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MYCORRHIZA AND STRESS TOLERANCE OF VEGETABLES: A REVIEW

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From year to year, the world growing area is being more poluted with heavy metals or excessive salt level and exposed to lack of moisture or avaiable nutrients in the soil. This resulting in a loss of agricultural land where vegetables were grown in the past. The producers must also fight with more resistant and new species or strains of soil pathogens, while chemical protection is not always the most suitable solution for human health and the environment. Our review focuses on the great importance of using arbuscular mycorrhizal fungi to alleviate abiotic and biotic stress, taking into account the use in vegetable production. The review is gradually focusing on individual stressors and defines the mechanisms of mycorrhizal fungi that contribute to the sustainable agriculture even under severe stress conditions.

Keywords: mycorrhizal fungi, abiotic stress, biotic stress, vegetables

Mycorrhizal symbiosis, a mutually beneficial relationship between soil fungi and plant roots, is one of the first symbiotic relationships that have evolved on our planet. Already more than fifty years ago, Balis in the New Zealand observed positive impact of mycorrhizae on the phosphorus uptake by the host plant. By observing nature around us, but also by controlled experiments, humans have gradually discovered many other benefits that mycorrhizal fungi bring to host plants. Nevertheless, even today we do not know everything about mycorrhiza. Mycorrhizal symbiosis is an extremely complicated relationship, and a lot of effort has to be made to obtain any new information about its functioning. Especially in Turkey, Italy, India and other Asian countries many scientists have been dealing with mycorrhiza and from year to year, increasing number of scientific papers describe the newly found positive effects it has on plants, or the possibility of its use in agriculture and forestry. Mycorrhizal symbiosis is widespread in nature. If we grow a plant in the absence of mycorrhizal fungi, we do something unnatural, something that is unusual in nature. Majority of plants host mycorrhizal fungi on their roots and finding a plant without them is quite difficult. As we cross the countryside, we constantly step on an invisible underground network of fine fibers (*hyphae*) of mycorrhizal fungi (Gryndler et al., 2004; Koide and Mosse, 2004). However, in Slovakia, mycorrhiza has not yet become known to the growers as much as it deserves. We believe that this review could also help to make this issue more visible and help to share the latest knowledge about the use of mycorrhizal fungi in horticulture.

For vegetable growers, the most important type of mycorrhiza is arbuscular mycorrhizal symbiosis. Arbuscular mycorrhiza (AM) is the most ancient and widespread form. Paleobotanical and molecular sequence data suggest that the first land plants formed associations with *Glomalean* fungi from the *Glomeromycota* about 460 million years ago. This is estimated to be some 300–400 million years before the appearance of root nodule symbioses with nitrogen-fixing bacteria (Finlay, 2008). Intercellular and intracellular fungal filaments are characteristic – *hyphae* and especially specific intracellular structures – arbuscules (these are tree-like structures that allow the interchange of nutrients between the fungus and the plant) and vesicles (cams that store the stock for both the fungi and the plant) (Figure 1). According to these unique structures, this type of endomycorrhiza is also called vesicular-arbuscular mycorrhiza (VAM). Fungal organisms that are involved in this symbiosis live exclusively in connection with plants and do not form any visible structures to the naked eye. These fungi are also referred to as biotrophic symbionts that are unable to exist without their plant partner and cannot be artificially cultivated *in vitro* (Kavková, 2014).

The infection of the roots occurs through the inoculum which is present in the soil as spores or mycelium. After the first mycorrhizal infection, AM fungi are massively spreading in root cells and colonize surrounding cells, especially in deeper layers of the root cortex and intercellular spaces. From the surrounding soil, it leads to further infections of root cells and the connection between extramatricular mycelium, and mycelium inside the cells is multiplied (Mejstřík, 1988).

Genus *Glomus* is the largest genera of AM fungi, with 85 described species. This includes the important species such as *G. claroideum*, *G. etunicatum*, *G. mosseae* (e.g. *Funneliformis mosseae*), *G. viscosum*, *G. fasciculatum* or the most important and used *Rhizophagus irregularis* (previously

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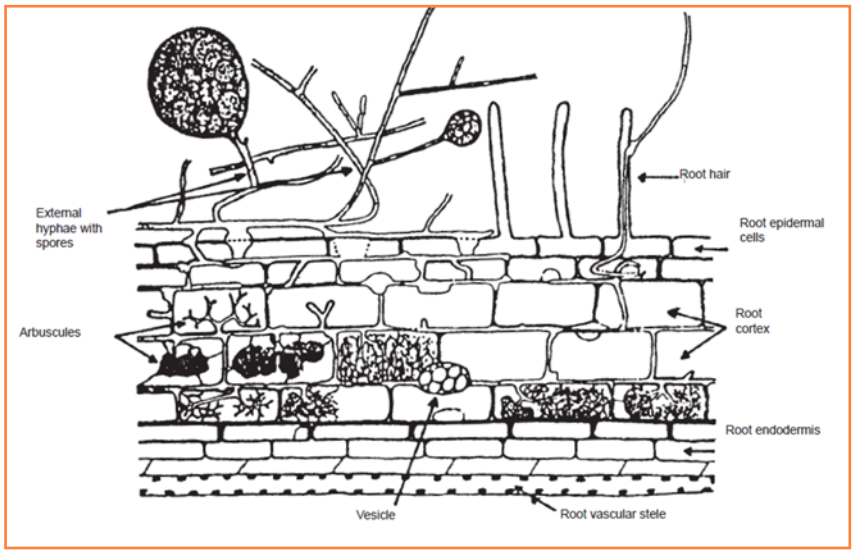

Figure 1 Diagram of a root colonized by AM fungi showing the diagnostic features of the fungi
Source: Habte, 2000

known as *Glomus intraradices*), which is also mentioned in several contexts of this article. *Rhizophagus irregularis* (*Glomeromycota*) has been found in different ecosystems around the world, including temperate and tropical regions. It is also the first AM fungi from which the genome was sequenced (Tisserant et al., 2012).

AM fungi are able to create a symbiosis with most vegetables including major crops of different families, such as: *Alliaceae*, *Apiaceae*, *Asteraceae*, *Fabaceae* and *Solanaceae* (Baum et al., 2015). In the cultivation of vegetables and other crops with AM fungi, it is also recommended in crop rotation to rotate only crops that are also able to form mycorrhizal symbiosis, which also applies to inter-crops. It is unsuitable to use fungicides for mycorrhizal cultivation, as along with pathogenic fungi we can kill mycorrhizal ones. Too frequent application of herbicides and insecticides is also unsuitable (Elbon and Whalen, 2015).

The importance of mycorrhizal fungi against abiotic factors

Drought

Drought is one of the major constraints on plant productivity worldwide and is expected to increase with climatic changes. Several ecophysiological studies have demonstrated that AM symbiosis is a key component in helping plants to cope with water stress and in increasing drought resistance (Figure 2). The alleviating effect of AM symbiosis in response to drought generally relies on the positive effects of AM fungi on the uptake and transport of water and on an improved uptake of nutrients, especially of available soil phosphorus (P) and other immobile mineral nutrients, resulting in the hydration of plant tissues, a sustainable physiology and a clear promotion of growth (Rapparini and Peñuelas, 2014).

An increased root length and density or an altered root system morphology (Fig. 3), as enhancing soil exploration and water extraction, have been hypothesized as potential mechanisms for the improved drought resistance of mycorrhized plants. Enhancement of plant stomatal control or root water uptake by mycorrhizal *hyphae*, as well as turgor maintenance by osmotic adjustment, have been documented. At the levels of both leaves and roots, the osmotic stress usually caused by drought is counteracted by mycorrhizal plants through biochemical changes that mostly include increased biosynthesis of metabolites (mainly proline and sugars) that act as osmolytes. These compounds contribute to the lowering of the osmotic potential, and in turn, of the leaf water potential. These lower potentials allow the plants to maintain high organ hydration and turgor that sustain overall cell physiological activity, mainly related to the photosynthetic machinery. AM plants withstand drought-induced oxidative stress with increasing the activities of antioxidant enzymes. Mycorrhizal fungi also affect the hydraulic conductivity and gas exchange in the root and leaves. Molecular mechanisms activated by AM symbiosis to counteract drought include gene activation of functional proteins, such as the membrane transporter aquaporins (Bethlenfalvay and Linderman, 1992; Candido et al., 2015; Rapparini and Peñuelas, 2014). Rob (1985) also stated that mycorrhizal plants restored their normal functions after a temporary shortage of water

Figure 2 Pepper plant with AM fungi on the right and non-AM plant on the left
Source: Davies, 2016

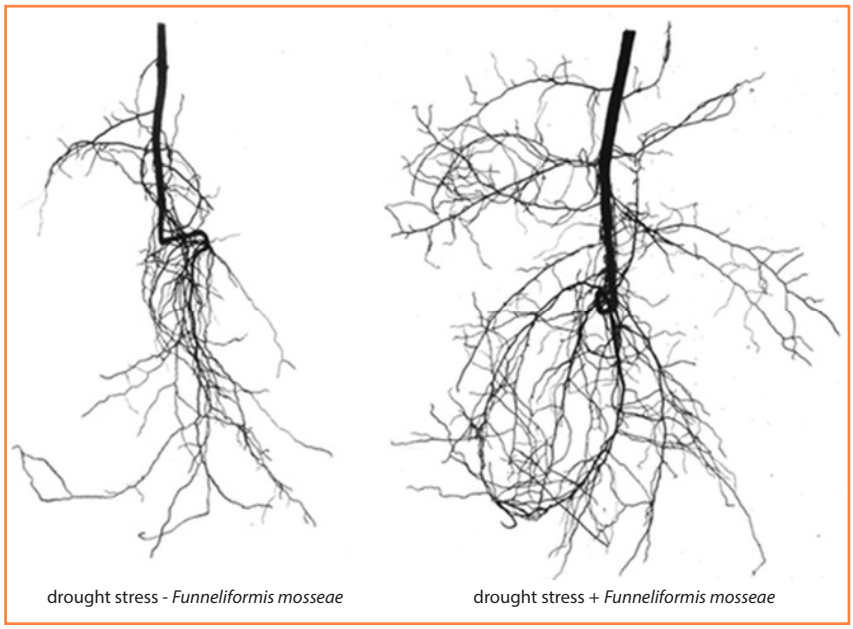

Figure 3 Root inoculated with *Funneliformis mosseae* under drought stress
Source: Wu and Zou, 2017

faster than non-mycorrhizal plants. He assumed that the cause was to reduce the resistance on the water transport pathways.

In addition, AM symbiosis can increase the resistance of plants to drought through secondary actions such as the improvement of soil structural stability that in turn increases the retention of soil water. For example, Tauschke et al. (2015) indicated a positive effect on corn, soybeans, onions or lettuce in their study. Hazzoumi et al. (2015), during their research, reported that basal plants inoculated with *G. intraradices* showed better growth than non-inoculated during drought stress, while mycorrhiza improved growth of the overground parts of the plant by 51% and the root part by up to 130%.

