Republic was the only country to see an increase of +12% from 2000 to 2017. The observed increase was caused by a 115% increase in cereals and an increase in 130% emissions. By contrast Hungary (-11.11%), Poland (-5.2%) Slovakia (-7.8%)was declined. Hungary, the decrease in GHG emissions intensity is due to a 139% increase in GHG emissions with an increase of 124%. For Poland, a 129% increase in emissions was accompanied by a 136% increase in crops and a 145% increase in emissions in Slovakia, coupled with a 158% increase in crops.

During the period under review, the values of the countries showed almost the same fluctuations. In the case of this product, it can therefore be concluded that the cereal production potential of the countries has increased, but the implementation of the related cultivation and management tasks has become greener.

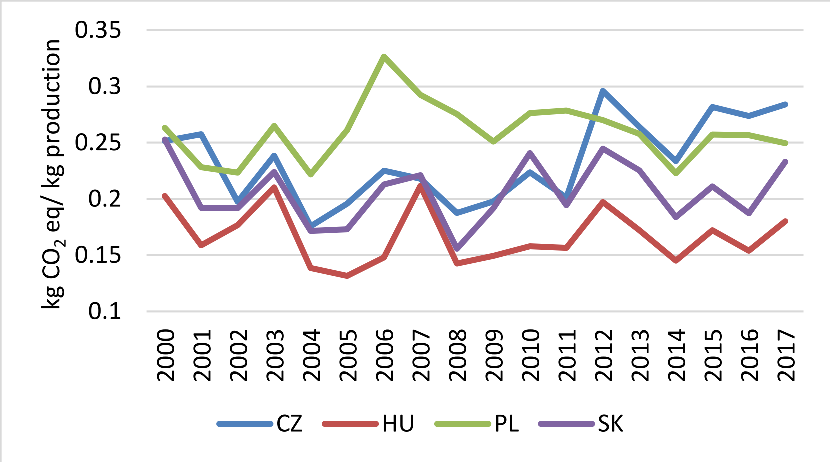

Figure 2 The intensity of GHG emission associated with cereal production without rice, kg CO_2 eq/ kg production. Source: (FAOSTAT. DATA, 2019)

We find a larger difference in the intensity of the GHG result per kilo of eggs, the Czech Republic lags far behind the trend of other member states. It produced lower values than the other member states and showed a steady decline with a minimum of 0.7073 kg CO_2 equivalent/kg of eggs in 2010. In contrast, for Slovakia, the highest emission intensity was measured at a maximum value of 1,465 kg CO_2 eq/kg of eggsin 2002. In terms of averages, Poland also has the highest value for this product at 1.1176 kg of CO_2 eq/ kg of eggs. Hungary average emissions intensity for egg production was 1.1062 kg CO_2 eq /kg of eggs, for Slovakia this value was 1.0955 kg CO_2 eq /kg of eggs, while for the Czech Republic only 0.7592 kg CO_2 eq/ kg of eggs.

The lowest variability is in Hungary with a CV of 5.33%, and the Czech Republic the second lowest with 5.73%. For Poland and Slovakia, we received higher CV results of 9.07% and 10.17%. In addition to variability, we observed a decrease from 2000 to 2017 in all countries except Hungary (+0.93%). Hungary's low growth was achieved while output decreased by -23.9% from 2000 to 2017 and product production by -23.96%. The Czech Republic reduced CO_2 eq emissions per kilo of eggs by -15.3%, Poland by -17.60% and Slovakia by -12.94%. In the Czech Republic, the decrease was achieved by a -55% decrease in product production and a -61.9% decrease in emissions from 2000 to 2017. In contrast, for Poland, the decrease in GHG emissions intensity was achieved with an increase in emissions of 115.6% and an increase in product production of 140%. In the case of Slovakia, we also observed a decrease with increasing output (106%) and increasing product production (122%). Overall, the values of Hungary, Poland and Slovakia developed similarly, while the Czech Republic lags significantly behind them. The extremely low value of the Czech Republic is because egg production is carried out in a very extensive way than in other Visegrad countries, and the share of imports is highest in this country.

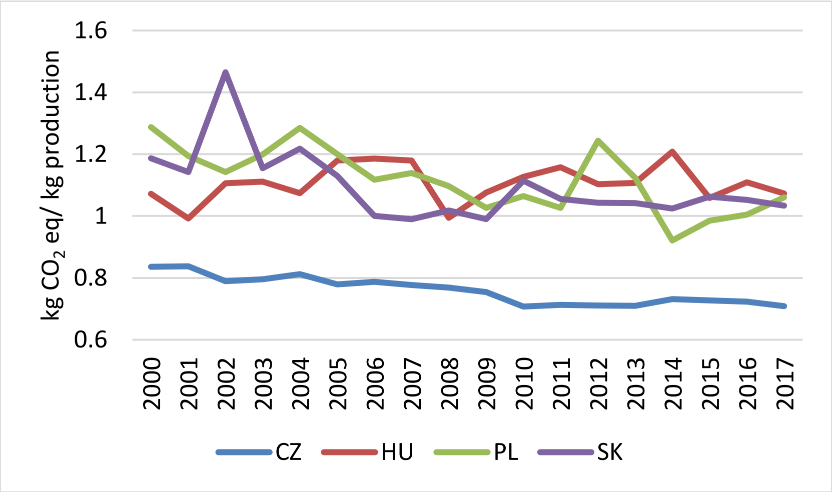

Figure 3 The intensity of GHG emissions associated with egg production, kg CO_2 eq/ kg production. Source: (FAOSTAT. DATA, 2019)

In terms of the intensity of GHG emissions per kilo of beef, the country group had similar values at the beginning of the period, followed by a significant increase in emissions per kg for Slovakia, Hungary and the Czech Republic. While in Poland we have observed a decrease. Slovakia, which grew the most, produced a maximum of 56.49 kg of CO2 eq/kg of beef by the end of the period (2016). While the lowest value was observed for Poland with a minimum value of 12.69 kg CO2 eg / kg beef in 2017. In terms of averages, Slovakia had the highest average emissions intensity over the time period of 34.7972 kg CO2 eq/ kg of beef. The second highest value was calculated for Hungary with a value of 25,798 kg CO_2 eq/ kg of beef. For the Czech Republic, the average for this product is 22.9635 kg CO_2 eq/ kg of beef, while for Poland it is only 14.99873 kg CO_2 eq/ kg of beef. While Poland, which is on a downward trend, has added a minimum of 12.69 kg of CO_2 eq/ kg of beef for 2017 by the end of the period. In terms of their variability, Poland has the lowest CV % of 8.05%, which is significantly different from other countries.

CV of Slovakia is 16.14%, CV of Hungary is 33.77%, and CV of Czech Republic is 38.99%. 2000 to 2017 Poland reduced GHG emissions per kilogram of beef production by -20.83%, while the Czech Republic increased emissions by +57.3%, Hungary by 200.4% and Slovakia by 244.6%. Poland achieved an emission reduction of 130% and product production by 164%. The Czech Republic growth was achieved by -1.4% and product production by -37.39%. In contrast Hungary, the increase was achieved by a 126% increase in emissions and a -57% decrease in product production. Slovakia showed a decrease in product production of -78% with an emission reduction of -24.2%. The decline in the production potential of countries is greatly influenced by the shortage of domestic demand. Thus, income is driven by the evolution of the world market price.

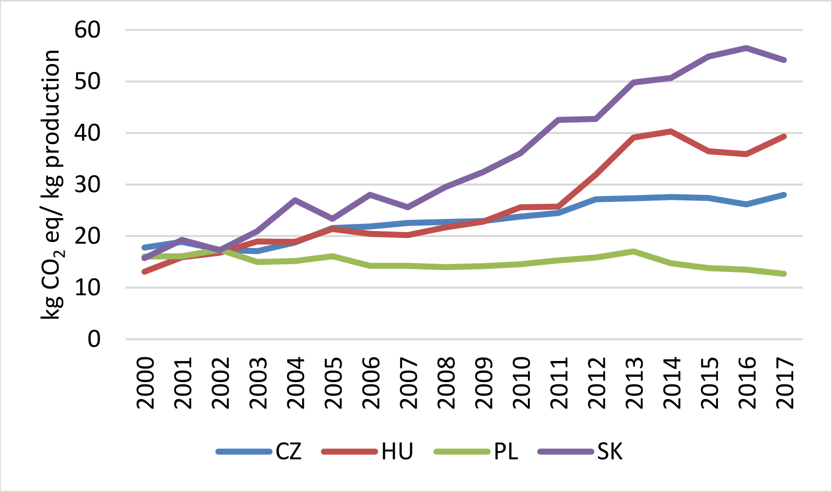

Figure 4 The intensity of GHG emissions associated with beef, kg CO_2 eq/ kg production. Source: (FAOSTAT. DATA, 2019)

As regards the production of chicken meat, Poland showed the highest emission intensity with a maximum of 0,6521 kg CO_2 eq/ kg of chicken meat in 2002. However, even the lowest value was among Poland's emission values with a minimum value of 0.033 kg CO_2 eq/kg chicken meat in 2000 . For the other member states, we have not seen such large differences. There was no significant differentiation between the averages of the countries.

The Czech Republic has an average emission intensity of 0.3848 kg CO_2 eq/ kg of chicken meat, 0.3493 kg CO_2 eq/ kg of chicken meat for Slovakia, 0.2750 kg CO_2 eq/ kg of chicken meat for Poland and 0.2679 kg CO_2 eq/ kg of chicken meat for Hungary. Observing the variability, it can be seen that Poland shows an outstanding deviation of 51.7%. While the Czech Republic was 11.54%, Hungary 18.06% and Slovakia 13.95% different from its average. Slovakia is the only country where we have observed a decrease from 2000 to 2017 by -16.65%. Slovakia's decrease was accompanied by a -5.4% decrease in GHG output and a 113.4% increase in product production. In the case of the Czech Republic (+18.8%), Hungary (+55.9%) and Poland (+594%), an increase was observed. The increase in the Czech Republic's emission intensity was due to a -7.5% decrease in emissions and a -22.22% decrease in product production.

In the case of Hungary, the increase was accompanied by a 189.6% increase in output and a 121.5% increase in product production. In the case of Poland, with an increase in output of 2609.6%, there was an increase in product production of 375.5% from 2000 to 2017. The larger jump observed in Poland and then the decrease in the intensity of continuous emissions is caused by the switch to GMO broiler chickens production. To this day, Poland is still one of the dominant producers of chicken meat in Europe.

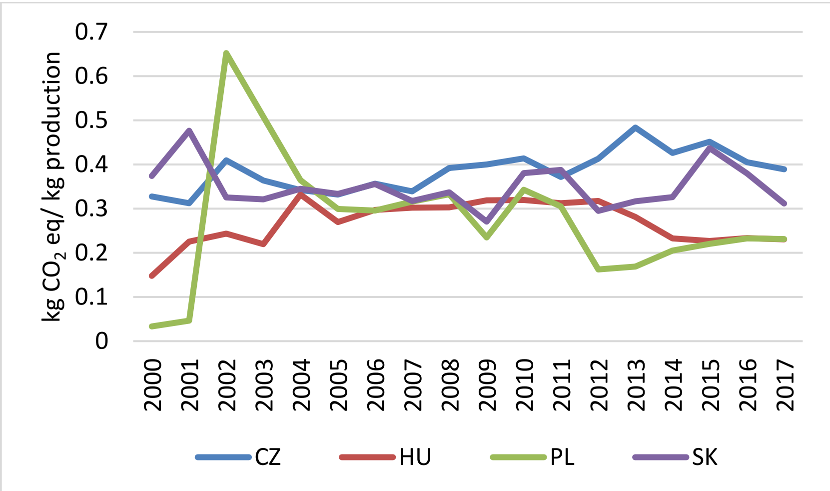

Figure 5 The intensity of GHG emissions associated with chicken meat, kg CO_2 eq/ kg production. Source: (FAOSTAT. DATA, 2019)

Concerning pork, the countries showed close values from 2000 to 2013, but from 2014 Slovakia, unlike the other countries, started to grow. The highest value for Slovakia was registered with a maximum value of 2.36 kg of CO_2 eq/ kg of pork in 2015. While the lowest value for Poland was observed at a minimum value of 1.04 kg CO_2 eq/kg of pork in 2017. In terms of averages, we did not expect large differences between countries. We calculated 1.8372 kg CO_2 eq/ kg of pork for Slovakia, 1.5499 kg CO_2 eq/ kg of pork for Hungary, 1.4816 kg CO_2 eq/ kg of pork for Poland and 1.4065 kg CO_2 eq/ kg of pork for the Czech Republic. In terms of variability, Poland has the highest difference of 17.53%, followed by Hungary the average of 12.61%. The third is the Czech Republic with a CV of 11.05% and Slovakia closes the line with a CV of 10.67%.

As already mentioned, Slovakia is the only member state where we have seen growth, from 2000 to 2017 at +5.91%. In contrast, the Czech Republic (-23.45%) Hungary (-28.85%) and Poland (-37.73%) reduced GHG emissions per kilogram of pork. Slovakia's increase in emissions intensity is due to an emission reduction of -62.4% and a decrease in product production of -64.59%. In contrast, for the Czech Republic, the decrease in emissions intensity was caused by an emission reduction of -59.5% and a decrease in product production of -47.1% Hungary's recession was accompanied by a -45.5% decrease in output and a -23.4% decrease in product production. In contrast, Poland's decrease is due to an emission reduction of -33.69% and a 106.4% increase in product production.

For all countries except Poland, the production potential of the sector has decreased due to the changed animal welfare and consumption patterns. Which also reduced the level of emissions proportionately. The constant rise in feed prices and the spread of swine fever pose additional threats to the sector in all countries. Which causes a further decrease in production and a further decrease in output in the sector.