Soil salinity

To deal with saline soils and minimize crop loss, scientists have searched for new salt-tolerant crops or varieties or breaded salt-tolerant cultivars through breeding etc. However, biological processes such as mycorrhizal application to alleviate salt stress could be a better option. AM fungi have been reported to promote plant growth and salinity tolerance by many researchers. It is found to vary with the isolates of fungus and species of plants (Heikham, Rupam and Bhoopander, 2009). Several authors reported (Heikham, Rupam and Bhoopander, 2009; Baum et al., 2015) that mycorrhizal fungi promote salinity tolerance by employing various mechanisms, such as enhancing nutrient acquisition, producing plant growth hormones, improving rhizospheric and soil conditions, altering the molecular (changes in gene expression and ultrastructural changes), biochemical (accumulation of antioxidants, proline, betaine or soluble carbohydrates) and physiological (photosynthetic activity, relative permeability, water relation or nodulation and nitrogen fixation in vegetables) properties of the host. In addition, AM fungi can improve host physiological processes like water absorption capacity of plants by increasing root hydraulic conductivity and favourably adjusting the osmotic balance and composition of carbohydrates. This may lead to increased plant growth and subsequent dilution of toxic ion effect. At experiments on soybean, bean, and vigna plants, mycorrhizal plants have been found to have a significantly increased accumulation of proline, non-toxic and protective osmolyte, which helps the plant maintain osmotic balance at low water potentials and acts as a reservoir of energy and nitrogen during salt stress. At higher salinity levels, the *glycine* betaine (quaternary ammonium compound) content in AM plants was about twice as large as in non-AM plants. AM ameliorated the negative influence of salinity stress which enhanced the activity of antioxidant enzymes such as catalase (CAT) and peroxidase (POD). What is more, AM inoculation further improved their activity, thus strengthening the defense system of plants (Heikham, Rupam and Bhoopander, 2009; Mohsen and Abdel-Rahman, 2016; El-Sarkassy, Ibrahim and Desoky, 2017). A good example would be Al-Karaki (2013), who observed greater size and weight of the root dry matter during salt stress, as well as the overall increased yield of tomato fruits in terms of both weight and quantity, for mycorrhiza plants, as in any variety of non-mycorrhiza tomato plants. Heikham, Rupam and Bhoopander (2009) reported improved growth, yield, water status, nutrient content and fruit quality of the pumpkin plant colonized with the species of the genus *Glomus* sp under salt stress. El-Sarkassy Ibrahim and Desoky (2017) also mentioned that application of mycorrhiza gave positive effect and enhanced all growth parameters of pepper plant at all levels of salt stress (2,000 ppm and 4,000 ppm), thus acting as a growth stimulant.

Heavy metals

Hildebrandt, Regvar and Bothe (2007) indicated that in the vegetable production, particularly *Glomus intraradices* isolates proved to be successful. Under conditions of optimal root infection they can provide protection against several heavy metals in the soil for many plant species. But until recently, it could not have been distinguished, whether elements in the inner root parenchyma cells were deposited in the fungal cells, plant cells or both types. Very recent results obtained from electron-dispersive X-ray spectrometry (EDXA) showed that Zn, Cu and Cd accumulated in the cell wall and in electron-dense granules in the cytoplasm of the fungi while their cytoplasm itself was essentially free of these elements. Other studies showed that glomalin produced by AM fungi and in large quantities released into the soil is also capable of immobilizing a significant amount of metal (Seguel, 2014). Increased tolerance to Cd stress was observed in carrots with inoculated AM fungi of

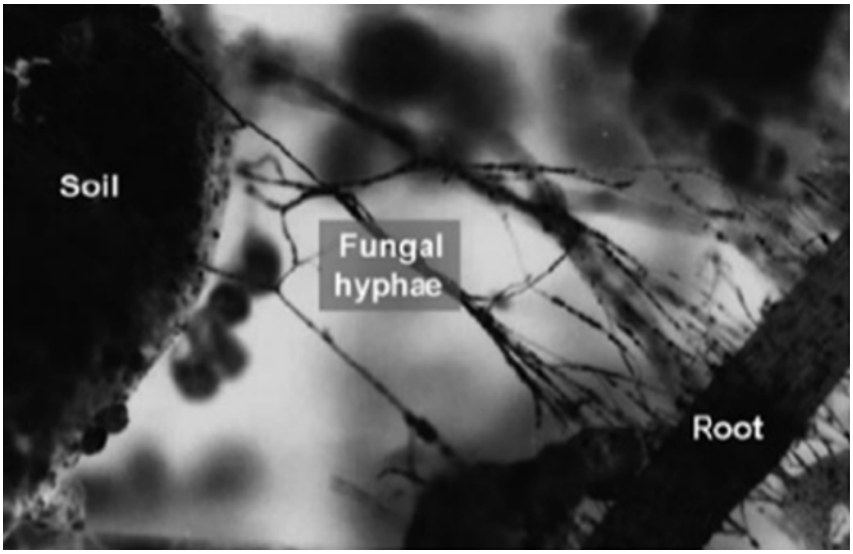

Figure 4 Mycorrhizal fungi filaments (*hyphae*) which enhance soil aggregation and play an important role in soil structure development
Source: Al-Karaki, 2013

Glomus intraradices. In this case, the concentration of polyamines in the roots was also measurably reduced (Baum, El-Tohamy and Gruda, 2015). Hildebrandt, Regvar and Bothe (2007) demonstrated the buffering effect of mycorrhizal colonization on pea and barrelclover plants under stress from Cd.

Cold

The impact of mycorrhiza on cold stress is the subject of many scientific studies. For non-AM plants there have been observed a decrease in photosynthetic activity due to the stress, but for AM plants the same photosynthetic activity proved no stress conditions. They conclude that in mycorrhizal plants the photosynthetic apparatus was less damaged due to stress, which also affects the tolerance of the plant. Oxidation of membrane lipids is a reliable indicator of uncontrolled production of free radicals, and thus oxidative stress. In case of plants without mycorrhiza, this oxidation was higher. For mycorrhizal plants, MDA content has increased only minimally or not at all. As a result, antioxidant protection was higher in AM plants than in non-AM. It indicated that vaccination with AM fungi results in a well-established defense mechanism against cold, and that the alleviation of oxidative stress can be an essential part of the real effect of symbiosis (Pedranzani et al., 2015).

Physical properties of the soil

AM fungi are considered as key determinants of soil quality. They develop an extensive network of external fungal filaments (*hyphae*) that act as an extension of the root absorbing area. Furthermore, AM fungi may play a role in the formation of stable soil aggregates, building up a macroporous structure of soil that allows penetration of water and air and prevents erosion, but also through elimination of sticky substances, such as the glycoprotein – glomalin, which bonds the soil particles into microaggregates, and then to more stable ones. As a result, inoculations of disturbed areas with mycorrhizal fungi could have a dual benefit: helping naturally indigenous or cultivated plants and improving soil stability macroaggregates (Figure 4) (Al-Karaki, 2013; Vogelsang et al., 2004).

The importance of mycorrhizal fungi against biotic factors

In addition to causing non-target effects, chemical pesticides are becoming more expensive year after year, loosing their effectiveness as a result of co-evolution and development of resistance to pathogens. Most of the new generation pesticides are systemic in their mode of action and leading to certain level of toxicity in the plant system and thus hazardous to health. Furthermore, they disturb the ecology of the microbial diversity in ecosystem and thus the whole environment. Therefore, many scientists have been currently working to find various alternative forms of vegetable protection against pests and pathogens. One of these, and not poorly observed, is mycorrhizal protection. Mycorrhiza inoculation has additional advantages as it is effective even against soil-borne pathogens, which are difficult and expensive to manage through chemical and physical treatments. Hence, mycorrhization has been proposed as an alternative for the management of soil-borne pathogens while AM fungi, such as biocontrol of plant diseases (biopesticides) are recommended by more and more experts and researchers in horticulture (Sharma et al., 2004).

Many studies have shown that AM fungi improve plant's resistance that consequently reduces incidence as well as severity of plant diseases caused by a wide range of attackers including viruses, bacteria, nematodes and fungi. Although well defined direct mechanisms are still unclear, several indirect mechanisms have been proposed by a number of researchers for increasing bioprotective ability of mycorrhizal plants (Singh and Giri, 2017).

AM fungi are known to improve plant growth and nutrient absorption and physiological responses of the host to environmental stresses resulting in more resistance or tolerance to pathogen attacks. These mycorrhiza-induced compensatory processes may explain the increased tolerance of mycorrhizal and P fertilized plants as they can compensate for loss of root mass or function caused by pathogens, including nematodes. Thanks to the influence of AM fungi on growth, absorption area and cellular processes in the roots, plants can compensate for losses of plant root mass and its functions caused by pathogens or nematodes. It has been suggested that nematode pathogens, on the other hand, require host nutrients for reproduction and development and direct competition with AM fungi has been hypothesized as a mechanism of their inhibitors. Since AM fungi, soil-borne fungal pathogens, and plant parasitic nematodes occupy similar root tissues, direct competition for

space has been postulated as a mechanism of pathogen inhibition by AM fungi (Sharma et al., 2004).

It is also important to remember that an indigenous fungus adapted to specific edifying conditions has the ability to survive and colonize plant roots better than a non-native species under similar conditions. It also has a greater inculcation potential and competitive ability (Gopal et al., 2015).

Singh and Giri (2017) reported many cases in which the positive effect of AM fungi on different types of vegetable pathogens has been demonstrated: effect on different pathogenic fungi such as: *Alternaria solani* or *Botrytis cinerea* on tomatoes; *Phytophthora capsici* on pepper; *Phytophthora parasitica* on tomatoes; *Phytophthora nicotianae* on tomatoes; *Phytophthora sojae* on soy; *Pythium ultimum* on cucumbers; *Rhizoctonia solani* on potatoes or tomatoes; *Sclerotium cepivorum* on onion. In the context of bacterial diseases, they mentioned positive effects on: *Pseudomonas syringae* pv. *glycine* on soy; *Ralstonia solanacearum* and *Stolbur phytoplasma* on tomatoes. As for nematodes, the following was presented: *Heterodera glycines* on soy; *Meloidogyne hapla* on tomatoes; *Nacobbus aberrans* and *Rotylenchulus reniformis* on tomatoes; but especially many *Meloidogyne incognita* studies on tomatoes.

Additionally, the magnitude of the AM-induced decline in disease severity/nematode suppression ranged from 30 to 42% and 44–57% for fungal and nematode pathogens respectively, irrespective of pathogens' identity or lifestyle suggesting that through AM fungi, plants possibly receive similar protection from all pathogens rendering AM formulations a potentially broadly effective biocontrol agent (Singh and Giri, 2017).

A suitable example would also be asparagus inoculated with AM fungi producing more shoots, and more dry matter in shoots and roots. Moreover, six weeks after the artificial infection of plants with *Fusarium*, root rot symptoms were manifested by only 20% of seedlings inoculated with *Glomus* sp. R10, while for non-AM plants, the symptoms were shown in up to 90% of plants. Similar experiments were made with pre-planted cucumber seedlings or roots of carrots, in which mycorrhiza fungi limited pathogen growth only to the outer bark of the roots (Sharma et al., 2004).

Conclusions

Recently, the use of mycorrhiza in vegetable production is addressed by many scientists, while there is also a gradually growing interest of farmers in practice. It can be concluded that inoculation of vegetables with arbuscular mycorrhizal fungi is a cultivation technique, which, when properly used, can protect plants against adverse abiotic factors, especially stress from drought, cold, salt soils, etc., but they also contribute to the protection of plants against various soil pathogens (bacteria, nematodes or insect pests). However, arbuscular mycorrhizal fungi also have a positive effect on physical properties of soil and its overall fertility, thus improving the conditions for growing the vegetables themselves. The use of AM as biopesticides has not only an impact on plant health, but also indirectly on the protection of the environment and our health as it reduces the need for harmful chemical pesticides. However, in order for the mycorrhiza itself to occur, it is necessary to know where and under what conditions we can apply the inoculum.

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COMPARATIVE STUDY OF THE PHENOLIC COMPLEX, THE RESVERATROL CONTENT AND THE ORGANOLEPTIC PROFILE OF BULGARIAN WINES

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A comparative study of the phenolic complex, the resveratrol content, the antioxidant activity and the organoleptic profile of Bulgarian wines from the local vine varieties Dimyat, Pamid, Gamza and from the hybrid varieties Plevenska rosa, Storgozia, Kaylashki rubin was carried out. Differences in the chemical composition and tasting characteristics of the experimental wines were identified. As for the white wines, Dimyat had higher sugar-free extract and titratable acidity, while concerning the red ones, Storgozia and Kaylashki rubin showed higher rates. The *trans*-resveratrol amount in the red wines was significantly higher compared to the white ones, as the samples from the red local varieties had a higher content than the hybrid varieties. Difference in the phenolic composition of the wines was also found. Gamza wine had the highest concentration of total phenolic compounds, total flavonoids and catechin. Storgozia sample contained the highest rates of monomeric anthocyanins and epicatechin. Pamid revealed the lowest concentrations of all analyzed phenolic components except catechin. The red wines had better antioxidant features than the white ones. From the red wines, the highest and the lowest activity were reported in the local varieties – Gamza and Pamid, respectively. There was no strict correlation between the effects of the studied phenolic components on the wine organoleptic profile.

Keywords: wine, chemical composition, antioxidant activity, resveratrol, organoleptic profile

Chemical composition of grapes and wine is complex and varied. It is determined by a number of factors such as variety, soil and weather conditions, cultivation practices, maturity, winemaking technology, etc.

The phenolic compounds are important components of wines. They have a significant effect on their organoleptic profile, especially in terms of colour intensity and taste characteristics such as density, tartness, and bitterness. Most of them pass from the grapes, so their content in wines is determined by the variety, and its potential and phenolic reserves (Ghiselli et al., 1998; Lee and Koh, 2001). They are extracted from the solid particles during the alcoholic fermentation, influenced by the technological factors as well under which the process takes place – temperature, yeast strain, etc. (Monagas et al., 2005; Savova, 2013). White wines contain a minimum amount of phenolic substances (up to 50 $mg.l^{-1}$), mainly non-flavonoids. Red wines are characterized by a high phenolic content (up to 4 $g.l^{-1}$), predominantly flavonoid components and anthocyanins, due to the specificity of the production technology (Carvalho et al., 1998; Burns et al., 2003).

The phenols content in wine is usually associated with their antioxidant activity. Almost all groups of phenolic compounds have the ability to interact with free radicals and to disable active oxygen particles in human body (Joubert and Beer, 2006; Polovka, 2006). Antioxidant properties depend not only on the total amount of phenols, but also on individual and fractional composition of the polyphenol complex (Rivero-Perez, Muniz and Gonzales-Sanjose, 2008). Therefore, wines, especially the red ones, containing high ratios of catechins, procyanidins, anthocyanins, gallic acid and other phenolic matters, refer to beverages having high antioxidant activity (Valkova et al., 2004; Monagas et al., 2005; Savova, 2013).

In recent years there has been a growing interest in resveratrol (3,5,4-trihydroxystilbene) – a natural antioxidant contained in grape skins, that pass from there into red wines during the process of maceration and fermentation (Fartsov et al., 2012). It belongs to the group of polyphenol compounds produced by some plant species in response to stress, damage, bacterial or fungal infection. Its quantity in wine varies depending on variety and the area of cultivation (Mihaylova et al., 2012; Videnova and Fartsov, 2012). It exists both as a *cis* and a *trans* isomer as well as in a glycosidic form (Gu et al., 2000; Mark et al., 2005). The *trans* isomer predominates in wine.

The objective of this study was to determine and compare phenolic composition, antioxidant activity, resveratrol content and organoleptic profile of six Bulgarian wines, obtained from local and hybrid vine varieties.

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Material and methods

The study was carried out at the Institute of Viticulture and Enology (IVE) – Pleven, Bulgaria, and at the Viticulture Research Institute (VRI) – Tekirdag, Turkey. The investigation was focused on six samples of wine, vintage 2014 – two white and four red. The wines were made from the local wine grape varieties Dimyat (white), Pamid (red) and Gamza (red), distributed in Bulgaria and most of the Balkan region, and the varieties Plevenska rosa (white), Kaylashki rubin (red) and Storgozia (red), selected at IVE – Pleven through interspecies hybridization, characterized by increased resistance to diseases and low winter temperatures.

The varieties were grown at the Experimental vineyards of IVE – Pleven (Central Northern Bulgaria). The process of ripening was monitored, and upon reaching technological maturity the grapes were harvested. The chemical composition of grapes of the studied varieties is given in Table 1. The following methods were applied for determining the grapes composition from the studied varieties (Ivanov et al., 1979): sugars ($g.l^{-1}$) – airmeter of Dujardin; total acids ($g.l^{-1}$) – titration with NaOH; pH – pH-meter.

Table 1 Chemical composition of grapes of the studied varieties

Indicator / Variety	Sugar ($g.l^{-1}$)	Total acids ($g.l^{-1}$)	pH
White varieties			
Dimyat	186.00	7.05	3.11
Plevenska rosa	198.00	5.30	3.27
Red varieties			
Pamid	174.00	5.13	3.35
Gamza	186.00	7.80	3.30
Storgozia	205.00	6.80	3.22
Kaylashki rubin	212.00	7.28	3.10

The grapes were processed at the Experimental Winery of IVE – Pleven under the conditions of micro-vinification. The classic white and red winemaking technology was applied (Amerine, Berg and Cruess, 1972):

- White wine – crushing, destemming, pressing, sulphuring (50 $mg.dm^{-3}$ SO_2), must clarification, adding pure culture dry wine yeast *Saccharomyces cerevisiae Vitilevure* B + C (20 $g.hl^{-1}$), fermentation temperature 18 °C, racking, further sulphuring, storage.
- Red wine – destemming, crushing, sulphuring (50 $mg.kg^{-1}$ SO_2), adding pure culture dry wine yeast *Saccharomyces cerevisiae Vitilevure* CSM (20 $g.hl^{-1}$), fermentation temperature 25 °C, separation of liquid part (young red wine) by pressing and racking, further sulphuring, storage.

After the completion of the process, the wines were decanted and further sulphured to 30 $mg.dm^{-3}$ free SO_2.

The basic indicators of wine chemical composition were analyzed in the laboratories of IVE – Pleven by conventional methods used in the winemaking practice (Ivanov et al., 1979): sugars ($g.dm^{-3}$) – Schoorl's method; alcohol (vol. %) – distillation method, Gibertini apparatus with densitometry of the distillate density; total extract (TE) ($g.dm^{-3}$) – Gibertini apparatus with densitometry, density of alcohol-free sample; sugar-free extract (SFE) ($g.dm^{-3}$) – calculation method (the difference between TE and sugars); total acids (TA) ($g.dm^{-3}$) – titration with NaOH; colour intensity I [abs. units] – method of Somers; pH – pH meter.

The indicators concerning phenol complex of the wines, antioxidant activity and *trans*-resveratrol content were analyzed in the laboratories of VRI – Tekirdag. The following methods were used:

- total phenolic content was determined using the Folin-Ciocalteu's colorimetric assay (Waterhouse, 2002) and results were expressed as gallic acid equivalents (mg $GAE.l^{-1}$);
- DPPH (1,1-diphenyl-2-picrylhydrazil) Radical Scavenging Activity assay was used based on the methods of Brand-Williams, Cuvelier and Berset (1995), as modified by Xu and Chang (2007). The free radical scavenging activity of wines was expressed as an equivalent of Trolox (µmol $TEAC.ml^{-1}$) using the calibration curve of Trolox. Linearity range of the calibration curve was 20 to 1,000 µM;
- ABTS [2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid)] Radical Scavenging Activity was determined according to the method described by Re et al. (1999). The calibration curve between % inhibition and known solutions (0.5; 1.0; 1.5; 2.0 mM) of Trolox was then established. The radical-scavenging activity of the wines were expressed as trolox equivalent antioxidant capacity (µmol $TEAC.ml^{-1}$);
- total monomeric anthocyanin content was determined by the pH differential method as described by Giusti and Wrolstad (2001) and the results were expressed as malvidin-3-glucoside equivalents ($mg.l^{-1}$);
- total flavonoid content of the samples was determined according to the method described by Zhishen, Mengcheng and Jianming (1999). The results were calculated and expressed as catechin equivalents (mg $CAE.l^{-1}$) using the calibration curve of catechin;
- catechin, epicatechin, syringic acid,vanillic acid and *trans*-resveratrol levels ($mg.l^{-1}$) in wine samples were determined using based on the methods of Anonymous (2010), as modified by Gülcü (2016). HPLC system (Shimadzu LC 20 A) was combined with a fluorescence detector in an Inertsil ODS-3(C18) column (5 µm, 4.6 × 250 mm). Mobile phase A: 0.2% Formic acid in Water, mobile phase B: 0.2% Formic acid in Acetonitrile. For separation to following gradient; B Conc. 23% (5 min), 26% (12 min), 40% (14 min), 100% (14–18 min), 23% (22 min); the flow rate was 1.5 $ml.min^{-1}$. Column temperature was 30 °C. The fluorescence detector was set at λex 278 nm and λem 360 nm for catechin, epicatechin, syringic acid and vanillic acid, λex 300 nm and λem 386 nm for *trans*-resveratrol. Samples of 5 µl of standard or wine were directly injected. The wine samples, standard solutions were filtered by a 0.45 µm pore size PTFE syringe filter.

The presented test results were the average of three independent replicates from the measurement of each

analyzed indicator. The organoleptic features of the experimental samples were determined according to 100-score scale for the indicators: colour, aroma, taste and general impression (Prodanova, 2008) by a nine-member tasting committee.

The data were subjected to correlation analysis using the statistical software package JMP (version 7, SAS Institute, Cary, NC, USA).

Results and discussion

Two white and four red Bulgarian wines obtained from local and hybrid varieties were selected for the study, which made it possible to determine and compare their chemical composition, phenolic complex, antioxidant activity, resveratrol content and organoleptic profile.

The chemical composition and the tasting assessments of the experimental wines are presented in Table 2. The results showed that the wines had no deviations from the normal rates of the investigated indicators. They were within the typical ranges for each variety, according to its specificity and varietal potential. The alcoholic fermentation in the samples occurred completely, with a full sugar fermentation and maximum alcohol accumulation, evidenced by the residual sugars content.

The amount of sugars in the samples varied from 1.06 to 1.98 g.l^{-1}. Plevenska rosa (12.57% vol.) and Kaylashki rubin (12.69% vol.) were distinguished with higher alcohol content. The differences in the sugar-free extract (SFE) were the result of the varietal features. That indicator had an influence on the taste properties of wine and determined its density. From the white wines, Dimyat had higher SFE, as for the red ones – Storgozia and Kaylashki rubin. However, the higher content of SFE was not related to the better wine tasting assessment. The total acids of the samples were within the typical ranges for the variety. Lower rates were recorded in the wine from the aromatic variety Plevenska rosa (5.15 g.l^{-1}) and Pamid (4.71 g.l^{-1}), characteristic for their variety, and the highest rates – in Kaylashki rubin (6.87 g.l^{-1}). The acids in the wine had an influence on taste freshness, but the results did not reveal any correlation with the wine tasting assessment. The samples from the hybrid varieties Storgozia and Kaylashki rubin showed higher SFE and titratable acidity in comparison with the samples from the local red varieties Pamid and Gamza. The *trans*-resveratrol content determined in the experimental wines showed that its amount in the red wines was considerably higher than in the white wines. Higher resveratrol rate was in the samples from the local red varieties, compared to the samples from the red hybrid varieties. Its concentration was the highest in Pamid wine (1.26 mg.l^{-1}) and in Gamza (1.23 mg.l^{-1}), followed by Kaylashki rubin and Storgozia (Table 2).

The phenolic compounds contained in wines also significantly influenced their sensory properties and were determining to their antioxidant properties.

Table 2 Chemical composition and tasting score of the experimental wines

Indicator / Wine	Alcohol (vol. %)	Sugar (g.l^{-1})	Total extract (g.l^{-1})	SFE (g.l^{-1})	Total acids (g.l^{-1})	Colour intensity (abs. un.)	pH	*Trans* -resveratrol (mg.l^{-1})	Tasting score
White wines									
Dimyat	12.44	1.45	21.00	19.55	6.70	0.16	3.26	0.09	76.60
Plevenska rosa	12.57	1.47	19.83	18.36	5.15	0.29	3.11	0.02	80.80
Red wines									
Pamid	12.17	1.06	21.80	20.74	4.71	7.75	3.10	1.26	79.60
Gamza	12.32	1.47	23.73	22.26	5.51	9.66	3.17	1.23	78.30
Storgozia	12.41	1.49	26.57	25.08	5.57	9.34	3.28	1.00	78.70
Kaylashki rubin	12.69	1.98	26.03	24.05	6.87	10.16	3.19	1.06	80.00

Table 3 Phenolic composition of the experimental wines.