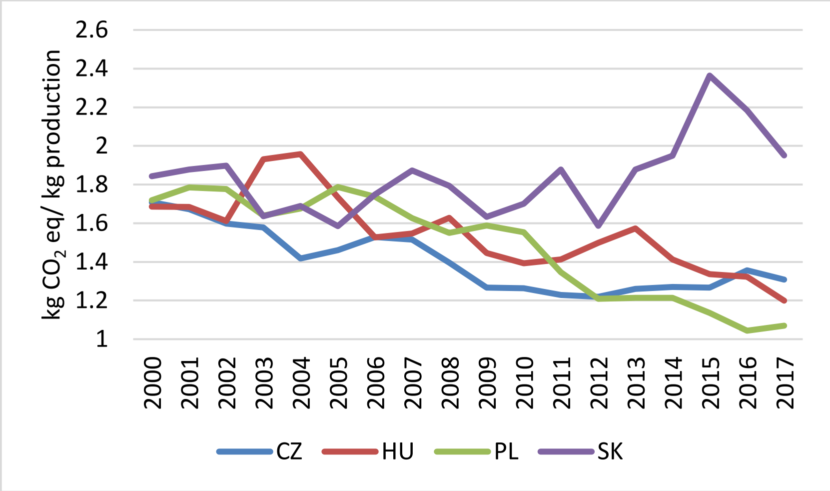

Figure 6. The intensity of GHG emissions associated with pork, kg CO_2 eq/ kg production. Source: (FAOSTAT. DATA, 2019)

In the case of the intensity of GHG emissions from lamb, we found outstanding values in Hungary. It is several times ahead of the development of emission intensities by members of the Visegrad Group of countries. The maximum value of the examined period is 537.65 kg CO_2 eq/kg lamb, which is significant in Hungary. The lowest value for the Czech Republic was 11.04 kg CO_2 eq/kg lamb. There was significant differentiation between each country. The averages were also the highest for this product.

In the case of Hungary, we calculated the highest average emission intensity with a value of 316.182 kg CO_2 eq/ kg lamb. The other member states lagged significantly behind this value, Poland averaged 68.2766 kg CO_2 eq/ kg lamb, Slovakia 48.2983 kg CO_2 eq/kg lamb, while the Czech Republic showed 17.6242 kg CO_2 eq/kg lamb. In terms of variability, Hungary CV % is the highest at 37.96%. Slovakia showed the second-highest variability with 29.03%.

The Czech Republic then deviated by 25.03% and Poland by 19.32% over the period under consideration. Hungary has significantly increased the intensity of GHG emissions per kilo of lamb production by +202.59% from 2000 to 2017. We also experienced an increase of +70%

for Slovakia. Czech Republic (-46.38%) and Poland (-38.39%) reduction was observed. Hungary increased by 132.5% due to a decrease in product production of -56.1%. In addition, for Slovakia, the increase in emissions intensity is due to an increase in emissions of 110.2% and a decrease in product production of -35.1%. The Czech Republic's decrease was caused by an increase in product production of 443.6% with an increase in emissions of 237.8%. In contrast, the recession in Poland was accompanied by a -33.65% decrease in output coupled with a 107.6% increase in product production.

The reason for the significant difference in Hungary may be the outdated livestock farming technology. Which resulted from inappropriate different genotypic mating and lack of grazing throughout the year. While in the case of Poland and the Czech Republic, the setting of successful different genotypic pairing was appropriate for pastures. In the case of Slovakia, the size of the pastures could allow the sector to develop, but the sector is struggling with a significant lack of resources, which also harms emission values.

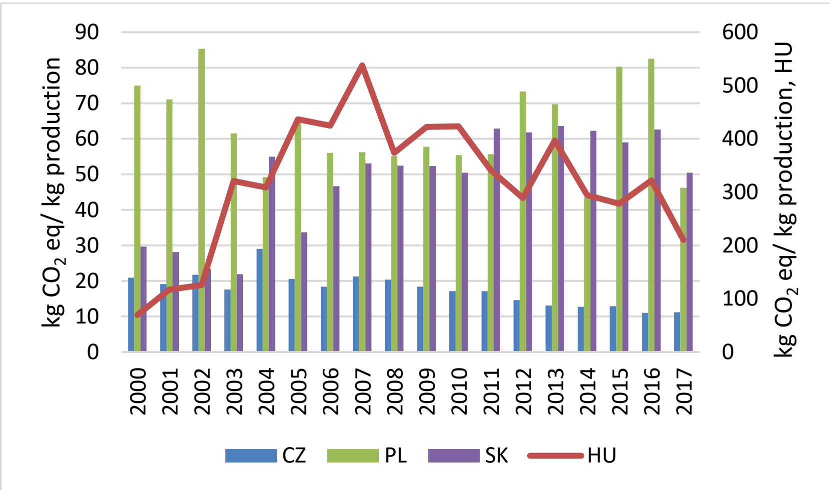

Figure 7 The intensity of GHG emissions associated with lamb, kg CO_2 eq/ kg production. Source: (FAOSTAT. DATA, 2019)

There has been a decrease in cow's milk in all countries. We have not seen any significant differences between countries. Poland showed the highest value with a maximum of 0.7497 kg of CO_2 eq/ kg cow's milk in 2000. While the lowest value was recorded for the Czech Republic with a minimum value of 0.3572 kg CO_2 eq/ kg cow's milk in 2016. Observing the bowls of countries, it can be said that there were no significant differences. The average in Poland is 0.6222 kg of CO_2 eq/ kg cow's milk, the average in Slovakia is 0.5245 kg of CO_2 eq /kg of cow's milk, Hungary average is 0.4828 kg of CO_2 eq/kg cow's milk and the Czech Republic average is 0.4300 kg of CO_2 eq/ kg cow's milk. By observing CV %, we can see that the countries have desisted from their averages by almost the same amount, with the Czech Republic by 13.40%, Hungary by 13.05%, Poland by 13.98% and Slovakia by 13.27%. From 2000 to 2017, Slovakia reduced the intensity of GHG emissions per kilogram of cow's milk by -39.59%.

Poland experienced the second-largest decrease with -37.94%. The Czech Republic followed with -33.58 and Hungary closed the line with a reduction of -27.50%. The recession in Slovakia was driven by a -47.1% decrease in output and a -12.53% decrease in product production. While in the case of Poland a -28.5% decrease in emissions, an increase of 115.18% in product production was observed. For the Czech Republic, the reduction in the intensity of emissions was achieved by a decrease of -26.4% and the production of products increased by 110.7%. Hungary’s intensity of emissions showed a decrease in product production by -10.48% with an emission reduction of -35.1%. For each country, a significant decrease was observed, which is due to the development of technology

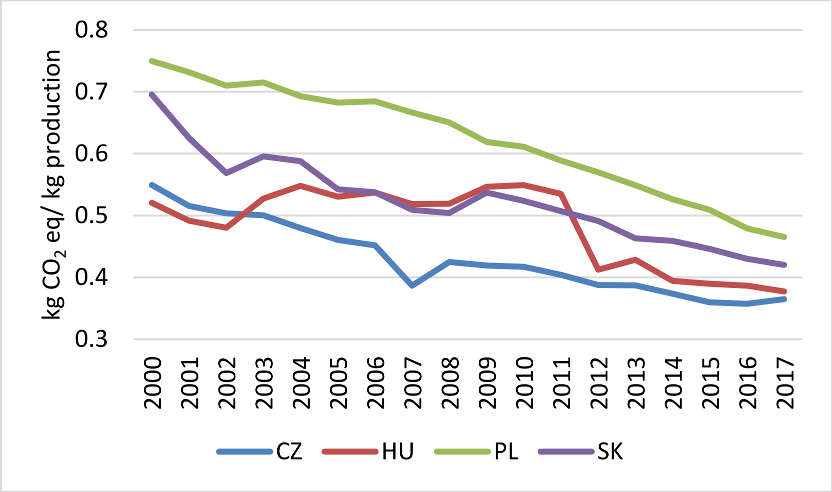

Figure 8 The intensity of GHG emissions associated with cow milk, kg CO_2 eq/ kg production. Source: (FAOSTAT. DATA, 2019)

Conclusion

Based on our studies, it can be said that prolific animal species have more favorable product production values, so intensity of the emission of GHG alterates more favorable. The highest GHG emission intensity is observed for the production of lamb. Due to the production of lamb, the high values for the annual useful production of herds, which is high in addition to the production of biomass per kilogram. Then the GHG emission intensity of beef production is also followed by a high proportion of biomass per kilogram. Pork production came in third place and egg production followed. For these products, the utility of the nutrient is more favourable and thus the intensity of GHG emissions is more favourable.

The production of cow's milk and the production of chicken meat represented similar emission intensity values. In the case of cow's milk, the low value of GHG intensity is due to the high production units, while the low value of chicken meat production is due to fertility). The lowest emission intensity was shown by the production of cereals. Furthermore, the established ranking is strongly influenced by the fact that lamb and cattle are ruminants and intestinal fermentation, faecal formation and urination emissions make up the largest proportion of agricultural GHG emissions.

The volume of emissions by pork production came in third place because the problems of handling slurry produced by animals make a significant contribution to the totality of agricultural emissions. In the case of the production of eggs and cow's milk, there were no such high values because dairy cows' daily milk drop-off and laying eggs per day significantly exceed their emissions. The production of chicken meat does not result in significant GHG emissions due to its intensive production. The differentiation between the volume of GHG emissions of products is influenced by the specific characteristics of the products and the evolution of market conditions and is also significantly influenced by the type of farming. In the case of Poland, an increase in the production of pork, lamb and cow's milk has been observed with decreasing emissions.

As regards Slovakia, the production of chicken meat has seen an upward trend in product production, in addition to the downward trend in output. While in Hungary and the Czech Republic, it has been observed a phenomenon in terms of the production of cow's milk. In contrast, in Hungary, increased emissions in terms of beef production and lamb production and were observed in the case of the product production decreased Furthermore, for Slovakia, in the case of lamb production, we have observed a decrease in product production with increasing emissions. On this basis, it can be said that the changes in Poland were the most favourable, while in Hungary the situation proved to be rather unfavourable. Overall, there is significant potential for reducing GHG emissions in member states agriculture. The key to reducing emissions is among other things such as developing the right feed systems, supporting the transition to organic farming and encouraging farms to become more environmentally friendly.

In the next period (2021-2030), this activity incentive will be used to exploit the potential for reducing GHG emissions in agriculture.

Acknowledgement

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INVESTIGATION OF THE RELATIONSHIP BETWEEN THE EVAPORATION AND METEOROLOGICAL VARIABLES FOR DIFFERENT CLASS A PAN TREATMENTS

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Abstract

Evaporation is a key member of the hydrological cycle. Climate change requires a more accurate understanding of the process. In addition to physical processes, the evaporation of open water is also influenced by biological factors (eg aquatic plants). To better understand the phenomenon, an experiment has been set up in 2020 growing season: in addition to the traditional use of Class A pan (WMO), the presence of sediment and submerged macrophyte (*Myriophyllum* sp., *Potamogeton* sp., and *Najas* sp.) was also ensured in the Class A pan's. The Class A pan's were located in an open area at the Agrometeorological Research Station in Keszthely. Meteorological variables were also measured at the Station (air temperature, precipitation, relative humidity, solar radiation, wind speed). The aim of the study was to determine the effect of sediment and macrophyte on evaporation and explore the relationship between evaporation and meteorological variables. The results showed that the presence of both

sediment and submerged macrophyte increased evaporation. Among the meteorological variables, solar radiation and air temperature showed the closest relationship with evaporation.
Keywords: evaporation, class A pan, sediment, macrophytes

Összefoglalás

A párolgás a hidrológiai ciklus kulcsfontosságú tagja. Az éghajlatváltozás következtében a folyamat pontosabb megértése nélkülözhetetlen. A fizikai folyamatok mellett a nyílt víz párolgását biológiai tényezők is befolyásolják (pl. vízi növények jelenléte). A jelenség jobb megértése érdekében egy kísérletet állítottunk be 2020 tenyészidőszakában: a párolgásmérő A kád (WMO) hagyományos alkalmazása mellett az A kádakba üledéket és alámerülten élő, gyökerező hínárnövényeket telepítettünk. Az A kádak a keszthelyi Agrometeorológiai Kutatóállomáson voltak elhelyezve, nyílt területen. Az állomáson a meteorológiai változókat is mértük (levegő hőmérséklete, csapadék, relatív páratartalom, sugárzás, szélsebesség). A vizsgálat célja az iszap és a hínárnövények párolgásra gyakorolt hatásának meghatározása volt. Célkitűzés volt továbbá a párolgás és a meteorológiai változók kapcsolatának vizsgálata is. Az eredmények azt mutatták, hogy mind az üledék, mind a hínárnövények jelenléte fokozta a párolgást. A meteorológiai változók közül a sugárzás és a levegő hőmérséklete mutatta a legszorosabb kapcsolatot a párolgással.
Kulcsszavak: párolgás, párolgásmérő A kád, iszap, hínár

Introduction

Evaporation is a key member of the hydrological cycle that is responsible for water loss. Accurate estimation of evaporation is of great importance, especially in regions with limited water resource. According to some estimates, 61% of the fallen precipitation evaporates

(Alsumaiei, 2020). Therefore, the accurate estimation of evaporation rates using is a vital task for hydrologic engineering, water resources management and agriculture (Deo & Samui, 2017). The effects of climate change are becoming more pronounced, and this process may also have an impact on evaporation.

Lake or water reservoir evaporation is rarely measured directly. Different evaporimeters instruments may be used in different countries to approximate the evaporation of natural water surfaces. The most common indirect method is the measurement of pan evaporation. The World Meteorological Organization (WMO) recommends Class A pan for measuring evaporation.

Since evaporation is a complex operation, a reliable formula to represent all the physical processes involved is difficult to obtain. Several researchers have tried to use meteorological variables to forecast pan evaporation values (Adnan et al., 2020; Alizamir et al., 2020). In addition to physical processes, biological phenomena (plants) present in water can also affect evaporation. Important evaporation differences among open water evaporation and aquatic plant evapo(trans)piration covers have been reported around the world (Pauliukonis & Schneider, 2001; Goulden et al., 2007).

In the present study, we sought to answer how the presence of litter sediment and aquatic macrophyte affects daily pan evaporation. Aim study was to determine the relationship between different pan treatments and meteorological variables in 2020 growing season.

Material and method

In this study, pan evaporation (E_p) and meteorological data in the Agrometeorological Research Station of Keszthely (latitude: 46° 44′ N, longitude: 17° 14′ E, elevation: 124 m above sea level) investigated. The station follows standard methods of observation for data collection as per the World Meteorological Organization guidelines (WMO, 2012). The combined sensor was placed at a standard height 2 m above ground level. Signals from air temperature (T_a, °C), relative humidity (*RH*, %), wind speed (*u*, m s^{-1}) and solar radiation (*Rs*, W m^{-2} day^{-1}) were collected every 2 s, and 10-min averages were logged. The height of the anemometer was 10.5 m above ground level. The Class A pan are circular cylinders of 1.21 m diameter and 0.255 m depth mounted on an elevated 0.15 m height open frame wooden grid set on the ground (Figure 1). Daily E_p rates, which were adjusted to precipitation, were measured manually at 7.00 a.m. every morning.