Indicator / Wine	Total phenolic compounds (mg GAE.l^{-1})	Total monomeric anthocyanins content (mg.l^{-1})	Total flavonoids content (mg CAE.l^{-1})	Catechin (mg.l^{-1})	Epicatechin (mg.l^{-1})	Syringic acid (mg.l^{-1})	Vanillic acid (mg.l^{-1})
White wines							
Dimyat	315.90	–	54.24	3.02	0.70	N.D.*	0.09
Plevenska rosa	538.40	–	81.80	2.29	1.69	N.D.*	0.24
Red wines							
Pamid	819.50	23.63	301.71	20.42	9.47	0.74	2.95
Gamza	2402.00	61.86	902.32	34.12	17.60	5.20	2.37
Storgozia	1144.50	160.53	396.83	14.58	29.49	6.37	3.18
Kaylashki rubin	1567.00	103.50	552.32	11.10	11.35	10.53	4.64

*N.D. – not detected

The data on the content of total phenols, total monomer anthocyanins, total flavonoids, catechin, epicatechin, syringic and vanillic acids in the studied wines are presented in Table 3. The red wines were distinguished by a higher rate of phenolic compounds. The difference was determined by the influence of a number of factors, mainly the grape variety specificity. The results demonstrated a significant difference in the phenolic composition of the wines obtained from the local varieties Dimyat, Pamid and Gamza and those made from the hybrid varieties of Plevenska rosa, Storgozia and Kaylashki rubin.

The sample from Plevenska rosa exceeded that of Dimyat in the amount of total phenols, total flavonoids, epicatechin and vanillic acid. Dimyat wines contained more catechin. In the white wine samples, no syringic acid was found.

The wines from the local varieties Pamid and Gamza had a lower content of monomeric anthocyanins, syringic and vanillic acids, but a significantly higher rate of catechins. The sample of Pamid showed the lowest concentrations of all analyzed phenolic components except catechin. The wine from Gamza variety contained approximately circa 3 times more total phenols, total monomeric anthocyanins and total flavonoids compared to Pamid. The rate of catechin and epicatechin was also higher. Gamza wine had a greater amount of syringic acid and less vanillic acid compared to Pamid. In the case of red wines from the hybrid varieties, the sample of Kaylashki rubin contained more total phenols and total flavonoids. Storgozia wine, however, surpassed Kaylashki rubin in terms of the concentration of total monomeric anthocyanins, catechin and epicatechin. Gamza wine was characterized by the highest rate of total phenolic compounds, total flavonoids and catechin, followed by Kaylashki rubin in terms of total phenolic compounds and total flavonoids. Storgozia sample had the highest content of monomeric anthocyanins and epicatechin. As for the analyzed phenolic acids all red wines contained different rates of syringic and vanillic acid. Their amount was significantly higher in the samples from the hybrid varieties Storgozia and Kaylashki rubin. The highest content of both phenolic acids was found in Kaylashki rubin wine. In all samples, except for Pamid, the syringic acid content exceeded that of vanillic acid.

Table 4 Antioxidant activity of the experimental wines.

Wine	Radical scavenging activity (μmol TEAC.ml^{-1})	
	DPPH	ABTS
White wines		
Dimyat	0.41	3.52
Plevenska rosa	1.16	7.69
Red wines		
Pamid	0.98	6.56
Gamza	1.91	23.70
Storgozia	1.14	15.63
Kaylashki rubin	1.27	14.68

The phenolic compounds content in wine was associated with their antioxidant capacity. Therefore, their antioxidant activity was determined using the two analytical tests DPPH and ABTS (Table 4). The data from the two analytical tests used revealed the better antioxidant properties of the red wines compared to the white ones. This was due to both the higher phenolic content and to the different degree of polymerization of the procyanidins in white and red wines, and to the different ratio of the individual catechins in the polymer phenolic molecule (Valkova et al., 2004). The probable cause for the differences between both methods was the presence of other components in the wine composition exhibiting antiradical properties (Kerchev, Yoncheva and Ivanov, 2005).

The obtained experimental data did not allow a specific comparison between the antioxidant properties of the wines made from the local and the hybrid varieties. In the case of the white wines, the sample of Plevenska rosa had a better antioxidant potential than Dimyat, but for the red wines, with both analytical methods, the highest and the lowest activity was recorded for the local varieties. The highest rate was found in Gamza, and the lowest – in Pamid which contain the highest and the lowest content of total phenols and total flavonoids, respectively. According to the DPPH method, Kaylashki rubin wine had better antioxidant

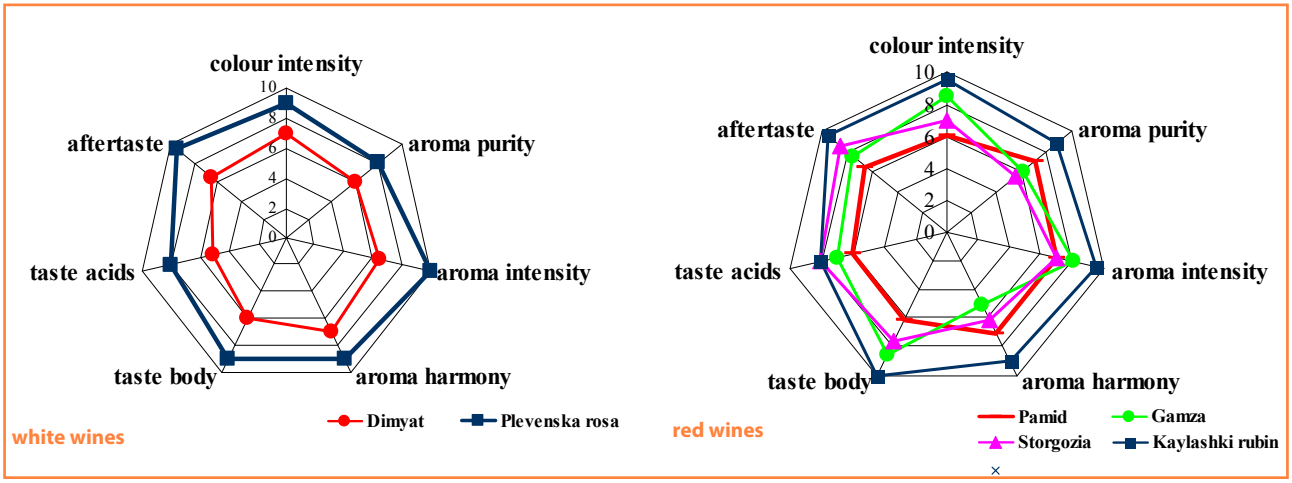

Figure 1 Organoleptic profile of the studied experimental wines

Table 5 Correlation analysis between the data determined with all experimental wine samples (n = 18)

	Alcohol	Total extract	Total acids	Colour intensity	pH	Total phenolic compounds	DPPH	ABTS	Total flavonoids	Catechin	Epicatechin	*Trans*-resveratrol
Alcohol												
Total extract	0.191 NS											
Total acids	0.658 **	0.324 NS										
Colour intensity	-0.163 NS	0.843 **	-0.038 NS									
pH	0.197 NS	0.521 *	0.615 **	0.071 NS								
Total phenolic compounds	-0.057 NS	0.587 *	0.023 NS	0.784 **	-0.023 NS							
DPPH	-0.028 NS	0.382 NS	-0.275 NS	0.631 **	-0.295 NS	0.900 **						
ABTS	0.009 NS	0.652 **	-0.009 NS	0.743 **	0.120 NS	0.956 **	0.904**					
Total flavonoids	-0.130 NS	0.608 **	0.011 NS	0.821 **	-0.007 NS	0.993 **	0.868 **	0.938 **				
Catechin	-0.587 *	0.360 NS	-0.356 NS	0.729 **	-0.169 NS	0.840 **	0.753 **	0.761 **	0.875 **			
Epicatechin	-0.206 NS	0.833 **	-0.155 NS	0.777 **	0.428 NS	0.565 *	0.486 *	0.702 **	0.590 *	0.552 *		
***Trans*-resveratrol**	-0.422 NS	0.662 **	-0.219 NS	0.952 **	-0.095 NS	0.724 **	0.578 *	0.632 **	0.780 **	0.826 **	0.675 **	
Tasting score	0.306 NS	-0.009 NS	-0.415 NS	0.154 NS	-0.687 **	0.044 NS	0.358 NS	0.048 NS	-0.001 NS	-0.091 NS	-0.035 NS	0.111 NS

NS: Non significant
** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

activity compared to Storgozia, while according to the ABTS test it was just the opposite. Regardless the lower content of total phenols, total flavonoids and phenolic acids, Storgozia sample had a higher antiradical activity. These results show that there is not always a correlation between the content of phenolic components in wines and their antioxidant capacity.

The organoleptic profiles of the studied experimental wines are presented in Figure 1.

The wines from the hybrid varieties Plevenska rosa (white) and Kaylashki rubin (from the red ones) were determined to have the best tasting features in terms of colour, aroma, taste and aftertaste. They had a pure, distinctive varietal aroma, harmony and balance of the tasting indicators. The data did not reveal a strict correlation between the influence of the studied phenolic components and wine organoleptic profile. The sample of Gamza variety, despite its higher ratio of total phenols and total flavonoids, had lower tasting assessment compared to Storgozia and Kaylashki rubin. Storgozia wine contained most monomeric anthocyanins, but received a lower tasting score than Kaylashki rubin. The reason was due to the difference in the total impact of the studied indicators of the wine composition on its sensory characteristics.

The relations between the determined parameters are statistically evaluated in Table 5. The significant correlations among total phenols, total flavonoids, catechin, resveratrol and different radical scavenging activity assays (DPPH and ABTS) were found in wine samples. The colour intensity of wines showed significant correlation with total extract, phenolic composition and radical scavenging activity. Tasting scores exhibited a significant correlation only with pH value, while it had no statistically significant correlations with all other analysis parameters.

Conclusions

- From the white wines, Dimyat had higher sugar-free extract and titratable acidity. From the red wines, the samples of the hybrid varieties Storgozia and Kaylashki rubin showed higher rates compared to the samples from the local varieties Pamid and Gamza.
- The *trans*-resveratrol amount in the red wines was significantly higher compared to the white ones, as the samples from the red local varieties had a higher content than the samples from the hybrid varieties. The highest rates of resveratrol concentration showed the wines Pamid (1.26 mg.l^{-1}) and Gamza (1.23 mg.l^{-1}).
- The red wines had more phenolic compounds. Difference in the phenolic composition of the samples obtained from the local varieties and those from the hybrid varieties was also found. Gamza wine had the highest concentration of total phenolic compounds, total flavonoids and catechin. Storgozia sample contained the highest rates

of monomeric anthocyanins and epicatechin. Pamid revealed the lowest concentrations of all analyzed phenolic components except of catechin. The content of phenolic acids in the wines from the hybrid varieties Storgozia and Kaylashki rubin was significantly higher. Syringic acid was not found in the white wines.

- The red wines had better antioxidant features than the white ones. From the red wines, the highest and the lowest activity was reported in the local varieties – Gamza and Pamid, respectively, which contain the highest and lowest content of total phenols and total flavonoids.
- Strict correlation between the effects of the studied phenolic components on the wine organoleptic profile was not found. The wines with the best tasting characteristics in terms of color, aroma, taste and aftertaste were the wines from the hybrid varieties Plevenska rosa and Kaylashki rubin.

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EFFECTS OF GIBBERELLIC ACID AND LOW TEMPERATURE ON GERMINATION OF SOME *PRUNUS* SPECIES EMBRYOS (WITHOUT COTYLEDONS) UNDER LABORATORY CONDITIONS

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Seeds of *Prunus* species do not germinate as a result of different mechanisms of dormancy such as physiological, physical and/or chemical ones. This study was carried out in order to determine the effects of three concentrations of Gibberellic acid (GA_3) 1, 3, and 5 mg.L^{-1} and low temperature at 5 °C on germination and on the length of isolated embryos from cotyledons of almond, apricot, plum, peach, mahaleb and sweet cherry on top of filter paper under laboratory conditions. The highest germination percentage (96.67%) was at 1 mg.L^{-1} GA_3 without cold treatment or 3 mg.L^{-1} GA_3 + cold treatment in almond, the highest length of embryos (15.47 mm) was also in almond at 1 mg.L^{-1} GA_3 without cold treatment. Embryos of mahaleb and sweet cherry germinated at low germination percentages of 31.16%, 33.33% respectively at 3 mg.L^{-1} GA_3 + cold treatment. It was concluded that embryos of almond, apricot, plum and peach were germinated successfully on top of filter paper under laboratory conditions and GA_3 increased significantly the germination percentages of the isolated embryos either after cold treatment or without compared with controls. A strong positive correlation was found between seed germination without testa, embryos germination and final embryos length simultaneously.

Keywords: *Prunus*, isolated embryos, GA_3, cold treatment, filter paper, laboratory conditions

Seeds of *Prunus* species do not germinate immediately when planted, either in the field or in the laboratory, as a result of different mechanisms of dormancy such as physiological (embryo), physical (endocarp), chemical (inhibitors) ones or the combinations of mentioned mechanisms; dormancy in these species may last for several months.

The germination inhibitors exist at different concentrations in various parts of seed, including pericarp, seedcoat, cotyledons, and embryo. The proportion of inhibitors could be decreased by removing either one or several parts of the seeds and thus germination percentage could be increased (San and Yildirim, 2009). On the other hand, cold stratification of seeds for several months could overcome dormancy. Also, some growth regulators such as GA_3 were used to induce the germination of the seeds of some species, for example *P. mahaleb* (Ghayyad et al., 2010; Gercekcioğlu and Cekic, 1999; Pipinis et al., 2012).

Overcoming seed dormancy *in vitro* germination was also successfully used in strawberry (Miller et al., 1992), citrus fruits (Hassanein and Azooz, 2003), walnut (Kaur et al., 2006), and almond (San and Yildirim, 2009). Germination of immature embryos has been successfully accomplished in some stone fruits including cherry (Hormaza, 1999), apricot (Ning et al., 2007), peach, almond, and peach×almond hybrids (Ledbetter et al., 1998).

A few studies have been carried out about *in vitro* germination of embryos isolated from cotyledons (Arbeloa et al., 2009; San and Yildirim, 2009), but all of the studies are based on MS medium and combinations of benzylaminopurine (BAP) and gibberellic acid (GA_3) and /or Indol butyric acid (IBA). San et al. (2014) reported that for successful *in vitro* embryo germination, the MS medium should be fortified with 0.5 mg·L^{-1} BAP + 3.0 mg·L^{-1} GA_3 in apricot, peach, and wild cherry. Payghamzadeh and Kazemitabar (2010a) reported that the best performing medium for immature embryos germination walnut was DKW basal medium supplemented with 1 mg L^{-1} alone and 1.5 mg L^{-1} BAP in conjunction with 0.01, 0.05 and 0.1 mg L^{-1} IBA (germination ratios vary between 49.32% and 67.76%). Kaur et al. (2006) reported that the best performing medium was MS with 0.5 mg.L^{-1} kinetin, 0.5 mg.L^{-1} BAP and 2 mg.L^{-1} GA_3 yielding 66.6% germination in Netar Akhrot cultivar of walnut after 12 days of culturing and the percent germination of immature embryos was higher when BAP and IBA were simultaneously applied as compared to those when applied separately (Kaur et al., 2006).

In addition, Kaur et al. (2006) used cold treatment in *in vitro* germination of immature walnut embryos, and the per cent germination of excised embryos was higher when GA_3 and cold treatments were simultaneously applied as compared to those when applied separately.

The object of this study is to determine the effects of Gibberellic acid (GA_3) and low temperature on germination of isolated embryos from cotyledons of almond, apricot, plum, peach, mahaleb and sweet cherry on top of paper under laboratory conditions without the need for nutritious medium and the easy and rapid assessment of embryos growth under simple conditions.

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Material and methods

This study was carried out in 2017 during Summer and Autumn in Damascus Countryside – Beit Tima Seed Scientific Research Laboratory.

Seeds of six *Prunus* species were collected: almond (*Prunus amygdalus* L.), apricot (*Prunus armeniaca* L.), plum (*Prunus domestica* L.), peach (*Prunus persica* L.), Mahaleb (*Prunus mahaleb* L.), and sweet cherry (*Prunus avium* L.). Fruits were collected in the same year of the study, then the seeds were isolated from mature fruits and dried at laboratory temperature (20–25 °C) for a month before they were used in the tests.

At first, hard endocarps were removed using a small hammer, then seeds were soaked in sterilized water for 48 hours for easy removal of testa and embryos, water being replaced every 12 hours.

After the testa (seedcoat) was removed, seeds with and without testa were incubated on top of filter paper under laboratory conditions in petri dishes without any pre-treatments as controls to compare with other treatments.

The embryos were carefully excised from the cotyledons under sterile conditions and were cultured on top of filter paper under laboratory conditions (temperature 20–25 °C, 16 hours a day and 8 hours a night) in petri dishes and submitted to the following treatments for three (3) weeks. Each treatment consisted of three (3) replications and each one contained tow (2) petri dishes with 10 embryos in each petri dish.

Gibberellic acid (GA_3)

Embryos were moistened with three (3) different concentrations (1,3, and 5 $mg.L^{-1}$) of GA_3 solutions during the three weeks test (when needed).

Cold treatment (low temperature)

Embryos were kept in the refrigerator for three (3) weeks at 5 °C and moistened with distilled water when needed, after that embryos were tested under laboratory conditions.

GA_3 + cold treatment

Embryos were moistened with the three (3) concentrations (1,3, and 5 $mg.L^{-1}$) of GA_3 solutions, then kept in the refrigerator for 3 weeks at 5 °C and moistened with GA_3 when needed, then tested in laboratory conditions.

At the end of the tests, the results of the germinated embryos were recorded in table (1) as percentages. Also, the final length of the germinated embryos (root and shoots) was measured in order to examine how different species of embryo growth are affected by the different treatments. The results were recorded in table (2).

Statistical analysis

Embryo germination percentages were transformed using ArcSin (Square root (X)). Since transformed data must meet the assumption of normality, data normality was determined by Kolmogorov-Smirnov test of variances, factorial ANOVA was performed to test the main effects and interactions between the studied factors (species 6 levels × GA_3 concentrations 4 levels × cold treatment/without cold treatment 2 levels × 3 replications). For comparing the germination of the isolated embryos with seed germination,

Table 1 Effects of GA_3 and cold treartments on embryos with and without cotyledons germination on top of filter paper under laboratory conditions

Seed germination percentage with cotyledons (%)								
Treatments		**species**						**average**
		almond	**apricot**	**plum**	**peach**	**mahaleb**	**cherry**	
Seed with testa		0	0	0	0	0	0	0
Seed without testa		81.67	60	70	66.67	35	28.33	56.94
Embryo germination percentage without cotyledons (%)								
Treatments		**species**						**average**
GA_3 ($mg.L^{-1}$)	**cold treatment 5 °C**	**almond**	**apricot**	**plum**	**peach**	**mahaleb**	**cherry**	
0	0 week	76.66bL	48.33bM	13.33cN	16.67bN	15.00bN	10.00bN	**30.00**
1	0 week	96.67aL	91.67aL	71.16aM	68.83aM	25.00aN	30.00aN	**63.88**
3	0 week	95.00aL	90.00aL	61.67aM	65.00aM	20.00aN	31.67aN	**60.55**
5	0 week	91.67aL	81.16aLM	73.33aLM	66.67aLM	21.67aN	20.00aN	**59.08**
0	3 week	40.00cL	40.00dL	36.67bL	35.00bL	13.33bM	11.67bM	**29.44**
1	3 week	83.33aL	80.00aL	80.00aL	76.67aL	31.16aM	33.33aM	**64.08**
3	3 week	96.67aL	51.67bcdN	80.00aL	70.00aM	15.00bO	11.67bO	**54.17**
5	3 week	90.00aL	60.00cbM	73.33aLM	71.67aLM	13.33bN	10.00bN	**53.05**
Average		**83.52**	**66.98**	**62.16**	**59.68**	**21.05**	**20.74**	

Means with the same letters of a, b, c, d in the same column are not significantly different at $\alpha = 0.05$. Means with the same letters of L, M, N, O in the same row are not significantly different at $\alpha = 0.05$

factorial ANOVA (species 6 levels × treatments 9 levels × 3 replications) was also performed. The Tukey HSD test at (α = 0.05) was used as a multiple comparison procedure after ANOVA analysis. Data analysis was performed by using Microsoft Excel 2007 and Statistical.8 StatSoft, Inc Program. The results were presented after back-transform data by using (Sin (*x*))2.

Results and discussion

Table 1 shows the germination percentages of seeds with cotyledons (without testa) and isolated embryos germinated on different concentrations of GA_3 and cold treatment under the laboratory conditions on top of filter paper in almond, apricot, plum, peach, mahaleb and sweet cherry.

Seeds with testa did not germinate on filter paper under laboratory conditions in all studied species. The highest germination percentage of the seeds without testa was recorded for almond, then for plum, peach, apricot, mahaleb and sweet cherry simultaneously, but according to the Tukey test at (α = 0.05) there were no significant differences between almond and plum, apricot and peach, and mahaleb and cherry.

Factorial ANOVA results showed that the final germination of the isolated embryos was significantly affected by the species ($F(5, 96) = 542.72$, $p < 0.001$), by the GA_3 concentrations ($F(3, 96) = 266.56$, $p < 0.001$), by the interaction of species and GA_3 ($F(15, 96) = 16.166$, $p < 0.001$), by the type of cold treatment ($F(1, 96) = 11.888$, $p < 0.001$), by the interaction of species and the type of cold treatment ($F(5, 96) = 30.238$, $p < 0.001$) and by the interaction of all of these factors ($F(15, 96) = 6.9483$, $p < 0.001$).

Results of the Tukey test (α = 0.05) showed that germination percentage of almond was significantly the highest followed by apricot, plum and peach, and mahaleb and cherry. Germination percentage at 1 $mg.L^{-1}$ of GA_3 was significantly the best. For the concentrations 3 and 5 $mg.L^{-1}$ there were no significant differences, control was the lowest. Germination percentages of the incubated embryos without cold treatment were significantly higher than after cold treatment.

There were no significant effects of 1, 3 or 5 $mg.L^{-1}$ concentrations of GA_3 for almond, plum and peach, meanwhile the concentration of 1 $mg.L^{-1}$ was significantly higher than the others on apricot, mahaleb and cherry.

Germination percentages of almond and apricot embryos without cold treatment were significantly higher than those after cold treatment and in reverse for plum and peach, whereas there were no significant effects on mahaleb and cherry.