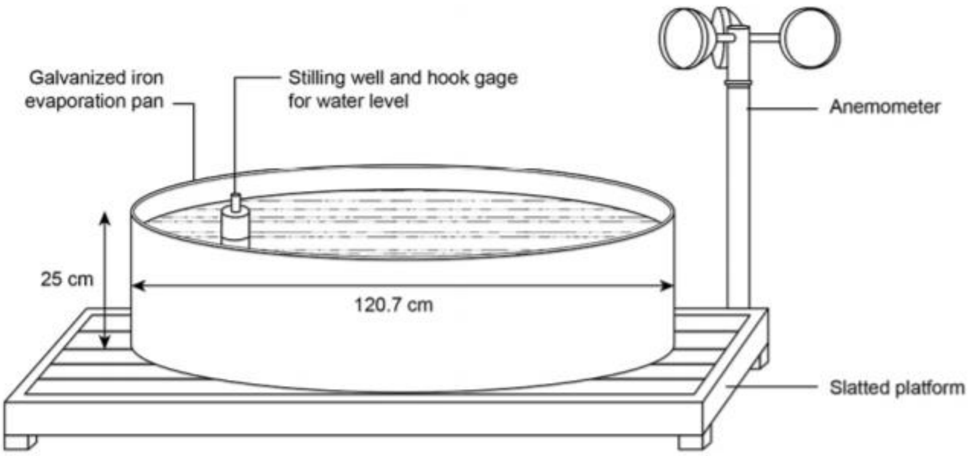

Figure 1 Illustration of a standard Class A pan (Alsumaiei, 2020)

Three pan treatments have been set:

- control pan, *C*;
- pan was supplemented with a 0.05 m thick littoral sediment layer that covered the bottom, *S*;
- pan was planted with littoral sediment and submerged macrophytes (*Myriophyllum* sp., *Potamogeton* sp., and *Najas* sp.), *SM*.

We used three submersed macrophyte species in the *SM* pan treatment, which can be found in the Keszthely-Bay in the summer season.

We used Microsoft Excel and SPSS software packages to evaluate the data.

Results and discussion

The highest daily maximum E_p rates were always measured in Class A pan with macrophytes, while the lowest in the "empty" pan (control). The daily measured E_p rate for *C*, *S* and *SM* averaged 3.17±0.95, 3.39±0.98 and 3.65±1.05 mm day^{-1}, respectively, during in 2020 growing season. A paired-type t-test was conducted to explore the impact of the studied pan treatments on E_p rates. There was significant difference neither between the E_p of *C* and *S* (p=0.1014) nor between E_p of *S* and *MS* (p=0.0700). However, E_p of *C* treatment was significantly different from that of *SM* (p<0.001). The E_p of *S* fitted better to E_p of *C* (R^2=0.9597), while in the case of *SM* the relationship is less close (R^2=0.9268) (Figure 2).

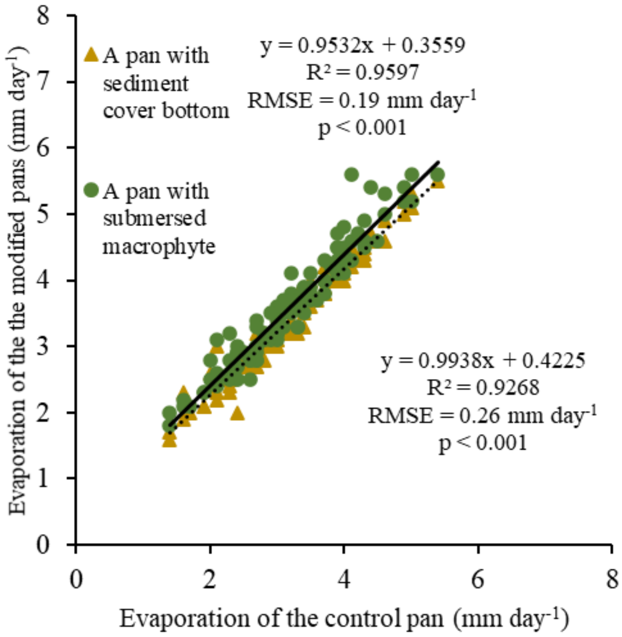

Figure 2. Relationship between evaporation of standard Class A pan and modified Class A pan's

To date, there is few information about the impact of submerged macrophytes on pan/open water E_p rate. According to a previous study aquatic plants evapotranspirated 26% more water than that of the free water surface (Brezny et al., 1973). Anda et al. (2016; 2018a,b) have shown that the presence of sediment increases the evaporation of the Class A pans by an average of 12.7% and the submerged aquatic macrophytes by an average of 21.3%. Jiménez-Rodríguez et al. (2019) reported that the observed E_p were higher for aquatic plants than the open water cover.

The relationship between E_p and meteorological variables (Figure 3) is very complicated, for that reason it's difficult to analyse (Wang et al. 2017, Kisi, 2015; Kim et al., 2015). The highest correlation coefficients were observed between E_p rates of *C* and *Rs* (R=0.6159). Meteorological variables related to available energy (such as *Rs*, T_a), the most relevant factor in E_p of Class A pan (Chen et al., 2019). A positive correlation was observed with most meteorological variables (T_a, *Rs*, *u*), while a negative correlation was observed with *RH* (R=-0.1942 for *SM* and R=-0.2286 for *C*). This result is supported by other research in the literature (Sheffield et al., 2006; An et al., 2017). In this study, *u* hardly affected the E_p rates of each treatment. This does not confirm the conclusions made by earlier studies (McVicar et al., 2012).

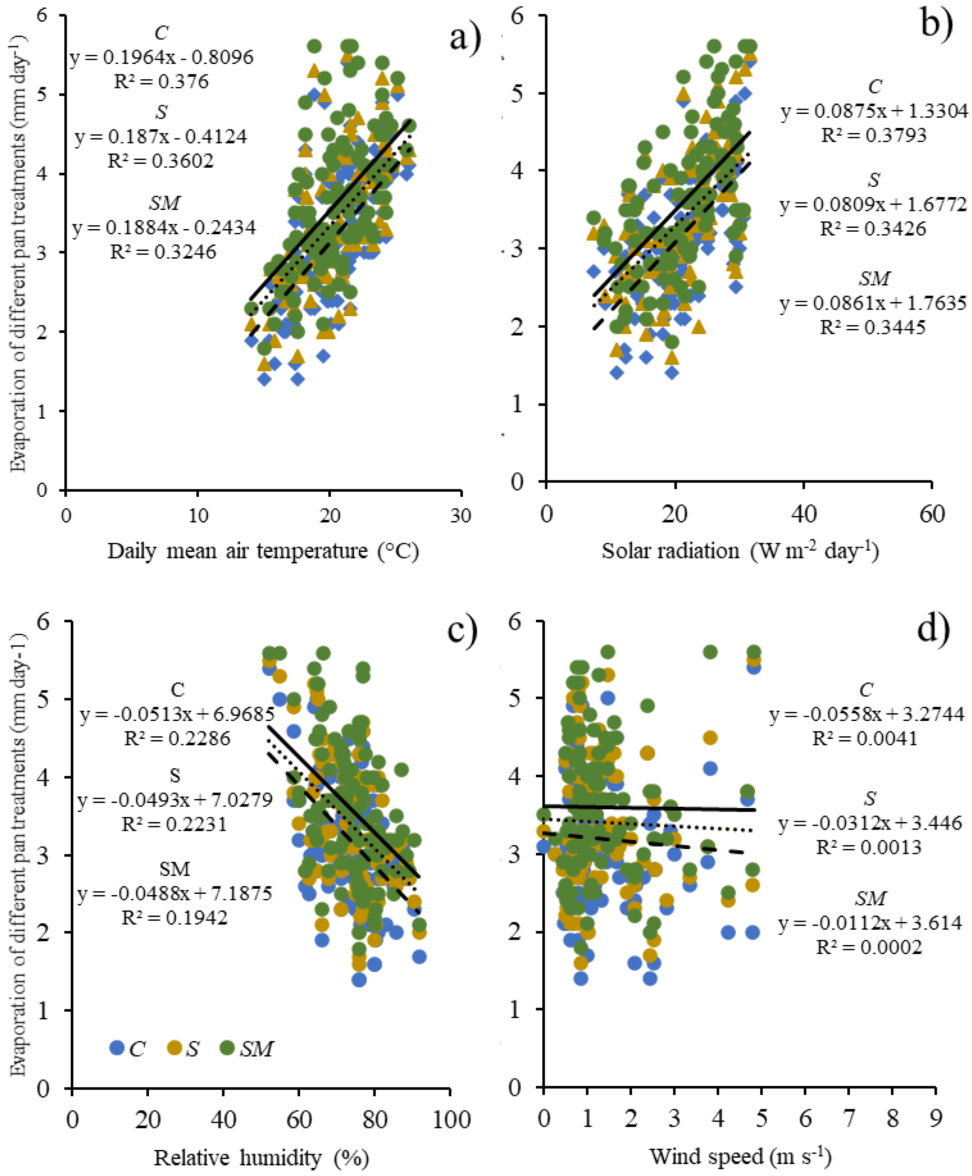

Figure 3. Relationship between evaporation of different pan treatments (control pan, C, pan with sediment cower bottom, S and pan with submersed macrophyte, SM) and meteorological variables (air temperature, °C – a), solar radiation, W m^{-2} day^{-1} – b), relative humidity, % – c), wind speed, m s^{-1} – d))

This may be due to the fact that Keszthely (and Agrometeorological Research Station) is sheltered by surrounded mountains causing lower wind speeds (Anda et al., 2016). Jiménez-Rodríguez et al. (2019) described a lower value for the correlation between evapotranspiration of *T. geniculata* and *u*. Stepwise regression analysis showed that *Rs* and *RH* impacted the E_p rate the most, regardless of treatment (Table 1).

Table 1 Multiple stepwise regression analysis between meteorological elements and measured Class A pan evaporation: "empty" pan (C), pan with sediment (S) and pan with macrophyte (SM) during 2020 growing season

	R^2	F	F sig.	SE	Regression equation
C					
Model 1	0.484	96.76	0.000	*Konst.* = 0.251 *Rs* = 0.011	$E_p = 0.111Rs + 0.796$
Model 2	0.552	62.73	0.000	*Konst.* = 0.937 *Rs* = 0.013 *RH* = 0.01	$E_p = 0.06Rs - 0.039RH + 4.34$
S					
Model 1	0.511	107.74	0.000	*Konst.* = 0.250 *Rs* = 0.011	$E_p = 0.116Rs + 0.892$
Model 2	0.571	67.89	0.000	*Konst.* = 0.938 *Rs* = 0.013 *RH* = 0.01	$E_p = 0.087Rs - 0.038RH + 4.315$
SM					
Model 1	0.585	154.46	0.000	*Konst.* = 0.247 *Rs* = 0.011	$E_p = 0.133Rs + 0.777$
Model 2	0.654	96.44	0.000	*Konst.* = 0.903 *Rs* = 0.014 *RH* = 0.01	$E_p = 0.099Rs - 0.043RH + 4.711$

Conclusion

In this study increased evaporation was measured in the modified Class A pans, a larger increment at the submerged macrophytes and lower at the sediment cover use. The relationship between evaporation of different Class A pan treatments and meteorological variables was similar. Higher R^2 values were usually observed in *MS*. Higher R^2 values between the E_p of *SM* and R_s and T_a implied that evaporation was mainly controlled by available energy in 2020 growing season. The regression equations in Table 1 provide an opportunity to estimate the E_p of different Class A pan treatments from meteorological variables.

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EFFECTS OF AGRICULTURE ON SURFACE AND SUBSURFACE WATER RESOURCES – A REVIEW

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Abstract

Surface and subsurface water resources are vital elements in the functioning of environmental processes and human society. In favour of sustainability, it is extremely important to maintain their good quality, quantity and exemption from harmful substances. In this literature review the agricultural activities - causing greater negative impact on surface and subsurface water resources - will be presented. The current regulatory status of surface- and groundwater in Hungary will be explained, just like agricultural activities, endangering water resources, and harmful substances derived therefrom, which move the state of the waters to a negative direction. Furthermore, currently available and long-term perspectives will be presented to eliminate the harmful effects of agricultural activities. The main aim of the review is to draw attention to the way and extent, how industrial agriculture damages and changes the quality, quantity and wildlife of water bodies.

Keywords: water resources, eutrophication, fertilizers, insecticides, erosion, irrigation

Összefoglalás

A felszíni és felszín alatti vízbázisok a környezeti folyamatok és az emberi társadalom működésében egyaránt létfontosságú elemek. Ezek mennyiségének, minőségének és káros anyagoktól való mentességének megőrzése, a fenntarthatóság érdekében kulcsfontosságú. Ebben a szakirodalmi áttekintésben a nagyobb negatív környezeti befolyást okozó mezőgazdasági tevékenységek, felszíni és felszín alatti vizekre gyakorolt hatása kerül kifejtésre. Áttekintést kaphatunk a Magyarországi felszíni és felszín alatti vizek jellemzőinek hatályos szabályzásáról, a mezőgazdaság vízbázisokat veszélyeztető tevékenységeiről, az ezekből kikerülő és károsító anyagokról, valamint ezek hatásairól, melyek negatív irányba mozdítják el a vizek állapotát. Továbbá, szó lesz még arról, hogy milyen jelenlegi és jövőbeni megoldások vannak, és lehetnek majd arra, hogy a mezőgazdaság vizeket károsító hatását mérsékeljük. Az áttekintés fő célja felhívni a figyelmet arra, hogy az iparszerű mezőgazdaság milyen módon és milyen mértékben károsítja és változtatja meg a vízbázisok minőségét, mennyiségét és élővilágát.

Kulcsszavak: vízkészletek, eutrofizáció, műtrágya, rovarölőszer, erózió, öntözés

Introduction

Until the 1950s, agricultural activities were carried out on smaller family farms that used organic fertilizers. After that, there was a worldwide shift in agricultural production, which was relocated to larger, monocultural, intensively operated farm units (Novotny, 1999). Along with the population explosion, the demand for agricultural production has also increased. The population of the world is now around 7.8 billion (INTERNET 1), and because of that, only industrial agriculture is appropriate to feed so many people.