The highest germination percentage (96.67%) was at 1 $mg.L^{-1}$ GA_3 without cold treatment and 3 $mg.L^{-1}$ GA_3 + cold treatment in almond. However, the differences between the germination percentages obtained from cold treatment and GA_3 were not statistically significant. The highest germination percentage (91.67%) was 1 $mg.L^{-1}$ GA_3 + cold treatment in apricot, in plum 1 or 3 $mg.L^{-1}$ GA_3 + cold

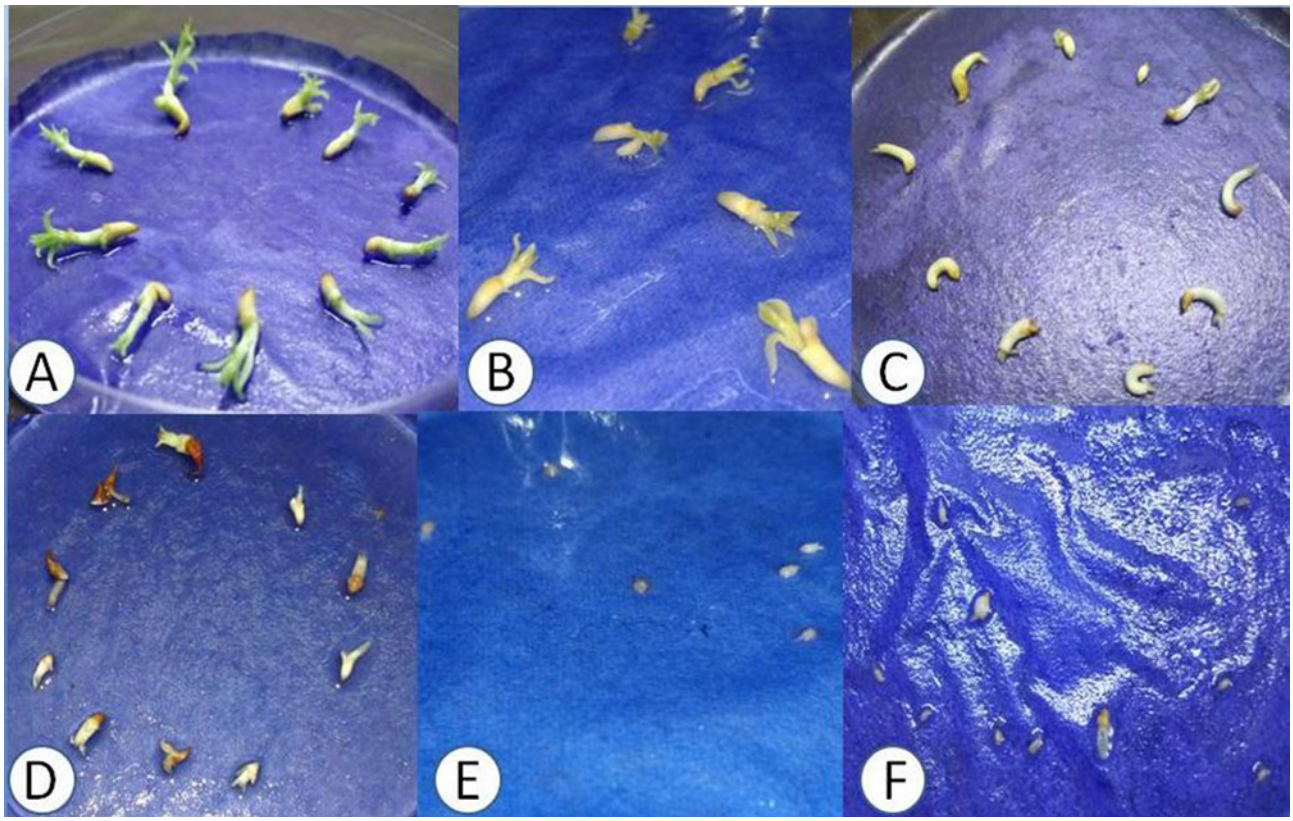

Figure 1 Embryos germination of some *Prunus* species without cotyledons on top of filter paper (FP) under laboratory conditions (A) almond embryos on FP + GA_3 1 $mg.L^{-1}$ without cold treatment. (B) apricot embryos on FP + GA_3 1 $mg.L^{-1}$ without cold treatment, (C) plum embryos on FP + GA_3 3 $mg.L^{-1}$ + cold treatment, (D) peach embryos on FP + GA_3 1 $mg.L^{-1}$ + cold treatment, (E) mahaleb embryos on FP + GA_3 1 $mg.L^{-1}$ + cold treatment, (F) sweet cherry embryos on FP + GA_3 1 $mg.L^{-1}$ + cold treatment

treatment (80%), meanwhile 1 mg.L^{-1} GA_3 + cold treatment in peach, mahaleb and cherry (76.67%, 31.16%, and 33.33% respectively), it was noted that only the root germinated in plum.

The results obtained in this study showed that seeds with testa did not germinate. Removing the testa increased the final germination percentage, this agrees with the fact that in some species, dormancy is overcomed by removing from seed coats that are likely to contain substances that inhibit embryo growth including *Malus*, *Prunus*, and *Pyrus* (Johnson and Chirco, 2003), while San et al. (2014) reported that seeds with cotyledons and/or testa of apricot, peach, and wild cherry did not germinate on the MS medium without cold treatment or stratification.

Isolated embryos of almond, apricot, plum and peach germinated on top of filter paper successfully in spite of the facts that tests were carried out under laboratory conditions (Figure 1 shows the isolated embryos from cotyledons germination of the studied species). Isolated embryos of mahaleb and sweet cherry did not germinate very satisfactorily. Likewise, seeds without testa also germinated at low germination ratios, that may be related to combined dormancy (Embryo not fully developed when seed shed with physiological germination block, Geneve, 1999). Roots of plum germinated, but shoots did not either after cold treatment or without, this may indicate to the Epicotyl dormancy in plum since the same was noted in seeds without testa test.

In all species, gibberellic acid (GA_3) significantly increased the germination percentages of the isolated embryos either after cold treatment or without comparing with controls; these results agree with Hartmann et al. (1997) in the rule of gibberellin in overcoming physiological dormancy in seeds with dormant embryos, but there was no significant effect of the addition of gibberellic acid on embryos germination; Payghamzadeh and Kazemitabar (2010a) reported that in the free PGR (plant growth regulators) medium embryos of walnut germinated, but did not induce any embryo development.

The interaction of species and cold treatment from statistical standpoint showed that germination of plum (only root) and peach isolated embryos was effected positively by the cold treatment, which means roots germinated after cold treatment, shoot remained dormant, and according to Geneve (1999) this may also indicate the Epicotyl and radicle dormancy. Meanwhile, embryos of almond and apricot were germinated better without cold treatment, there was no effect of cold treatment on mahaleb and sweet cherry embryos, the changes in the last two species were only turgidity of the root, that may relate to embryo dormancy which did not overcome completely. In all species and in general, there was no significant effect of GA_3 + cold treatment on germination embryos compared with only GA_3; this means that the main effect on germination was for GA_3, and for plum, peach, mahaleb and sweet cherry there was maximum embryo germination percentages of 80%, 76%, 67%, 31.16% and 33.33% respectively.

Embryo length (shoots and roots)

Table 2 shows the mean length of the germinated embryos for almond, apricot, plum, peach, mahaleb and sweet cherry on filter paper under laboratory conditions at the end of the tests.

Factorial ANOVA results showed that there were significant differences betweeen the length of the germinated embryos of the studied species ($F(5, 96) = 1{,}711.4$, $p < 0.001$), GA_3 concentrations ($F(3, 96) = 137.01$, $p < 0.001$) The type of cold treatment $F(15, 96) = 17.873$, $p < 0.001$), the interaction of species and the type of cold treatment ($F(5, 96) = 90.297$, $p < 0.001$), and the interaction of all of these factors ($F(15, 96) = 22.348$, $p < 0.001$).

Results of the Tukey test ($\alpha = 0.05$) showed that the highest length of germinated embryos was significant in almonds, followed by apricot and peach, plum, cherry and then mahaleb.

The highest length of the germinated embryos was shown at 1 or 3 mg.L^{-1} of GA_3 concentration, whereas the control reached the lowest length. The length of the incubated embryos without cold treatment was significantly higher compared to the cold treatment, and the length of almond, apricot, peach and cherry embryos without cold treatment was significantly higher than after cold treatment,

Table 2 The mean final length of the germinated embryos (root and shoots mm) after 3 weeks incubation

Treatments		Species						average
GA_3 (mg.L^{-1})	cold treatment 5 °C	almond	apricot	plum	peach	mahaleb	cherry	
0	0 week	11.50bL	7.14bM	6.00bM	6.17bM	1.67abN	2.67bN	**6.19**
1	0 week	15.47aL	8.50aM	6.17bN	8.33aM	3.00aO	3.56aO	**7.51**
3	0 week	14.77aL	9.10aM	50.80bN	5.58aM	2.89aP	3.89aO	**7.51**
5	0 week	12.56bL	6.92bM	7.11abM	7.61aM	2.83aN	3.5aN	**6.75**
0	3 week	7.33dL	7.25bL	4.00cM	3.42cM	2.00nN	2.00aN	**4.33**
1	3 week	9.75bcL	5.87bcN	8.13aM	6.67bN	2.56aO	2.33aO	**5.88**
3	3 week	10.70bL	4.80cO	8.25aM	6.73bN	2.33aP	3.00aP	**5.97**
5	3 week	11.11bL	5.28cM	6.19bM	6.28bM	2.67aN	2.67bN	**5.70**
Average		**11.65**	**6.86**	**6.46**	**6.72**	**2.50**	**2.95**	

Means with the same letters of a, b, c, d in the same column are not significantly different at $\alpha = 0.05$; means with the same letters of L, M, N, O, P in the same row are not significantly different at $\alpha = 0.05$

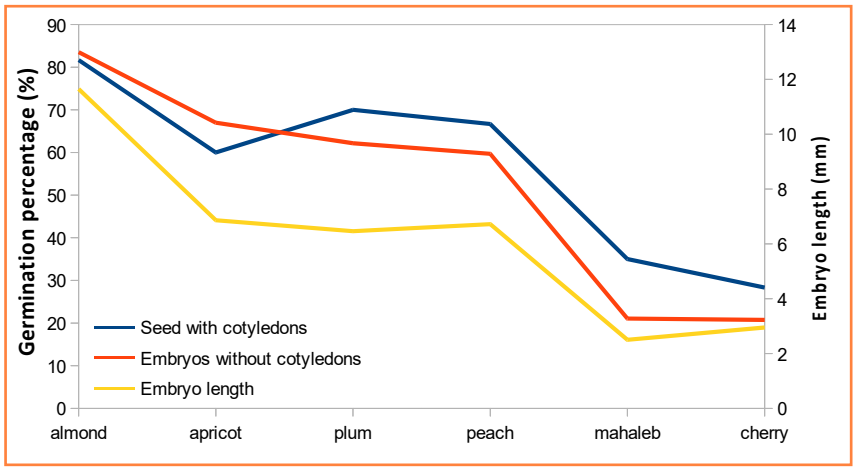

Figure 2 The mean seed germination percentages of the species with cotyledons, the mean isolated embryos germination percentages without cotyledons and the mean final embryos length

whereas there were nt significant differences in plum and mahaleb.

The highest length was recorded in almond at 1 or 3 mg.L^{-1} GA_3 without cold treatment (15.47 mm, 14.77 mm), the same for apricot, peach, mahaleb and cherry the highest length was at 3 mg.L^{-1} without cold treatment. However, there were no significant differences between 1, 3, and 5 mg.L^{-1} without cold treatment for the three previous species, for plum the highest length was at 3 or 1 mg.L^{-1} + cold treatment (8.25 mm, 8.13 mm).

Gibberellin acid (GA_3) without cold treatment had a positive effect on the development of the embryos, except for plum embryos (only root) which had the best length after cold treatment with presence of GA_3. In general, low temperatures seem to be favourable to the germination and embryo growth of plum because of the epicotyl dormancy. Payghamzadeh and Kazemitabar (2010b) reported that high frequency of plantlet of pecan embryos was reported in modified DKW basal medium supplemented with 1 mg L^{-1} BAP, 0.05 mg L^{-1} IBA and 2 mg L^{-1} GA_3 and dark culture condition.