The intensification of agriculture and population growth went hand in hand. The quality and quantity of agricultural products are very important, but this sector has an inevitable impact on water quality, which has direct and indirect effects on fertility, so it can be said, that the purity of water and fertility are closely related. Agriculture pollutes water resources as a result of the usage of agrochemicals, organic substances, saltwater drainage and pollution with toxic materials threatening human health and water ecosystems (Stoyanova et al., 2019). The agricultural sector is mainly responsible for nitrate, phosphorus, pesticide, salt, pathogen and soil sediment pollution of surface and subsurface waters, especially from crop and livestock activities (Parris, 2011). From these, eutrophication (the accumulation of the two key nutrients – nitrogen (N) and phosphorus (P)) and the contamination of subsurface and drinking water sources are the main problems in most of the countries. Unsustainable land use can also lead to water pollution.

The entire land surface functions as a catchment area for rivers, and almost every event on the catchment area have a large effect on freshwaters (Moss, 2008). Freshwater and marine ecosystems have all been degraded because of production, but freshwaters are the most vulnerable. The surface and subsurface water bodies are widely exploited (Withers et. al., 2014).

Water classification in Hungary

In Hungary, the MSZ 12749 standard - effective from 1. January 1994 - classifies surface waters into five quality classes, based on specific water characteristics. Table 1 shows the main parameters that can be released into water, as a result of agricultural activities, according to the standard.

Table 1 The main surface water quality parameters that change as a result of agricultural activities (MSZ 12749 standard)

Water quality characteristics	Unit of measure	Excellent (I.)	Good (II.)	Bearable (III.)	Contaminated (IV.)	Heavily contaminated (V.)
Dissolved oxygen	mg/l	7	6	4	3	<3
Oxygen saturation	%	80-100	70-80	50-70	20-50	<20
Biological oxygen demand	mg/l	4	6	10	15	>15
Chemical oxygen demand	mg/l	5	8	15	20	>20
NH_4^+	mg/l	0.26	0.64	1.29	2.57	>2.57
NO_2^-	mg/l	0.033	0.1	0.329	0.986	>0.986
NO_3^-	mg/l	4.43	22.14	44.28	110.7	>110.7
total phosphorus	µg/l	100	200	400	1000	>1000
Chlorophyll-A	µg/l	10	25	75	250	>250
Coliform count in 1 ml	-	1	10	100	1000	>1000
Cyanide	µg/l	10	25	50	100	>100
Zinc	µg/l	50	75	100	300	>300
Mercury	µg/l	0.1	0.2	0.5	1	>1
Cadmium	µg/l	0.5	1	2	5	>5
Lead	µg/l	5	20	50	100	>100
Copper	µg/l	5	10	50	100	>100
Phenols	µg/l	2	5	10	20	>20
Petroleum and products thereof	µg/l	20	50	100	250	>250
Polychlorinated biphenyls	µg/l	0.01	0.05	0.2	2	>2
pH	-	6.5-8	6.5-8.5	6-6.5	5.5-6	<5.5
Iron	mg/l	0.1	0.2	0.5	1	>1
Manganese	mg/l	0.05	0.1	0.1	0.5	>0.5

In Hungary, the quality of 109 watercourses and 4 lakes is currently regularly monitored, at a total of 241 locations. Environmental inspectorates take thousands of water samples every year.

Agricultural pollution of water resources

Fertilization

Chemical substances (artificial fertilizers) are used extensively in industrial agriculture in order to improve crop yield (Divja et al., 2012). Nutrients and other even harmful substances released from the fertilizers can easily enter surface- and groundwater through precipitation and runoff. The leading problem of fertilizers is that, if their main nutrients like nitrogen and phosphorus are not utilized and leached, they pollute water. These two nutrients are predominant pollution sources, causing harmful algal blooms and eutrophication (Wu, 2017). Eutrophication is a feature of surface waters. In 1995, Nixon formulated the definition of eutrophication as ”an increase in the rate of supply of organic matter to an ecosystem”. Thanks to the widespread use of fertilizers, agricultural nutrient pollution became an essential problem across the developed agricultural countries (Parris, 2011). Eutrophication occurs naturally over decades or centuries as lakes are filled with sediments, under the influence of natural processes (Carpenter, 1981). These processes are accelerated by human activities. Agricultural scientist and pond managers often eutrophy experimental water bodies and fish ponds by adding fertilizers to enhance primary productivity and increase the density and biomass, to grow economically important fish faster (Boyd and Tucker, 1998). Extra nutrients that can be consumed under artificial conditions cannot be utilized by natural water bodies, so it will cause undesired algal blooms. Algal blooms limit light penetration with which they lower the success of predators that need light to catch prey, and causing die-offs of plants in littoral zones (Lehtiniemi et al., 2005). Another impact of eutrophication causing dissolved nutrients like nitrogen and phosphorus, is when a water body becomes enriched in them, the increased growth of aquatic plant life can lead to the depletion of dissolved oxygen (Vallero, 2006). Beside the oxygen consumption of algae, metabolic breakdown of organic matter can also cause depletion of dissolved oxygen concentration, leading to hypoxia (very low oxygen level) or anoxia (no oxygen), which is fatal to the natural flora and fauna (Sharp, 2001).

Artificial yield enhancers has also a relevant negative effect on subsurface and drinking waters as nitrate transforms into nitrite. It is a dangerous contaminant, because it can cause methaemoglobinaemia (blue baby syndrome). The maximum allowable concentration of nitrate and nitrite in order to prevent human diseases has been set by the World Health Organization. Due to the 201/2001. (X.25.) Government decree, in Hungary the maximum concentration of nitrate could not be above 50 mg/l and nitrite could not be above 0.5 mg/l. To prevent the contamination of subsurface waters and damages to human health, the application of fertilizers, containing nitrogen compounds is highly restricted. In their study Parvizishad et. al. (2006) examined the adverse effects and benefits of nitrite and nitrate in drinking water to reconsider the specified limits. They came to the conclusion that the risk is not clear, because as these substances can cause methaemoglobinaemia, cancer, enlargement of the thyroid gland, or diabetes mellitus in high doses, they are also advantageous in the protection of the vascular system, blood pressure regulation and maintaining the homeostasis in small quantities.

Fertilizers often contain heavy metals and microelements which can also be harmful to water ecosystems and human health. These heavy metals may accumulate in soil with repeatable fertilizer applications and they can easily reach surface and groundwater through the water cycle. Possibly occurring and most important heavy metals in fertilizers are cadmium (Cd), arsenic (As), chromium (Cd), lead (Pb), mercury (Hg), nickel (Ni) and vanadium (V) (Mortvedt, 1995).

Chemical plant protection

Nowadays the use of pesticides, herbicides, insecticides, fungicides and other artificial plant protectants became common all around the world, not only on agricultural farms, but in backyard gardens as well. These chemicals have allowed farmers and gardeners to exercise greater control over the plants they want to grow, by warding off pests. But this high degree of chemicalization have not come without environmental costs like pollution of surface and subsurface waters. In the history of mankind, a plenty of substances were used in rudimentary- and developed agriculture. In the 15th century, toxic chemicals containing arsenic, lead and mercury were used. In the 17th century, nicotine-sulphate was used as an insecticide (Miller, 2002). In the 1960's triazine, carboxyl-acids such as glyphosate and other nitrogen-containing herbicides and pesticides became common (Ritter, 2009). Artificial chemicals threat waters and their biodiversity seriously. A good example of contamination of plant protection is DDT (dichloro-diphenyl-trichloroethane) which was a highly effective insecticide and was used massively worldwide until 1972, when it was banned internationally under the Stockholm Convention on Persistent Organic Pollutants, due to its persistence and accumulation mainly in water food chains (Yang et al., 2008). The major problem of DDT and similar plant protectans is their capacity to accumulate in the food chain and thus threatening both human health and the environment (Ochoa-Rivero et al., 2017). This substance has accumulated in higher order organizations with biomagnification, and in the 1960s it was discovered, that it prevented a number of fish-eating birds from reproduction (Anamika et al., 2018). Unfortunately, DDT is still used in some developing countries, to prevent malaria and other tropical diseases (Lobe, 2006). Nowadays we have thousands of new ingredients for chemization, but we know little about their long-term environmental and health effects and persistence.

Irrigation

As a consequence of climate change, irrigation of agricultural and horticultural crops is becoming increasingly inevitable. As a result of climate change, less and less rain is expected, with more unpredictable frequency. Irrigated agriculture has many advantages, such as increased production and reliable harvests (Duncan et al., 2008). On the other side of the coin, this intervention has also negative effects on the quality and quantity of water resources. For example, aside from the positive impact of irrigation, in the downstream part of the river basin, it can cause salinity to build up with the increasing level of the groundwater (Kume et al., 2007). Salinity means the accumulation of salt in water to levels that impact on human and environmental assets. Irrigation salinity is a common unfavourable situation in irrigated landscapes. Primary salinity means the natural occurrence of salt in water (for example: salt lakes, salt marshes). Secondary salinity is the problem, which is a consequence of human activity, such as agricultural irrigation.

Many irrigation water sources have high content of salt (mostly magnesium, calcium and bicarbonates), so the reaction of these waters is alkaline (pH 7.2-8.5) (Mengel, 1994). Acidifying irrigation water is an available, but valuable technique for lowering the pH of irrigation water, but it has a number of benefits, especially for the receiving water bodies. Untreated and partially treated wastewater is also widely used for irrigation in agriculture. While the nutrients of the wastewater are considered as beneficial in agriculture, their other contaminants like heavy metals and bacteria means environmental (and also health) risks in water resources (Srinivasan et al., 2009). In their study Srinivasan et al. (2009) examined the impact of irrigation water quality on human health in India. They found that the morbidity rates were significantly higher in wastewater irrigated villages, than in the control villages, and its reason may be the quality change of drinking water resources.

Irrigation has also a mobilizing effect on nutrients, heavy metals and other elements and substances which can easily lead to the eutrophication or contamination of water sources. The most important task is to prevent the deterioration of water sources with proper water management and agrotechnology. To preserve the good quality of natural resources, irrigation is also strictly regulated. In Hungary, 2019 CXIII. Law on irrigation management is the newest and most up-to-date regulator on the subject of irrigation.

Animal husbandry

Just like other polluting agricultural sectors (fertilization or chemicalization) the livestock sector is also growing and intensifying unstoppably. One of the most dangerous pollution sources is livestock farming, whose production waste, including manure and slurry has serious implications for water quality (FAO, 2006). As a consequence of development, the quantity of produced waste has reached huge levels, because of the increasing number of farm animals housed per unit area (Polat et al., 2018).

The mixing of livestock wastes with surface waters lowers water quality and may cause the death of sole living organisms in water, by poisoning (Mielke, 1992). Livestock waste pollutes water, because it contains a high amount of nitrate, phosphorus, pathogenic microorganisms and high biological oxygen demanded organic materials (Polat et al., 2018). From these, the two main pollutants are nitrogen and phosphorus. The high concentration of nitrogen, in drinking water, in nitrate form cause methaemoglobinaemia and other forms of nitrogen, like nitrite are considered to be potentially carcinogenic. Nitrogen also causes significant environmental impacts in form of ammonia by land spreading of slurry.

Phosphorus is the second main pollutant, because it may cause eutrophication in surface water bodies (Hubbard et al., 2004). The pollution of phosphorus may be caused by runoff to surface water sources, when application to land is in excess of crop requirements (Knowlton et al., 2004). In the past decades, a new class of pollutants has emerged in livestock waste in the form of veterinary medicines, like antibiotics and growth promoters which also move to water sources by runoff (WHO, 2012). The pollution of this sector depends on the technology of animal husbandry.

Livestock sector is separated into two systems. The first and most effective system is indoor- (pigs and poultry), and the second, but more natural system is pasture-based (sheep and livestock) animal husbandry (Česonienė et al., 2019). Concentrated livestock breeding has been identified as a significant source of contaminations of surface water. In their study, Česonienė et al., (2019) were monitoring surface water sources, next to potential agricultural pollution sources. In their results they highlighted, that the activity of pig farms affected the surface water quality more than that on livestock farms. They reported, that as the number of animals increased on the farms, pH decreased and the quantity of suspended harmful materials increased in surface water. In another study Hubbard et al. (2004) carried out experiments to examine the impact of pasture-based animal husbandry on surface water sources. They reported, that grazing animals can affect water quality both positively and negatively. Pasture management is advantageous in protecting the soil surface from erosion, compared with conventionally produced crops, but on the other side pasture animals can affect water quality through erosion and sediment and nutrient transport to surface waters.

Erosion

The impact of soil erosion on surface water quality is not negligible. Erosion is the geological process in which earthen materials are worn away and transported by natural forces, like wind or water. On rugged surface, water erosion is inevitable, but the cultivation of sloping lands or vegetation alteration accelerates this natural process. Soil erosion prevails mostly on sloping areas.

The main factor that cause soil erosion is poor land management which causes damage to the soil and results water runoff, instead of adequate infiltration (Liu, 2016). Soil erosion firstly causes soil loosening on the affected area, than, after the transport of soil, deposition means the main problem, especially in surface waters. These processes usually result in the relocation of top soil, which is rich in nutrients. Pollution of nearby waters and the reduction in cropland productivity are the direct consequences of soil erosion process (Issaka et al., 2016).

Not only nutrients from eutrophication are transported with erosion, but heavy metals and chemicals are also transported with soil particles, causing the disturbance of delicate aquatic ecosystems (Bing et al., 2013). The first visible consequence of erosion on surface waters is the suspending sediment, but the most harmful are not the visible factors, but rather the invisible ones, when water becomes unsafe for human consumption, irrigation, recreation and livestock activities, because of the above mentioned toxic substances (McCool et al., 1990).

Existing and conceivable solutions

It can be determined from the above, that almost all interventions of agriculture has a negative influence on water sources. Because of that, it is really important to preserve the good chemical, physical and biological quality and quantity of surface and subsurface water sources.

In Hungary, “123/1997. (VII. 18.) Government Decree - regulation on the protection of water bodies, long-term water bodies and water installations for the supply of drinking water” regulates the usage of water sources. The regulation determines hydrogeological protection zones, based on the access time of precipitation, in order to protect water resources from precipitation delivered harmful substances, derived from human activities, like agriculture.

Besides that, it determines the range of activities that can be performed within the zones. Another important regulator, to preserve the biodiversity and purity of water sources is the National Agri-Environmental Program, which aims to keep off vulnerable areas - like water sources - from agricultural utilization, based on the principle of the land use pyramid (Ángyán et al., 1999). Environmental policy principles are also important to regulate agricultural activities and pollutant emissions. These are the prevention-, partnership-, subsidiarity- and the polluter pays principles.

The amount of fertilizers, pesticides, and other chemicals, and the time of their application is also laid down in laws and government decrees.

Beside legal regulation, scientifically based good agricultural practice is also very important in the protection of water bases. There are a number of advanced agricultural technologies, in order to avoid the pollution of water sources, but the Hungarian practice shows, that financial resources are not always available to make investments in order to avoid pollution.

Conclusion

Taking everything into account, it can be stated, that agricultural interventions often have polluting effects on water bodies. Based on the above considerations, harmful substances of chemical interventions, like fertilization, chemical plant protection and veterinary preparations in animal manure pose the greatest threat on the environment and human health, as they enter the surface and subsurface water sources. Irrigation can also have negative effects on water by causing salinity rise and by mobilizing nutrients and other substances, just like soil erosion which also plays role in sedimentation of water bodies. It becomes more common, that the values of harmful substances exceeds the prescribed limits in waters. There are many studies dealing with the harmful effects of agricultural interventions on water resources. Because of seeing water resources becoming more and more polluted with macro- and micro-pollutants, it became an urgent research field. There are a number of studies and researches investigating the contamination content of agricultural close-up water resources and the effects of these substances, but as far as I am concerned it would be expedient to perform further to assess the impact of agricultural activities on surface and subsurface water resources, taking the progress of global climate change into account, because it may have serious effects on both agriculture and water supply.

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DETECTION OF VIRAL INFECTIONS IN A HUNGARIAN VINEYARD

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Abstract

Grape (*Vitis vinifera*) belonging to the family of *Vitaceae*, is one of the most important economic fruit crops in the world. For thousands of years in Hungry, grapevine has been cultivated in the Carpathian basin, where climatic and soil conditions are suitable for grape vine production. However, it is facing adversity of virus infections that influenced negatively the performance of the cultivars at an extent, which cause premature death of the stocks and generating great yield losses. The study was conducted in a Grapevine plantation from the area of Central Transdanubia (Lesencefalu) to diagnose important viral diseases that infect Othello variety and suggest measures possible for enhancing disease control and management strategies. Othello is also a commonly cultivated variety in Hungary with the assumption of possible tolerance to important grapevine diseases. 60 samples were collected from Othello grapevine variety and analysed in the laboratory to investigate virus infection using DAS-ELISA. Six viruses (GLRaV1, GLRaV2, GLRaV3, GLRaV6, GLRaV7 and GFkV) were found to infect this variety. Among those 60 samples, 27 samples were infected with viruses, almost half of the investigated leaf samples. The highest infection was found to be caused by GLRaV1 whilst the lowest infection was found to be associated with GLRaV3. However, 13 samples were found to be infected with more than one virus (multiple infection) whilst 14 samples were found to be infected with only one virus (single infection).

Keywords: DAS-ELISA, grapevine, virus, plant protection

Összefoglalás

A Vitaceae családba tartozó szőlő (*Vitis vinifera*) a világ egyik legfontosabb gazdasági gyümölcsnövénye. Magyarországon több ezer éve termesztenek szőlőt a Kárpát -medencében, ahol az éghajlati és talajviszonyok kedvezőek a szőlő termesztéséhez. A vírusfertőzések negatívan befolyásolták a fajták produktivitását és az állományok idő előtti leromlását és pusztulását okozzák. A Közép-dunántúli régió Lesencefalu szőlőültetvényein végeztünk vizsgálatokat, amelynek a célja a szőlőt fertőző fontos vírusos betegségek azonosítása, és a védekezési stratégia javítása volt. Az othello hazánkban is gyakori termesztésű fajta, feltételezve, hogy tolerálja a fontos szőlőbetegségeket. 60 mintát gyűjtöttünk Othello szőlőfajtából, és laboratóriumban DAS-ELISA segítségével azonosítottuk a kórokozókat. A vizsgálatok során hat vírus (GLRaV1, GLRaV2, GLRaV3, GLRaV6, GLRaV7 és GFkV) jelenlétét azonosítottuk. A 60 Othello szőlőminta közül 27 volt fertőzött. A legnagyobb arányban a GLRaV1 jelenlétét igazoltuk, ezzel szemben a GLRaV3 előfordulása volt a legalacsonyabb. Összesen 13 minta esetében mutattunk ki komplex vírusfertőzést.

Kulcsszavak: DAS-ELISA, szőlő, vírus, növényvédelem

Introduction

Grape (*Vitis vinifera*) belonging to the family of *Vitaceae,* is one of the most important economic fruit crops in the world (Kumar, 2010). From Western Europe to the Persian shores of the Caspian Sea, the vine has demonstrated high levels of adaptability to most environments (Senthil *et al.*, 2011). The general classification of grapevines is largely divided into red- and white-berried cultivars dependent on their fruit skin colour, although other colours such as yellow, pink, crimson, dark blue and black-berried cultivars also exist. Red berried cultivars have anthocyanin pigments in berry skin, whilst white-fruited cultivars lack this pigment due to non-functional of regulatory genes of the anthocyanin biosynthetic pathway (Walker *et al*., 2007).

The fruit is processed into different products including juice, wine and raisins (dried fruit). It is also consumed as fresh (Buyukbay et al., 2011). Large percentage of grape production of the world is used to make wine (FAO, 2012). Grapes peel are essential source of oil and pectin. They also serve as raw material for production of cattle feed and used for preparation of candies (Kumar, 2010). Grapes are very useful in fighting diseases like dyspepsia, hemorrhoids, stones in the urinary tract and bile ducts. It also helps to activate the functions of the liver, support easy digestion, helps to reduce blood cholesterol level and eliminate uric acid (Kumar, 2010).

In 2012 according to FAO, the total world production of grapes stood at 51.42% with countries such as Spain, China, France, Italy, Turkey and Hungary contributing their quota in the production of grapes (FAO 2012; OIV, 2016). Spain cultivated with a vineyard area of 1038kha contributing to about 38.2mhL of wine production whilst Hungary cultivated 78kha which contributed to about 2.6mhL of wine production (OIV, 2016).

The first grape vine in Hungary was introduced by the Romans to Pannonia, and by the 5th century AD, there were records of extensive vineyards in what is now Hungary (Smithsonian, 2013). The main varieties in Hungary which are cultivated for white wine production includes; Furmint, Welschriesling, Bianca, Chardonnay, Cserszegi fűszeres and Rajna Riesling whilst red wine varieties are: Cabernet Franc, Blaufränkisch, Blauer Portugieser, Merlot and Zweigelt (Hajdu, 2018).

Viral diseases of grapes are common throughout all viticultural regions of the world. Infections resulting in damage to the vineyards causes a decrease in the yield and quality of grapes. Viruses disrupt various aspects of plant metabolism such photosynthesis, depress plant growth and development, transfer of respiration assimilants, reduce winter survival, drought resistance of the vine and its life cycle which directly affects the quality of wine, causing economic damage to the industry (Meng et al., 2017; Mannini and Digiaro 2017). Some important virus diseases that infect grapevines include Grapevine virus A (GVA), Grapevine virus B (GVB), Grapevine fanleaf virus (GFLV), grapevine fleck virus (GFkV) and grapevine leafroll-associated viruses. Leafroll is a damaging disease of the grapevine causing yield loss of up to 40% (Naidu et al., 2014).

Viruses are able to infect grapevines systemically. Their infection frequently is not promptly followed by symptom development that is, the pathogen remains latent for variable time intervals due to the low level of initial pathogen concentration in the host plant, to unfavourable environmental conditions or to the defense reactions of the host plant. Thus, these visually healthy, symptomless plants may carry the pathogens which are spread by vegetative propagating material. This phenomenon frequently triggers epidemic disease outbreaks in new plantations leading to significant economic losses and serious legal repercussions. Therefore, early and accurate diagnosis of plant virus diseases is a crucial component of all grapevine-management systems and also ensuring certification of propagating material as being pathogen-free are strongly regulated in most grape growing countries (Frison and Ikin 1991; OEPP/EPPO

2008; Rowhani et al. 2005). The aim of the experiment was to diagnose or test for the presence of most important grapevine infecting viruses so as to be able to make recommendations that would help improve disease management systems on Hungarian vineyards.

Material and method

The study was conducted in a grapevine plantation from the area of Central Transdanubia (Lesencefalu) (Figure 2). Lesencefalu is a small town with an area of 718 hectares in Tapolca District, Veszprém County, Hungary. It is situated 8 km north of Lake Balaton between Lesencetomaj and Várvölgy with Latitudes 46° 50′ 39.48″ N and Longitudes 17° 20′ 36.24″ E (Figure 1). This town takes its name from the Lesence stream that runs through it towards Lake Balaton.

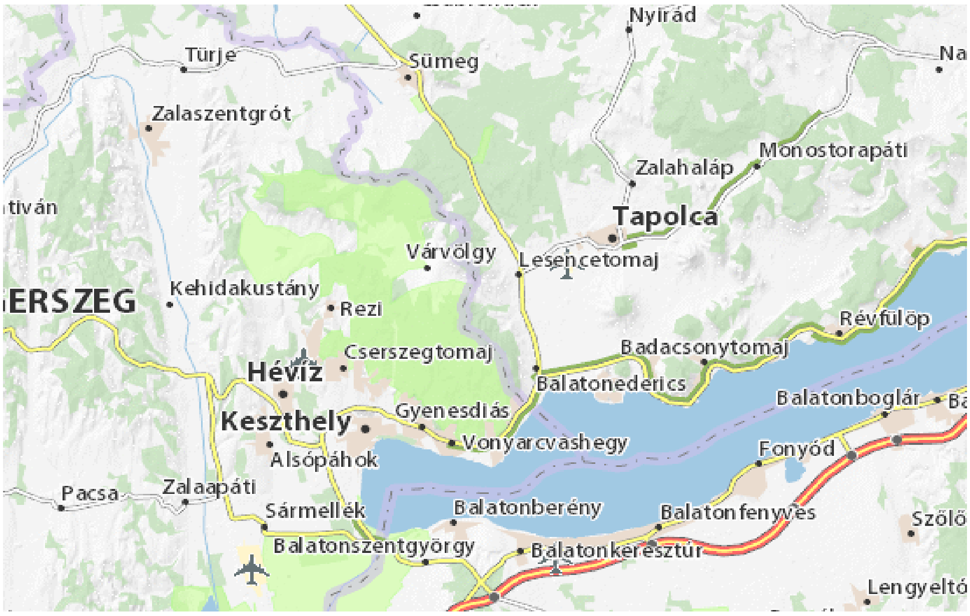

Figure 1: A map showing the study location (Google maps, 2021).

Figure 2. A map showing the exact study area (Google maps, 2021).

The samples were collected in July 2019 from one plantation (Lesencefalu) in the end of the summer. Othello is a widely cultivated variety in Hungary. It is also a direct growing cultivar with its cultivation mostly unsuitable. This is because during wine making, there is production of not only ethanol but also rising of methanol during fermentation process, making the variety not cultivated for wine making purposes. But there is an assumption that, because the Othello cultivar is not *Vitis vinifera* (*Vitis labrusca* x *Vitis riparia* x *Vitis vinifera*), it is tolerant or resistant against some of the main viral diseases of grapevine, hence virological studies about this variety is important. The number of samples collected were 60, and the plant part collected were the leaves. After collection, the samples were individually wrapped in polyethylene bags and stored at -20 C degrees until processed.

The samples were analysed or tested in the laboratory using serological Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS- ELISA) (Clark et al.,1976). The investigated viruses, Grapevine Leafroll associated virus 1 (GLRaV1), Grapevine Leafroll associated virus 2 (GLRaV2), Grapevine Leafroll associated virus 3 (GLRaV3), Grapevine Leafroll associated virus 6 (GLRaV6), Grapevine Leafroll associated virus 7 (GLRaV7) and

Grapevine fleck virus (GFkV) reagents were used from the LOEWE Biochemica. DAS-ELISA allows the reliable detection of the virus by using the available polyclonal antibodies prepared against the protein (Kritzman *et al*., 2001).

Figure 3. Preparation of experiments (Photo: György Pasztor).

Positive reactions are clearly visible, but we can get reliable results using an ELISA photometer (ELISA reader). The degree of color change was evaluated with a Labsystems Multiscan RC ELISA reader at 405 nm wavelength (Figure 3). We considered positive samples whose extinction values exceeded three times the negative control extinction value.

Results and discussion

Single virus infections are associated with one of the tested grapevine viruses infecting investigated samples. Among 60 samples, 27 (45%) samples were found to be positive/infected with at least one virus from 6 viruses (GLRaV1, GLRaV2, GLRaV3, GLRaV6, GLRaV7 and Grapevine fleck virus (GFkV), whilst 33 (55%) samples were not infected with any virus (showed negative virus infection). The magnitude of infection was different for each virus. 27 were positive/infected with GLRaV1, 12 were positive/infected with GLRaV2, 1 were positive/infected with GLRaV3, 5 were positive/infected with GLRaV6, 6 were positive/infected with GLRaV7 and 11 were positive/infected with GFkV (Table 1). This illustrates that, the Othello grapevine variety was found to be more susceptible to GLRaV1, the magnitude of infection was high 45% followed by GLRaV2 20%, GFkV 18.33%, GLRaV7 10%, GLRaV6 8.33% and GLRaV3 1.67% (Table 1). Only one sample was found to be infected with GLRaV3 indicating that, Othello grapevine variety was resistant/ not susceptible to GLRaV3. The highest infection numbers associated with GLRaV1 is in line with Martelli, 2014 and Habili et al., 2007 who reported that, GLRaV1 is one of the most widely spread and economically important viruses that infect grapevines. The virus can be found frequently in grapevines singly or in coinfection with other grapevine viruses.