In other study, Payghamzadeh and Kazemitabar (2010a) found out that the longest main shoot length of immature embryos of walnut was achieved in DKW medium supplemented with 1 and 1.5 mg.L^{-1} BAP.

Scaltsoyiannes et al. (1997) reported the effects of cytokinin (BA) and auxin (IBA) on shoot development.

A strong positive correlation was found between seed germination without testa (with cotyledons *x*) and embryos germination percentages of the species (*y*) ($R = 0.97$), the simple linear correlation equation was ($y = 1.19x - 15.53$). On the other hand, a positive correlation was found between embryos germination (*x*) (means of the species) and the final length of the germinated embryos (*y*) ($R = 0.95$, $y = 0.12x - 0.2$). (Figure 2 shows the relationship between seed germination with cotyledons, without cotyledons and embryos length). Subsequently, a positive correlation was found between seed germination without testa (with cotyledons *x*) and the final length of the germinated embryos (*y*) ($R = 0.92$, $y = 0.15x - 2.13$).

In this study, the strong positive correlation was found between seed germination percentage without testa (with cotyledons) and isolated embryos germination percentage, and the final length of germinated embryos; the more germination without testa, the more germination embryos without cotyledons, and the more length of embryos. These relationships may lead to the conclusion that inhibitors do not necessarily present in the cotyledons since the seeds were not stored and the inhibitors did not move from testa to the cotyledons or embryos according to the theory of the inhibitors movement during storage; in this case, the closest probability is that inhibitors are present in the embryos. Moreover, it was concluded that culturing isolated embryos from cotyledons depend on the range of the mother seed dormancy and viability.

These relationships give a clear idea about the nature of the dormancy and the approximate prediction of the embryos growth.

Conclusions

In conclusion, isolated embryos from cotyledons of almond, apricot, peach and plum (only root) were germinated successfully on top of filter paper under laboratory conditions without the need for nutritious medium.

In all species, gibberellic acid (GA_3) significantly increased the germination percentages of the isolated embryos either after cold treatment or without comparing with controls.

A strong positive correlation was found between seed germination without testa (with cotyledons), embryos germination and the final embryos length at the same time.

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IMPACT OF ORGANIC FERTILIZERS ON MORPHOLOGICAL AND PHENOLOGICAL PROPERTIES AND YIELD OF TOMATOES

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The field trial was carried out in 2016 and 2017 on the grounds of the Botanical Garden of the Slovak University of Agriculture in Nitra. The aim of the paper was to test the impact of new organic fertilizers from Company Rokosan on the yield parameters and the morphological and phenological properties of tomatoes. In the experiments, we observed two determinant varieties, namely 'Brixol F1' and 'Uno Rosso F1'. We used the bulk organic fertilizer Rokosan P, designed for fruiting vegetables, and the liquid fertilizer Rokohumin Z. Both forms of fertilizers are produced as organic biomineral fertilizers, their main ingredient being keratin. They contain 9% N, 9% P_2O_5, 9% K_2O, 3% MgO and trace elements. The total harvest in the control variant without fertilization was the lowest compared to the fertilized variants. The second variant was fertilized with the Rokosan P fertilizer, and the harvests were in both years and in both varieties higher than in the variant 1. The third variant was fertilized with the Rokohumin Z, the liquid fertilizer and achieved the best crop yields per plant. For the 'Uno Rosso F1' the best total harvest weight was 7.2 kg per plant in 2016 and 8.96 in 2017. For the 'Brixol F1', the highest harvest was 8.14 kg per plant in 2016 and 9.24 kg in 2017. In terms of yields and the number of fruits, combined fertilization with the bulk fertilizer Rokosan P and the liquid fertilizer Rokohumin Z reached the second highest values.

Keywords: tomato, organic fertilizers

Solanum lycopersicum syn. *Lycopersicon esculentum* is originally a multi-year plant that is used in our growing conditions as a one-year crop. Tomato is growing worldwide in many climatic zones (Rubatzky and Yamaguchi, 1997; Heuvelink, 2005).

The largest producers of tomatoes in the world include China, India, USA, Turkey and Egypt. Annually, they produce tens of millions of tons (Faostat, 2014).

In Slovakia, tomatoes are grown on the total area of 700 hectares of arable land. This area produces about 19,000 tons of tomatoes. In addition, tomatoes are grown in home gardens and in protected areas. The annual consumption of tomatoes per capita represents almost 18 kg, which approximately corresponds to the recommended dose (Meravá et al., 2017). Most tomatoes are grown in the Nitra, Trnava and Košice regions (Rozborilová et al., 2017).

Considering the demands of consumers to expand the range of organic vegetable products, we focused on growing tomatoes without the use of industrial fertilizers.

Material and methods

The field experiment was based on the grounds of the Botanical Garden of the Slovak University of Agriculture in Nitra (BG SUA in Nitra). We studied the impact of selected organic fertilizers on the yield and morphological and phenological properties of tomatoes. We observed two determinant varieties, namely 'Brixol F1' and ,Uno Rosso F1.

Tomato seeds were sowed on March 14, 2016 and on March 13, 2017 in the heated greenhouse and seedlings were grown. One week before planting of seedlings in the field, the soil was fertilized according to the variants. Planting of seedlings took place on May 25, 2016 and May 23, 2017.

The crops were harvested, weighed separately according to the variants, repetitions and varieties. The first harvest was on August 8, 2016 and on August 7, 2017, about 75 days after the planting. The second harvest was on August 18, 2016 and on August 21, 2017. The third time we collected the yield on August 30, 2016 and on September 4, 2017.

Each variety was grown in four variants. Before the planting, the land was fertilized on May 20, 2016 and on May 16, 2017. During the vegetation, we fertilized twice on June 20, 2016 and on July 25, 2016. In 2017, we fertilized on June 15 and on July 24.

The used fertilizers included humic acids. The dry, loose fertilizer Rokosan P is made on the basis of organominerals, the main component being keratin. It gradually releases nitrogen and meets the requirements for fruiting vegetables nutrition.

The second fertilizer was Rokohumin Z in the liquid state. It also has a keratin base, made from organic waste. The fertilizer encourages growth start of seedlings after

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planting. It can also be used for hydroponics. This fertilizer contains macro elements and a wide range of micro elements (Rokosan, 2017).

Data of field experiment

Number of varieties: 2, Number of repeats: 3, Number of variants: 4, Number of plants per variant: 18, Spacing: 0.8 × 0.6 m.

Used variants:

1. Variant – control, without fertilization.
2. Variant – loose fertilizer (Rokosan P) containing humic acids. Fertilization

of 150 g.m^2 before planting and at the same dose during vegetation after 25 days.

3. Variant – liquid fertilizer (Rokohumin Z) containing humic acids. Fertilization before planting at a dose of 15 ml fertilizer per 1 liter of water. In the same amount during vegetation there was repeated fertilization after 25 days.
4. Variant – a combination of loose and liquid fertilizer containing humic acids. Fertilization at a dose 150 g.m^2 of bulk fertilizer and a liquid fertilizer at a dose 15 ml.l^{-1} of water before planting. The combined fertilizations were repeated during vegetation 25 days after first application.

Brief description of varieties

'Uno Rosso F1' – medium-early determinant variety (102-days vegetation period), suitable for mechanized harvesting. It grows strongly with high fertility. The fruits are slightly elongated, reaching the weight of 60–70 grams. By more frequent rainfall and irrigation they are resistant to cracking. The storage time is at least 20 days. It comes from Unigen Seeds – USA (Orosco, 2013).

'Brixol F1' – a determinant medium-early variety suitable for combine harvesting. It has a compact moderate growth. The fruits are elongated and resistant to cracking after frequent rainfall and irrigation. Thanks to the high content of lycopene, they are quickly coloured into a deep red color. The average fruit weight is 65–75 g. The variety is very fertile if grown under irrigation and in warm areas. It comes from Unigen Seeds – USA (Orosco, 2013).

Evaluation of the growing area

The territory is set in a warm agroclimatic area. It is a dry sub-region with a mild winter and a longer sunshine. The Botanical Garden (BG) is located at the altitude of 130 m. The average wind speed is 2.4 m.s^{-1}. The meteorological measurements were carried out at a meteorological station at the BG area in Nitra (Table 1).

Soil conditions

The land is located on an open plain. The land belongs to the category of heavy soil and clay species. The soil analysis to determine the nutrient content was done at the Department of Agrochemistry and Plant Nutrition before the experiment was established (Table 2).

Used organic fertilizers

Rokosan P is loose, intended for fertilization of fruiting vegetables. It is produced as an organic biomineral fertilizer and the main ingredient is keratin. It contains 9% N, 9% P_2O_5, 9% K_2O, 3% MgO + trace elements. The content of the hazardous substances does not exceed the applicable limits according to the STN 654 804 (Slovak Technical Standard). The fertilizer is a harmonious and balanced source of nutrients that are evenly released during the growing season. It supports the creation of flowers, increases fertility and biological activity of soil. It is recommended to be applied once before planting in the amount of 120–190 g.m^{-2} and the fertilized land shall be intensively irrigated. The fertilizer can be used before planting and during vegetation (Rokosan, 2017).

Rokohumin Z is a universally liquid fertilizer suitable for vegetables. The fertilizer is a unique one, considering its production of keratin basis from organic waste. The fertilizer is enriched with calcium, magnesium, iron and trace elements such as molybdenum, copper, boron, manganese, and zinc. The fertilizer is also suitable for hydroponic cultivation.

Statistical evaluation

The results were evaluated by Statgraphic Centurion XVII (StatPoint Inc. USA). An analysis of variance was performed and the mean values were tested by LSD at 95% significance.

Table 1 Average temperatures and rainfall during the growing season

2016/month	May	June	July	August	September
T (°C)	15.0	20.3	21.4	19.5	17.5
R (mm)	91.0	14.0	13.5	35.0	37.0
2017/month	**May**	**June**	**July**	**August**	**September**
T (°C)	16.6	21.2	21.7	22.4	14.6
R (mm)	14.0	26.1	60.0	23.2	93.0

Table 2 Results of agrochemical analysis of soil

Year	Humus (%)	pH/KCl	Nutrient content (mg.kg^{-1}) of soil					
			Nan	P	K	S	Ca	Mg
2016	4.14 H	7.17 Ne	13 M	142.5 H	565 VH	16.3 L	14,750 VH	740.9 VH
2017	3.75 G	7.18 Ne	10.1 M	147.5 H	477.5 VH	91.3 H	5,850 H	765.6 VH

H – high content, G – good content, pH: Ne – neutral, M – medium content, L – low content, VH – very high

Results and discussion

Effect of fertilization on tomato yield

In the first variant where the fertilizer was not used before planting and during vegetation, the results of the 'Brixol F1' variety were better. The ´Brixol F1´ variety had a higher yield in all three collections. The total harvest of the 'Uno Rosso F1' in the control variant was 2.19 kg in 2016 and 3.79 kg per plant in 2017. For the 'Brixol F1' variety in the control variant, the yield was 3.19 kg in 2016 and 7.38 kg per plant in 2017. These harvests were lower than in other variants where different fertilization methods were used. Crop yields are generally related to the amount of nutrients received during vegetation (Hlušek, 2004).

The second variant was fertilized with bulk fertilizer and the harvest was higher in both years and at both varieties than in the control variant. The third variant was fertilized with Rokohumin Z, the liquid fertilizer and the best yields were achieved per plant. For the 'Uno Rosso F1 ' variety, three harvests consisted of the total fruit weight of 7.2 kg per plant in 2016 and 8.4 in 2017. The 'Brixol F1' had the highest yield in the third variant, namely 8.14 kg per plant in 2016 and 9.24 kg in 2017.