The magnitude of infection associated with GLRaV1 was found to be high, followed with GLRaV2 and GFkV, this is because GLRaV1 is the most common virus in Hungary infecting grape vine, followed by GLRaV2 and GFkV. These viruses are the most common in Hungary which infect grapevine and cause great losses of yield and quality. These grapevine diseases are recognised as important viral diseases across grapevine-growing regions, and they are known to cause a wide range of negative impacts including overall resulting effects on vine performance, significant reduction in fruit yield and wine quality (Alabi et al., 2016; Lázár, 2003).

Table 1. Single virus infections of investigated Othello grapevine

TYPES OF VIRUSES	GLRaV1	GLRaV2	GLRaV3	GLRaV6	GLRaV7	GFKV
NUMBER OF INFECTED SAMPLES	27	12	1	5	6	11
PERCENTAGE (%)	45	20	1.67	8.33	10	18.33

For multiple infections, the results obtained from the research indicated that, 13 samples were found to be infected with more than one virus. Some samples were detected to be infected with either 2 or 3, 4, 5 viruses and other sample was found to be infected with all the 6 viruses (Figure 4). The result shows that 3 samples were infected with 2 viruses (1 sample was detected to be infected with both GLRaV1 and GFkV, whilst 2 samples were found to be infected both with GLRaV1 and GLRaV2). Also 3 samples were diagnosed to be infected with 3 viruses (GLRaV1, GLRaV2 and GFkV) (Figure 4). Likewise, the result demonstrates that 3 samples were infected with 4 viruses (2 samples were infected with GLRaV1, GLRaV2, GLRaV7 and GFkV whilst 1 sample was infected with GLRaV1, GLRaV2, GLRaV6 and GFkV). Similarly, 3 samples were diagnosed to be infected with 5 viruses (GLRaV1, GLRaV2, GLRaV6, GLRaV7 and GFkV) (Figure 4). However, only 1 sample was detected to be positive/infected with all the six viruses (GLRaV1, GLRaV2, GLRaV3, GLRaV6, GLRaV7 and GFkV). The result reiterates an occurrence reported by Al Rwahnih et al., 2009 and Seguin et al., 2014 who stated that, multiple virus infections are common in grapevine. Interestingly, GFkV was recorded in most multiple infection occurrences. The causal agent of fleck is widespread in grapevines. GFkV mostly exhibit symptomless infection in grapevine cultivars and is very often found in mixed infection with other harmful viruses such has GLRaVs, GVA, etc making it very difficult to singly point out its specific impact. Fleck seems to have a major effect on rootstocks, reducing growth and propagation attitudes (Credi, 2001). Indeed, the challenge and target of future research should be geared towards the design of dependable strategies for preventing a quick sanitary deterioration of vineyards planted with costly certified materials. The realization of this goal would be largely dependent upon an integrated approach which involves a deeper understanding of the mechanisms for the replication of important viruses, their pathogenesis, interactions between these viruses and the grapevine host, as well as the interactions among different viruses in mixed infections (Maliogka et al., 2015).

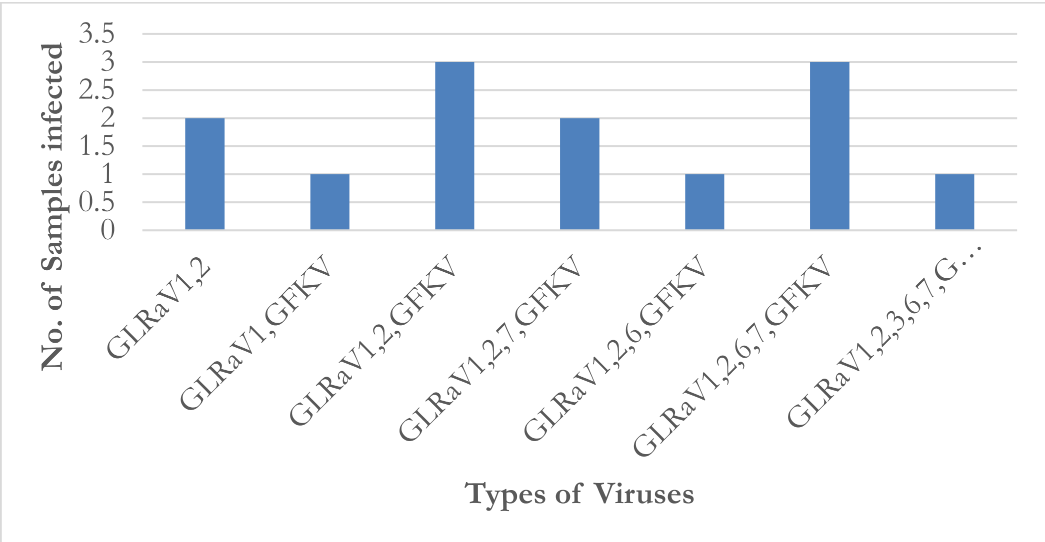

Figure 4. The Graph of Multiple virus Infection of Othello grapevine.

Conclusion

Grapevine has been operated for the purpose of propagation material, table grape and wine grape. Considering these importance and other benefits associated with its cultivation, and the fact that, viral diseases contribute to significant yield losses, studies continue to expand to enable us unravel the situation and intensify the methods of control and detection of viral diseases. This would help to limit the dissemination of viral pathogens by infected propagation and grafting materials as it is the most common way of virus spreading.

After testing the presence of infection by 6 viruses of the Othello variety by using DAS- ELISA, the results have demonstrated that, this variety is prone to important grapevine diseases more especially GLRaV1. For effective control and ultimately protection of grapevines against these diseases, early and frequent detection or diagnosis remains the initial driving force for revealing grapevine virus infestations and consequently the best control strategy. Moreover, the epidemiological characteristics of GLRaV1 appear to be similar to other *ampeloviruses* and integrated control strategies, involving a combination of virus-tested planting stock, cultural practices and vector control could be employed to minimize the spread of the viral diseases.
More studies could also be conducted to develop the immune resistant of the variety against those viruses. Breeders could select Othello grapevine variety as a breeding cultivar against GLRaV3, and it could be improved to be resistant/tolerant against other detected viruses. Furthermore, we need generic application which is improved in association with more precise and innovative techniques. However, Next generation sequencing is important for deepening the knowledge on the infection mechanism of the different viruses and the immune system response of the host. This will open new perspectives in the diagnostic field to detect the presence of viruses and identify other viruses which may have not been described yet in Hungary.

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COMPARISON OF CLASS A PAN EVAPORATION WITH ESTIMATED REFERENCE EVAPORATION AND EVAPOTRANSPIRATION

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Abstract

Aquatic plants (macrophytes) can have a large effect on water evaporation, yet they are ignored when determining evaporation from lakes and reservoirs. The aim of the experiment was to determine the effect of a floating leaf macrophyte (*Nuphar lutea*, yellow water lily) on evaporation. The measured evaporation data were also compared with the calculated from the evaporation/evapotranspiration formulas used in the literature (Shuttleworth, FAO56 Penman-Monteith, Hargreaves-Samani and Priestley-Taylor formula). The results showed that the presence of yellow water lily increase Class A pan evaporation. Of the empirical formulas, the FAO-56 Penman-Monteith reference evapotranspiration values were closest to the observed evaporation values.

Keywords: evaporation, class A pan, floating leaf macrophyte

Összefoglalás

A vízi növények nagy hatással lehetnek a víz párolgására, ennek ellenére figyelmen kívül hagyják őket a tavak és víztározók párolgásának meghatározásakor. A kísérletben célul tűztük ki egy úszó levelű hínár (Nuohar lutea, sárga vízitök) párolgásra gyakorolt hatásának meghatározását. A mért párolgás adatokat a szakirodalomban alkalmazott párolgási formulákból számolt párolgásértékekkel is összehasonlítottuk (Shuttleworth, FAO56 Penman-Monteith, Hargreaves-Samani and Priestley-Taylor formula). Az eredmények azt mutatták, hogy a sárga vízitök növeli az A kád párolgását. Az empirikus formulák közül a FAO-56 Penman- Monteith referencia párolgás értékei álltak a legközelebb a mért párolgásértékekhez.

Kulcsszavak: párolgás, párolgásmérő A kád, úszó levelű hínár

Introduction

Evaporation measurement is cumbersome and impractical in many locations in the World. Therefore, solutions are needed that can be used to estimate evaporation without measurement. Originally, the Dalton types of equations were the mainstream formulas used to estimate evaporation. The subsequent amendments improved the accuracy of estimating evaporation (Xiang et al., 2020). Evaporation refers to the evaporation of the soil surface and the open water. In nature, in addition to the evaporation of surfaces, transpiration of plants is also present. The two phenomena (evaporation and transpiration) together were called evapotranspiration.

However, it is important to distinguish between two closely related concepts: potential evapotranspiration and reference crop evapotranspiration (Xiang et al., 2020). Potential evapotranspiration represents "the combined evaporation from the soil surface and transpiration from plants, represents the transport of water from the earth back to the atmosphere, the reverse of precipitation (Thornthwaite, 1948)." It can be estimated, for example, by the Hargareves-

Samani and the Priestley-Taylor formula. The reference crop evapotranspiration defines "the rate of evapotranspiration from an extensive surface of 8 to 15 cm tall, green grass cover of uniform height, actively growing, completely shading the ground and not short of water" (Doorenbos and Pruitt, 1977). A good example to estimate reference crop evapotranspiration is the FAO56 Penman-Monteith formula recommended by FAO as a standardized method.

Aquatic macrophytes play an important role in water and wetlands balance (Said et al., 2018). Macrophytes can be divided depending on the connection with water and air: free floating; floating leaf but rooted; submerged and amphibian plants. Many of them are aggressive species that accelerate evaporation and contributes to the reduction of water resources (Soloviy& Malovanyy, 2019).

Nuphar lutea L. (yellow water lily) is common in the temperate regions of the northern hemisphere. They prefer water depths between 0.6 m and 2.4 m (Heslop-Harrison, 1955), so it also occurs in Hungarian lakes and reservoir, such as Lake Balaton. The floating leaves of yellow water lily shade most of the submerged leaves hereby limiting light penetration and photosynthesis (Schoelynck et al., 2014). Unlike the yellow water lily, other rooted, floating-leaved species like *Potamogeton natans* L. concentrate all leaves at the water surface which maximises their photosynthetic success (Bal et al., 2011). Nevertheless, transpiration of yellow water lily is not negligible, in lakes and reservoirs, where it occurs.

There are few studies in the literature that account impact of floating leaf macrophytes in estimating evaporation. Aim of study was determine the effect of floating leaf macrophyte on open water evaporation. A further aim is to determine which formula (hereinafter referred to as E/ET) is the most appropriate to estimate the actual evapotraspiration of yellow water lily. Investigating the effect of macrophyte on evaporation is particularly relevant, as any change in evaporation leads to change in water level and water quality.

Material and method

The location of the study is the experimental area of the Georgikon Campus, Hungarian University of Agriculture and Life Sciences. The Agrometeorological Research Station belongs to the observation network of the Hungarian Meteorological Service. Three different pan treatments were set in the study: Class A pan as control pan (*C*); Class A pan with sediment covered bottom (*S*) and Class A pan planted with yellow waterlily (*YWL*).

The observed daily pan evaporation values (the daily water loss was measured every morning at 7.00 am LMT) were compared to estimate the daily E/ET from the formulas below.

Daily evaporation rate, E_0 (mm day^{-1}) of water bodies was computed by the Shuttleworth formula (Shuttleworth, 1992), which was adapted from the original Penman equation (Penman, 1948):

$$E_0 = \frac{e_s R_n + \gamma * 6,43(1+0,536 * u_2)VPD}{LE(\Delta+\gamma)} \quad (1)$$

where R_n is net radiation (MJ m^{-2} day^{-1}), *m* is the slope of the saturation vapor pressure curve (kPa K^{-1}), u_2 is wind speed (m s^{-1}) at 2 m height, *VPD* is the vapor pressure deficit (kPa), *LE* is the latent heat of vaporization (MJ kg^{-1}), and γ is a psychrometric constant (kPa °C^{-1}).

The evapotranspiration was estimated using Penman- Monteith FAO-56 equation (Allen et al., 1998) for every day (mm day^{-1}) as follows (ET_{PM}):

$$ET_{PM} = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T_a+273} u(e_s - e_a)}{\Delta + \gamma(1+0.34u)} \quad (2)$$

where *G* is the soil heat flux density (MJ m^{-2} day^{-1}), T_a is the mean daily air temperature at 2 m height (°C), e_s is the saturation vapor pressure (kPa), e_a is the actual vapor pressure (kPa), Δ is the slope of the vapor pressure curve (kPa °C^{-1}) and 0.408 is a conversion factor from MJ m^{-2} day^{-1} to equivalent evaporation in mm day^{-1}.

Hargreaves and Samani (1985) developed a simplified equation requiring only temperature, day of year and latitude for calculating evapotranspiration (ET_{HS}):

$$ET_{HS} = 0.00135\ K_T(T_a + 17.78)(T_{max} - T_{min})^{0.5} R_a \quad (3)$$

where, T_{max} is the daily maximum air temperature at 2 m height (°C), T_{min} is the daily minimum air temperature at 2 m height (°C), R_a is extraterrestrial radiation in millimetres per day and can be obtained from tables (Samani, 2000). Because *KT* (empirical coefficient) assumes a value of 0.17, the 0.0135*KT* constant can be replaced by 0.0023 (Hargreaves and Samani, 1985).

The Priestley-Taylor equation (Priestley and Taylor, 1972), (ET_{PT}) is given by

$$ET_{PT} = \alpha \frac{R_n - G}{\Delta - \gamma} \quad (4)$$

where α are parameter:

$$\alpha = 1 + \frac{r_H^{-1}}{\Delta + \gamma^*}\left[\gamma r_c + (\Delta + \gamma)\Delta^{-1}\rho C_p \frac{VPD}{R_n - G}\right] \quad (5)$$

where *rH* and *rc* are the aerodynamic and bulk surface resistances, respectively, ρ is the atmospheric density, C_p is the specific heat of moist air and γ^* is a modified psychrometric constant (Monteith and Unsworth, 1990).

Alltests were carried out with Microsoft Excel (paired-type t-test) and SPSS Statistics version 17.0 software (repeated measure ANOVA).

Results and discussion

The presence of sediment had no significant effect on daily mean evaporation, although it increased evaporation by 6.1% compared to the *C* (Figure 1). Yellow waterlily further increased daily mean evaporation by 10.6% compared to the *C* and 4.8% compared to the *S*. A significant difference was found between *C* and *YWL* ($p<0.001$). According to Waheeb Youssef and Khodzinskaya (2019), some floating aquatic plants such as water lily, can reduce the evaporation of water reservoirs. The reason for the decrease in evaporation is that the aquatic plant preventing the connection between the air and the boundary layer of water. In a previous study, Snyder & Boyd (1987) also came to this conclusion. However, these authors did not consider the process of transpiration (Jiménez-Rodríguez et al., 2019). In contrast to the former, several have reported that the presence of aquatic plants increases evaporation (Brezny et al., 1973; Anda et al., 2018; Jiménez-Rodríguez et al., 2019).

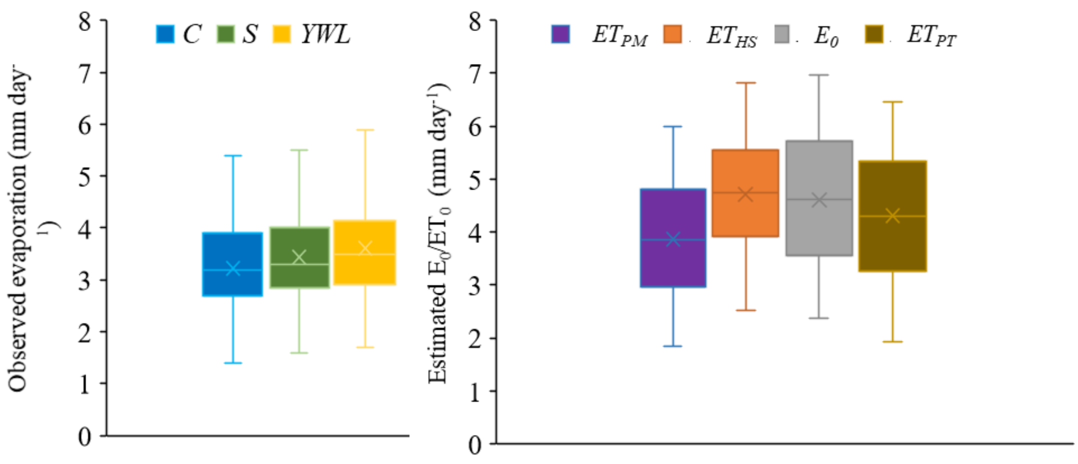

Figure 1. a) Daily mean pan evaporation (mm day^{-1}) of treated Class A pans: control pan (C), Class A pan with sediment cover bottom (S) and Class A pan with yellow water lily (YWL). b) Estimated daily mean evaporation (Shuttleworth formula, E_0) and evapotranspiration (ET_{PM} - FAO56 Penman- Monteith formula, ET_{HS} - Hargreaves-Samani formula and ET_{PT} - Priestley-Taylor formula)

The estimated *E/ET* was 16.6 – 31.4% higher than the E_p of *C* in 2020 growing season. In the case of *S*, there were low differences between the measured E_p and estimated *E/ET* (11.2-27.0%). E_p of *YWL* was closest to the estimated *E/ET* values, the estimated values were 6.7-23.3% higher than the measured rates. Irrespective of the Class A pan treatment, the ET_{PM} deviated the least from the measured E_p.

Irrespective of the pan treatments, there was a deviation in measured E_p rates and estimated *E/ET* values (Figure 2). The estimated *E/ET* values represent a different correlation (R^2 ranged from 0.437 and 0.5765, regardless of pan treatment). The highest R^2 value was for ET_{PM} for all pan treatments (*C*: 0.5765, *S*: 0.5395, *YWL*: 0.5386). Consistent with this, RMSE values were lowest for ET_{PM} regardless of pan treatment (*C*: 0.59 mm day^{-1}, *S*: 0.63 mm day^{-1}, *YWL*: 0.67 mm day^{-1}). Of the pan treatments, *C* was the highest R^2 values for all four estimated *E/ET* (R^2=0.5235-0.5765). Comparing the pan treatments, the lowest R^2 value was for ET_{PM}, ET_{HS} and ET_{PT} for *YWL* (0.5386, 0.437 and 0.4663, respectively). For E_0, R^2 was lowest for *S* (0.4957). RMSE values for all estimated *E/ET* were highest for *YWL* pan treatment (see Figure 2).

Tukey post-hoc comparisons revealed no significant difference between the E_p rate of *YWL* and ET_{PM} rate (p=0.510) (Table 1.). Therefore, of the formulas examined the Penman-Monteith assumption may be most suitable in a lake's estimation of evaporation when composed of floating leaf macrophytes (e.g. yellow water lily). Anda et al. (2018) concluded that estimated E_0 rates overestimated the measured E_p rates, when the Class A pan contained submerged macrophytes and sediments. Furthermore, similar to the results of current study, the ET_{PM} rate was closest to the measured E_p rate of submerged macrophyte in Class A pan.

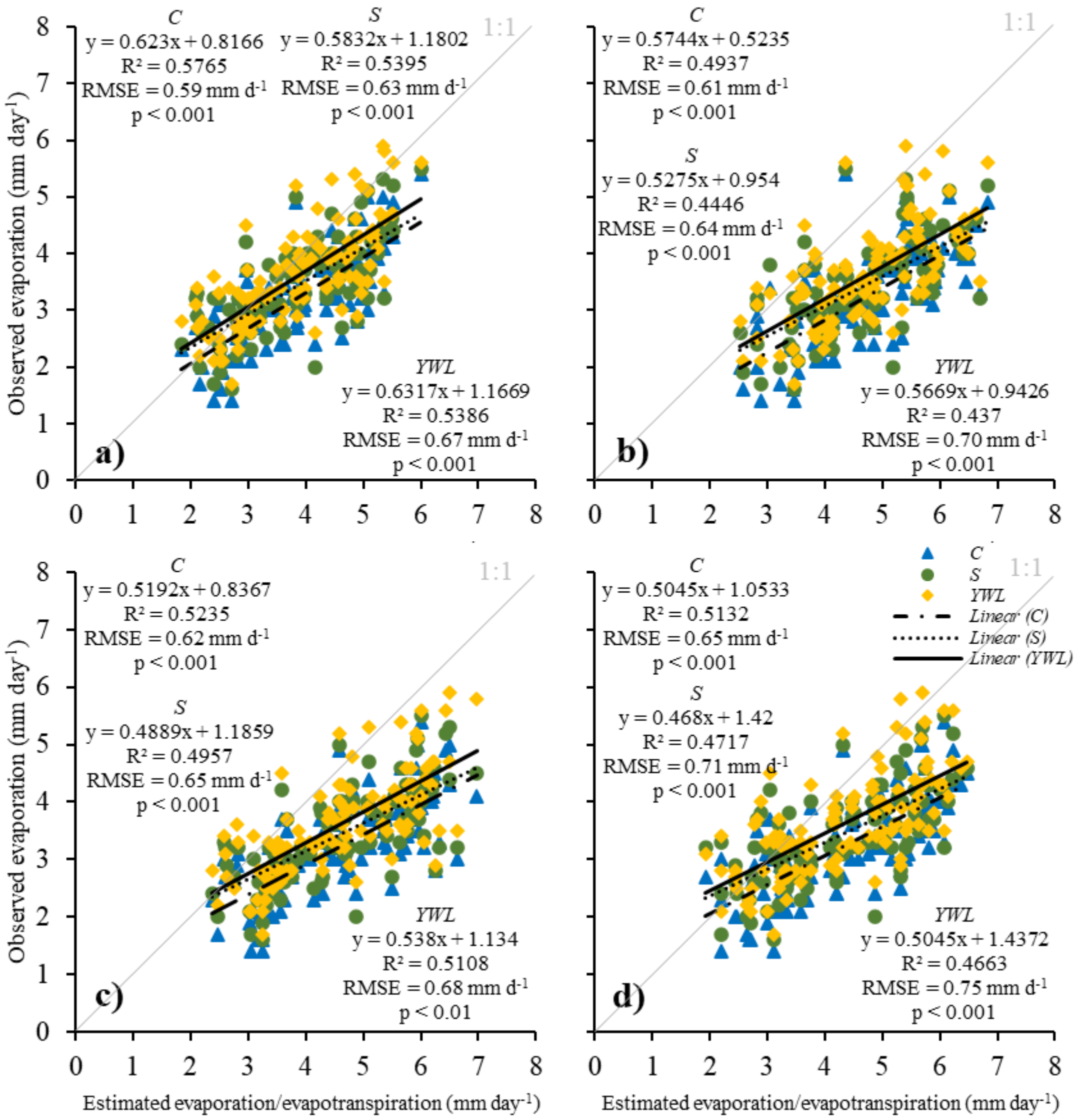

Figure 2. Relationship between the daily measured Class A pan evaporations (observed evaporation in different pan treatments) and daily reference evaporations and daily reference evapotranspirations computed by the FAO56 Penman- Monteith formula (a), Hargreaves-Samani formula (b), Shuttleworth formula (c) and Priestley-Taylor formula (d). C, S, and YWL denotes control, sediment covered, and yellow water lily implemented Class A pans, respectively.

Table 1 Effect on the observed evaporation (control pan - C, Class A pan with sediment cover bottom - S and Class A pan with yellow water lily - YWL) and estimated evaporation/evapotranspiration rates (E_0 - Shuttleworth formula, ET_{PM} - FAO56 Penman- Monteith formula, ET_{HS} - Hargreaves-Samani formula and ET_{PT} - Priestley-Taylor formula)

(I) treatment	(J) formula	Mean Difference (I-J)	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
C	ET_{HS}	-1.480*	**0.000**	-1.892	-1.068
	ET_{PM}	-0.642*	**0.000**	-1.054	-0.231
	ET_{PT}	-1.082*	**0.000**	-1.494	-0.671
	E_0	-1.378*	**0.000**	-1.789	-0.966
S	ET_{HS}	-1.270*	**0.000**	-1.682	-0.859
	ET_{PM}	-0.433*	**0.032**	-0.844	-0.021
	ET_{PT}	-0.873*	**0.000**	-1.284	-0.461
	E_0	-1.168*	**0.000**	-1.580	-0.757
YWL	ET_{HS}	-1.096*	**0.000**	-1.508	-0.685
	ET_{PM}	-0.258	0.510	-0.670	0.153
	ET_{PT}	-0.699*	**0.000**	-1.110	-0.287
	E_0	-0.994*	**0.000**	-1.405	-0.582

Based on observed means.
The error term is Mean Square(Error) = 1.017.
The error term is Mean Square(Error) = 1.065.
The error term is Mean Square(Error) = 1.327.
*. The mean difference is significant at the 0.05 level.

Conclusion

The change in E_p of a standard Class A pan with a sediment-covered bottom and with a floating leaf macrophytes (yellow water lily) was observed at Keszthely, in 2020 growing seasons. For *S* and *YWL* pan treatments increased E_p with a larger increment when floating leaf macrophytes were used but a smaller increment when sediment was used compared to *C*. Estimated ET_{PT}, ET_{HS} and E_0 rates overestimated the measured E_p rates during this study for all three pan treatments, therefore we do not propose the use of them to estimate evaporation of lake or reservoir with floating leaf macrophytes. However, ET_{PM} may be considered appropriate for estimating a lakes and reservoirs evaporation with floating leaf plants.

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A PRELIMINARY STUDY IN MODELLING EVAPOTRANSPIRATION OF COMMON REED STANDS IN THE KIS-BALATON WETLAND

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Abstract

During the examination of the water balance of Kis-Balaton wetland, the direct measurement and determination of evaporation and evapotranspiration is difficult. Evapotranspiration - as outcome parameter - is essential for the operation of the Kis-Balaton Water Protection System (KBVR). The evapotranspiration of the common reed (*Phragmites australis*), which plays a decisive role in ecosystems of KBVR marshes is a significant factor due to the fact that the area of reed canopies exceeds 2,000 hectares. The actual evapotranspiration from the heat balance and the Bowen ratio can be modelled indirectly by using microclimate measurements. Modelling can be performed with resistances expressed by meteorological parameters, whose values are temporally variable. Examinations in a reed canopy of Ingói-berek was carried out with a Bowen mast from July to August 2019. The following meteorological parameters were measured every 10 minutes: surface water temperature, air temperature and air humidity in the canopy, air temperature and humidity and wind speed at two levels above the canopy. Our measurements were supplemented weekly with canopy heights and leaf surface measurements (LAI), which are important model inputs. Hourly and daily evapotranspiration data were counted from the 10-minute microclimate probes. Our goal was to examine how Bowen-ratio modelling can be used to estimate reed evapotranspiration.

Keywords: wetland, common reed, evapotranspiration, Bowen-ratio

Összefoglalás

A Kis-Balaton vizes élőhely vízháztartásának vizsgálata során a párolgás és az evapotranszspiráció mérése és meghatározása közvetlen méréssel nehezen megoldható. A párolgás - mint eredményparaméter - a Kis-Balaton Vízvédelmi Rendszer (KBVR) működéséhez elengedhetetlen. A KBVR mocsarak ökoszisztémájában meghatározó szerepet játszó közönséges nád (*Phragmites australis*) párolgása jelentős tényező, mivel a nádasok területe meghaladja a 2000 hektárt. A hőháztartási egyenletből és a Bowen-arányból származó tényleges evapotranszspiráció mikroklímamérésekkel közvetve modellezhető. A modellezés meteorológiai paraméterekkel kifejezett ellenállásokkal végezhető, amelyek értékei időben változóak. Az Ingói-berek nádállományában 2019 júliusától augusztusáig Bowen-oszloppal végeztünk vizsgálatokat. A meteorológiai paramétereket 10 percenként mértük az alábbiak szerint: felszíni vízhőmérséklet, az állományban a levegő hőmérséklete és páratartalma, az állomány feletti két szintben a levegő hőmérséklete és páratartalma, valamint a szélsebesség. Méréseinket hetente kiegészítettük az állomány magasság és a levélfelület (LAI) mérésével, amelyek fontos modellbemeneti adatok. Az óránkénti és napi evapotranszspirációs adatokat a 10 perces szenzoradatokból számoltuk. Célunk megvizsgálni, hogy a Bowen-aránnyal történő modellezés hogyan használható a nád párolgásának becslésére.

Kulcsszavak: vizes élőhely, nád, evapotranszspiráció, Bowen-arány

Introduction

Evapotranspiration (ET) is the main component of wetlands' water loss, the highest energy consumer of incoming solar radiation, considered latent heat flux as its energy equivalent (Priban & Ondok 1985). The reed (*Phragmites australis* L.) is the dominant macrophyte plant of the Kis-Balaton. The area of its contiguous stands in the Kis-Balaton Water Protection System (KBWPS) is estimated to be about 2,000 ha. The presence of small or large coherent reed stands are very common in wetland habitats in the whole world (Struyf et al., 2007). The main objective of our study was to estimate the in-situ evapotranspiration of reed beds. A domestic precedent on reed evaporation can be found in Walkowszky (1973), in which the study site was Lake Fertő. Walkowszky tried to keep the reeds alive in evapotranspirometers, which could not survive more than 1 month, so that permanent replanting was necessary. In the case of the Ingói-berek of KBWPS, there is no possibility to use site-installed evapotranspirometers, so as an indirect method we tried to model the actual evapotranspiration by microclimate measurements based on the heat balance equation and the Bowen ratio.

Material and method

The site of our investigations was the Ingói-berek of the Lake Fenéki of the KBWPS Phase II, where the Bowen station was installed (N 46° 38' 8.6", E 17° 11' 57.6") (Figure 1). The station measures the following meteorological parameters at 10-minute resolution: surface water temperature and mean water temperature, canopy air temperature and humidity, above-stand air temperature and humidity at height of 1 and 2 m, above-stand wind speed at height of 2 m and sun radiation.

Figure 1. Measuring site in the Ingói-berek of Lake Kis-Balaton and the reference Agrometeorological Station in Keszthely (Google Earth)

Boreas type wind sensor at 2 m above the canopy were installed. Radiance was measured with Delta Ohm HD 2102.2 and Delta Ohm HD 2302.0 RAD sensors (Figure 2).

Figure 2: Bowen station in the Ingói-berek (July 23, 2019, photo bí Gábor Soós)

The hourly evapotranspiration was modelled using the Massman and Burba method (Massman, 1992; Burba et al.,1999). The basis is the net energy balance, R_n (the difference between shortwave and longwave radiation), which is the source of the energy-intensive processes in the reed stand. From the energy balance equation:

$$R_n - G - \lambda E - H \approx 0 \qquad (1)$$

where: H: sensible heat flux, λE: latent heat flux, G: ground heat flux.

Bowen ratio: expressed as the ratio of sensible (H) to latent heat fluxes (λE):

$$\beta = H/\lambda E \qquad (2)$$

The Bowen ratio can be calculated from the vertical change in air temperature and vapour pressure:

$$\beta = \gamma \frac{\Delta T}{\Delta e} \tag{3}$$

The latent heat flux:

$$\lambda E = \frac{R_n - G}{1 + \beta} \tag{4}$$

In our case, G is calculated from the change in water temperature:

$$G = c_w d \left(\frac{dT}{dt}\right) \tag{5}$$

where c_w is the specific heat of water, d is the water depth, dT is the change in temperature with respect to dt.

The sensible heat flux (H):

$$H = \beta \frac{R_n - G}{1 + \beta} \tag{6}$$

The surface energy budget is (R_{ns}):

$$R_{ns} - G - \lambda E_s - H_s \approx 0 \tag{7}$$

where: λE_s: surface latent heat flux, H_s: surface sensible heat flux.

The energy budget of a given thickness of vegetation (R_{nv}):

$$R_{nv} - \lambda E_v - H_v \approx 0 \tag{8}$$

where: λE_v: vegetation latent heat flux, H_v: vegetation sensible heat flux.

The net radiation is the combined energy of the surface and the stand:

$$R_n = R_{ns} + R_{nv} \tag{9}$$

The sums of latent and sensible heat:

$$\lambda E = \lambda E_s + \lambda E_v \tag{10}$$

and

$$H = H_v + H_s \quad (11)$$

The surface net radiation is calculated from the Monsi-Saeki (1953) formula. The extinction coefficient (k) is determined using digital image processing by weighing the incident radiation by the area ratio of sunlit and shadowed spots:

$$R_{ns} = (R_n)\exp(-kLAI) \quad (12)$$

Surface Bowen ratio, (β_s) latent heat flux, and sensible heat flux are approximated as follows:

$$\beta_s = \frac{H_s}{\lambda E_s} \quad (13)$$

$$\lambda E_s = \frac{R_{ns} - G}{1 + \beta_s} \quad (14)$$

$$H_s = R_{ns} - G - \lambda E_s \quad (15)$$

β_s is needed to calculate λEs, Hs, λEv and Hv. derived by Massman (1992), taking into account that in our case the resistance to surface water vapor transport is zero.

$$\beta_s = \frac{\rho_a C_p (T_{ws} - T_a) - Hr_a}{\left[(\lambda\gamma\rho_a / P)(e_{*Tws} - e_a) - \lambda E r_a\right]} \quad (16)$$

Where: ρ_a: wet air density, C_p: air heat capacity, T_{ws}: water surface temperature (measured), T_a: air temperature (measured), r_a: aerodynamic resistance,

λ: latent heat capacity of vapour, γ: psychrometric constant, P: atmospheric pressure, e_{Tws}: surface vapour pressure (measured), e_a: above-canopy vapour pressure (measured).

The reference evapotranspiration (ET_0) is calculated from data of the nearby (distance from measuring site: 7 km) Agrometeorological Station in Keszthely (N 46°44'; E 17°14') (Figure 1). The FAO-56 Penman-Monteith ET_0 [mm day^{-1}] equation (Allen et al., 1998) is as follows:

$$ET_0 = \frac{0.408\,\Delta(R_n - G) + \gamma \frac{900}{T+273} u_2 (e_s - e_a)}{\Delta + \gamma\,(1 + 0.34\,u_2)} \quad (17)$$

where T is mean daily air temperature at 2 m height, u_2 is wind speed at 2 m height, e_s is saturation vapour pressure, (e_s-e_a) is saturation vapour pressure deficit, Δ is slope vapour pressure curve, 0.408 is a conversion factor.

The calculation of the new daily crop coefficients as a dimensionless indicators using reference evapotranspiration (Eq. 17) and the modelled actual evapotranspiration λE (Eq. 10):

$$K_c = \frac{ET_c}{ET_0} \quad (18)$$

The monthly weather of 2019 July and August were characterized by the Thorthwaite Index (TI) based on the World Meteorological Organisation (WMO) Report (1975):

$$TI = 1.65\left(P / T_a + 12.2\right)^{10/9} \quad (19)$$

where P and T_a are the monthly sum of precipitation and the monthly mean of air temperatures, respectively.

Anda et al. (2014) suggested the next categories after calculating TI for monthly climate norms (1971-2000), and assuming 20% deviation:

Hot (dry) month (h): $TI_{month} > TI_{norm} \times 0.8$,

Cool (wet) month (c): $TI_{month} > TI_{norm} \times 1.2$,

Month with normal weather (n): $TI_{norm} \times 0.8 \leq TI_{month} \leq TI_{norm} \times 1.2$.

Anda et al. (2014) published the K_c values (Table 1) due to TI for *P. australis*.

Table 1 Values of reed crop coefficients (K_c) for July and August with different weather (Anda et al., 2014).

Season	July	Aug.
Cool	0.77	0.8
Normal	1.51	0.99
Hot	1.62	1.39
Average	1.46	1.22

Results and discussion

Characterizing the investigated period with the TI, July (Ta: 22.8 °C P: 92.1 mm) was 19% higher and August (Ta: 22.6 °C P: 25.9 mm) was with 67% lower than TI_{norm} of 1971-2000. At the same time, 1,51 and 1.39 K_c values were computed for "Normal" July and "Hot" August, with respectively. Due to weather conditions in summer 2019, high monthly mean ET_0 of 4.55 mm day^{-1}were computed in July, while somewhat lower monthly mean ET_0 of 3.98 mm day^{-1} for August were detected. With these counted K_c values, the $ET_{a\text{-}Kc}$ for reed was calculated using equation 18 and ET_0. The modelled data series $ET_{a\text{-}Bowen}$ were compared with these $ET_{a\text{-}Kc}$ data series.

For the modelled data the measured LAI was between 3.9 and 4.4 and the weighing average extinction coefficient (k) was 0.4. Hourly and then daily total evapotranspiration ($ET_{a\text{-}Bowen}$) was produced from the ten minutes of measurements. The model allows the calculation of evaporation and transpiration, separately. The daily values of mature vegetation in July and August 2019 are shown in Figure 3.

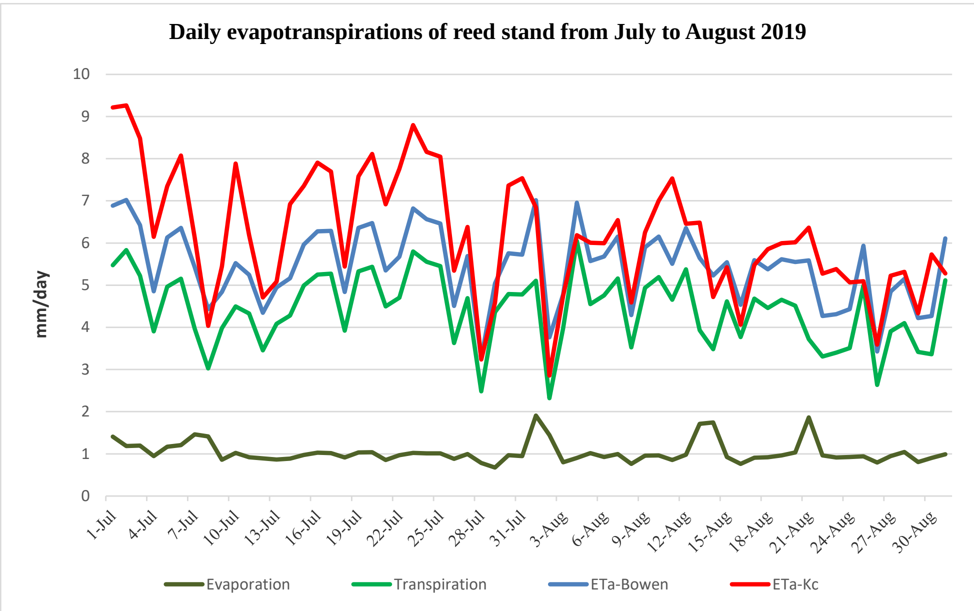

Figure 3 Daily evapotranspiration of reed stand ($ET_{a\text{-}Bowen}$) in the Ingói-berek and $ET_{a\text{-}Kc}$. $ET_{a\text{-}Kc}$ for reed was calculated using equation 18 and ET0.

The daily $ET_{a\text{-}Kc}$ ranged from 3.24 to 9.26 mm in July and from 2.86 to 7.53 mm in August. The daily $ET_{a\text{-}Bowen}$ values ranged from 3.27 to 7.02 mm in July and from 3.43 to 7.01 in August. The $ET_{a\text{-}Kc}$ values were 13.7% higher than that of the modelled $ET_{a\text{-}Bowen}$. The pattern of evapotranspiration curves was similar in the two ET assumptions, although, with smoother distribution in $ET_{a\text{-}Bowen}$.

The average portion of Evaporation and Transpiration to Evapotranspiration ($ET_{a\text{-}Bowen}$) are 0.19 (19%) and 0.81 (81%), respectively. The time series, with a normal data distribution, a paired-sample t-test was applied to compare differences between $ET_{a\text{-}Bowen}$ and $ET_{a\text{-}Kc}$ from ET_0 and Kc counted by Anda et al. (2014). The difference between daily modelled $ET_{a\text{-}Bowen}$ and $ET_{a\text{-}Kc}$ with average values of 5.46 and 6.21 mm respectively, was significant: $P(T<=t) = 0.000$. The average deviation was 0.75 mm.

We also counted new daily Kc values using the modelled reed evapotranspiration ($ET_{a\text{-}Bowen}$) and ET_0 from the Agrometeorological Station in Keszthely (Figure 4). The average values for July and August were 1.26 and 1.34 respectively. The August K_c value was closer to 1.39 suggested by Anda et al. (2014) than the July’s to the extreme 1.51. So the wetland microclimate could be more balanced as it was thought earlier. The explanation could be the large water table characterized with the high value of specific heat of water. This could result in smoothing the original K_c curve, suggested by Anda et al. (2014).

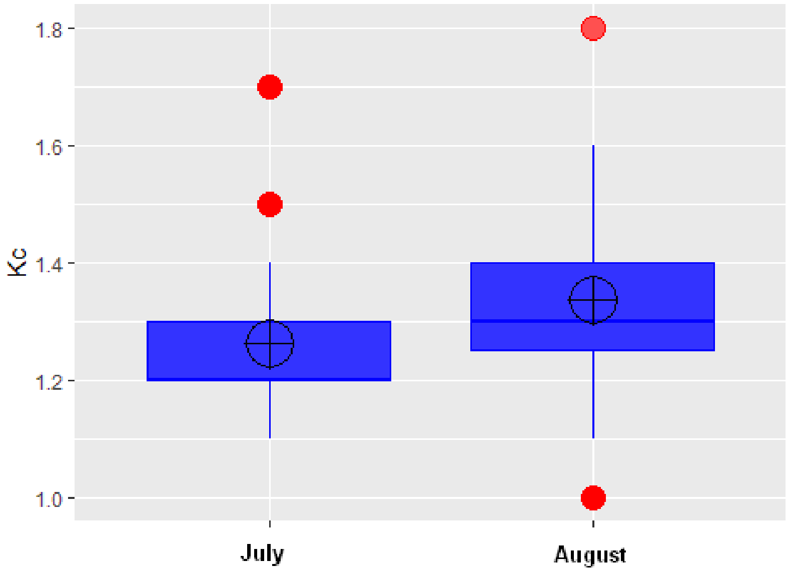

Figure 4 Boxplot of the new crop coefficient values for July and August with outliers. The crosshairs represent the mean

Conclusion

The crop coefficient values (K_c) were a good estimator of the reed water demand as published by Anda et al. (2014) through evapotranspirometer installed at the Agrometeorological Station in Keszthely. The method is also suitable for estimating the evapotranspiration of the large reed beds of Lake Kis-Balaton. We also should take into consideration the new discussion to get more precise outcome. Using this assumption, the largest outcome of the water balance, the ET can be determined more accurately.

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