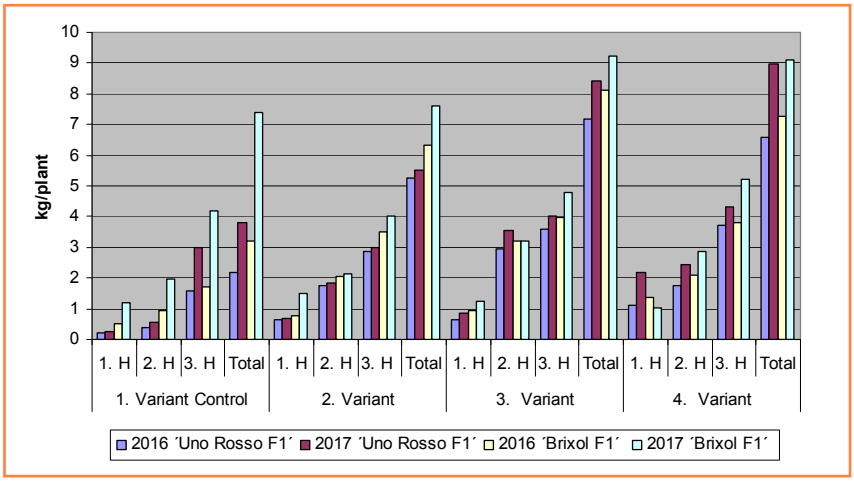

Figure 1 Yield of tomato in kg per plant

The fourth variant in crop yields per kg of plant was mostly weaker than variant 3, but still, the second best in the ranking (Table 3, Figure 1).

We found differences between variants in the number of fruits per plant. In 2016, there were harvested 34 pieces (control variant) to 111 pieces (third variant) from 'Uno Rosso F1' variety. The 'Brixol F1' variety produced larger number of fruits in 2016, ranging from 46 to 116 pieces. In 2017, more fruits were found on plants than in 2016 (Figure 2).

Figure 3 shows that the year 2017 was generally more favourable for tomato crops than 2016. The reason was probably the weather with higher temperatures and rainfall in July, which is a period of intense fruit production.

The authors Patane and Cosentino (2010) noted that not only soil water deficiency, but also soil and climatic characteristics affect the tomato yield and quality. Helyes, Lugasi and Pek (2012) also identified the irrigation effect on the yield and the content of the antioxidant components of industrial tomatoes.

Table 3 Yield of tomato in kg per plant and number of fruits per plant

Variant	Harvest	Variety ´Uno Rosso F1´				Variety ´Brixol F1´			
		year 2016		year 2017		year 2016		year 2017	
		kg/p	n/p	kg/p	n/p	kg/p	n/p	kg/p	n/p
1. Variant (Control)	first	0.2		0.25		0.52		1.2	
	second	0.4		0.54		0.95		1.98	
	third	1.59		3		1.72		4.2	
	together	2.19	34	3.79	58	3.19	46	7.38	105
2. Variant	first	0.66		0.70		0.79		1.5	
	second	1.75		1.85		2.05		2.12	
	third	2.85		2.98		3.5		4	
	together	5.26	81	5.53	84	6.34	91	7.62	109
3. Variant	first	0.65		0.85		0.96		1.24	
	second	2.94		3.55		3.19		3.2	
	third	3.61		4		3.99		4.8	
	together	7.2	111	8.4	110	8.14	116	9.24	132
4. Variant	first	1.12		2.19		1.35		1.02	
	second	1.74		2,45		2.1		2.88	
	third	3.7		4.32		3.81		5.2	
	together	6.56	101	8.96	119	7.26	104	9.1	130

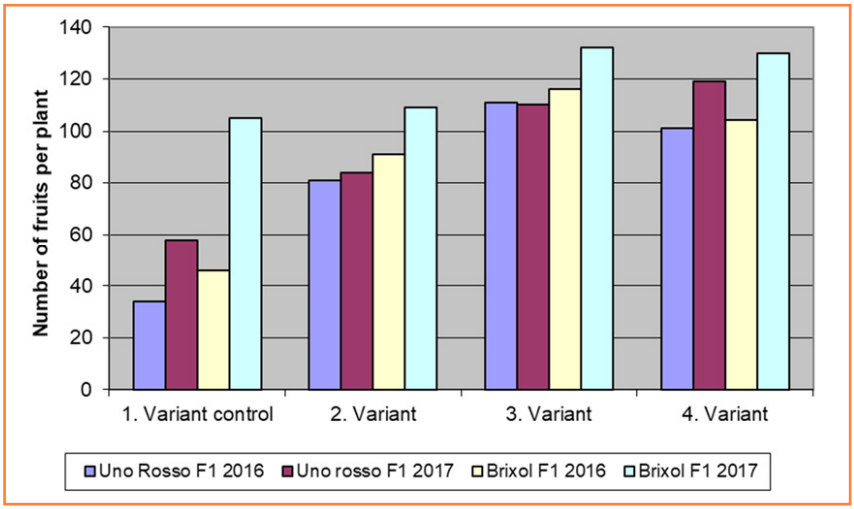

Figure 2 Number of fruits per plan
H = harvest

Evaluation of the fertilizers' impact

The yield and number of tomato fruits were highest in the variant with Rokohumin Z fertilizer. It is also evidenced by the highest total harvest in variant 3 in 2016. In 2017, the best harvests of 'Uno Rosso F1' were in the fourth variant and of 'Brixol F1' in 3rd variant.

The liquid form of the fertilizer worked better than the powder. In general, organic fertilizers in combination with irrigations result in faster incorporation and decomposition of nutrients in the soil (Ložek et al., 2000).

In terms of yields and the number of fruit, the combined fertilization with bulk fertilizer Rokosan P and liquid fertilizer Rokohumin Z reached the second highest results.

The variant fertilized only with the bulk fertilizer Rokosan P placed third in the crop level. The reason may be the fact that loose organic fertilizers release nutrients more slowly than liquid or industrial fertilizers (Flowerdew, 2011).

Nevertheless, in the variety 'Uno Rosso F1' in 2016, of 2nd variant, the harvest was higher (5.26 kg per plant) than the unmanaged control variant (2.19 kg per plant). In 2017, the yield in the control variant reached 3.7 kg per plant and 5.53 kg per plant in the second variant. As for the ´Brixol F1' variety, the bulk fertilizer also increased the yield from 3.19 kg per plant (control) to 6.34 kg per plant.in 2016 and from 7.38 to 7.62 kg per plant in 2017.

According to the authors Taiwo, Adediran and Sonubi (2008), organic fertilizers are important sources of plant nutrition and can be alternatives to synthetic fertilizers. Even a recent research (Wang and Xing, 2017) confirmed the positive effects of irrigation and fertilization on tomato fruit yield and quality.

Impact assessment of years

There were marked differences between the yields in individual years of the experiment in favour of 2017 in both varieties. In the 'Uno Rosso F1' variety, crop yields in 2016 ranged from 2.19 to 7.2 kg per plant and in 2017 from 3.79 to 8.96 kg per plant. The 'Brixol F1' variety also showed higher yield in 2017. The yield ranged from 3.19 to 7.26 kg per plant in 2016 and from 7.38 to 9.24 kg per plant in 2017.

Impact assessment of varieties

The 'Brixol F1' variety was more fertile in both years and in all variants. Even when evaluating the number of fruits per plant, the 'Brixol F1' was more successful in both years. These differences can be seen in Table 3. Tigist, Workneh and Woldetsadik (2012) confirmed the variability of the properties of different varieties of tomatoes when their research focused

Table 4 Multiple Range Tests for Yield kg / plant by varieties, years, variant and harvest

Varieties	Count	LS Mean	LS Sigma	Homogeneous Groups		
Uno Rosso	24	1.99542	0.103177	X		
Brixol	24	2.42792	0.103177		X	
Years	**Count**	**LS Mean**	**LS Sigma**	**Homogeneous Groups**		
2016	24	1.9225	0.103177	X		
2017	24	2.50083	0.103177		X	
Variants	**Count**	**LS Mean**	**LS Sigma**	**Homogeneous Groups**		
1. V	12	1.37917	0.145914	X		
2. V	12	2.0625	0.145914		X	
4. V	12	2.65667	0.145914			X
3. V	12	2.74833	0.145914			X
Harvest	**Count**	**LS Mean**	**LS Sigma**	**Homogeneous Groups**		
First	16	0.95	0.126365	X		
second	16	2.10562	0.126365		X	
Third	16	3.57938	0.126365			X

Method: 95.0% LSD

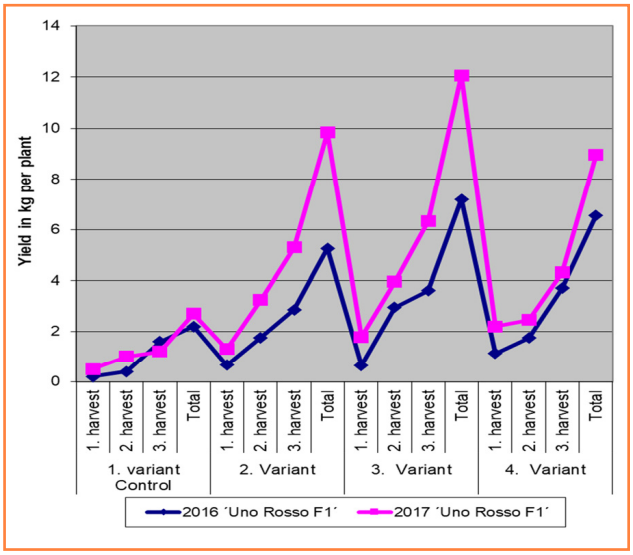

Figure 3 Comparison of 'Uno Rosso F1' variety yield in 2016 and 2017

on the influence of varieties on the crop, the physical and storage properties of tomatoes.

Results of statistical evaluation

The tests for crops in kg per plant and fruit numbers per plant showed significant differences between varieties, years, variants and collection dates (Table 4 and 5).

Evaluation of morphological and phenological properties

Selected morphological and phenological properties were evaluated according to the international tomato descriptor (IPGRI, 1996). This evaluation system is used by researchers, gene resources investigators and breeders. The morphological features of tomatoes should not change significantly over the years and, therefore, we only evaluated these indicators in 2016. Differences in the observed properties were found among varieties but not among variants. This means that the use of different fertilizers did not have any significant effects on the morphology and phenology of tomatoes.

Morphological properties

Plant and leaf

Both varieties are of determinate type, suitable for field cultivation with the possibility of mechanized harvesting (Uher et al., 2016). The variety of 'Uno Rosso F1' ranged up to the height of 0.3 m and the variety of 'Brixol F1' reached the height of 0.5 m. The stronger stem was found in the 'Brixol F1' variety with the diameter of 20–30 mm, which was likely to have a positive effect on crop production. Valšíková (1987) reported the colour of the stem as dark green, covered with small hairs, which was confirmed in the results of our varieties. Both varieties had dense and mighty foliage. The leaf type was normal, slightly curly and covered with truncated trichomes (Zimolka, 2008). The colour of the leaves was classified as green.

Vine

Vine was moderately dense for both varieties. For the 'Uno Rosso F1' variety, the length of vine was up to 0.25 m and for 'Brixol F1' from 0.25 to 0.30 m. The average number of flowers in vine ranged from 14 to 16 for 'Uno Rosso F1 ' and 17 to 19 for ' Brixol F1 '.

Inflorescence

As a part of floral morphology, we observed the colour, size and frequency of the flowers. Authors Andrejiová and Kóňa (2010) claimed that flowers of the new varieties have yellow colour and have six digits. The variety 'Uno Rosso F1' had larger flowers with 6–7 tips in comparison with 'Brixol F1'. From the morphology of inflorescence we evaluated the creations of flowering, the relative length and shape of the stigma and anther. These characters were the same for both varieties. The first inflorescence is usually based on sixth to eighth sheet (Uher et al., 2016). The 'Brixol F1' variety is based on inflorescences on 8^{th} to 9^{th} leaves and 'Brixol F1' at 6^{th}–7^{th} leaves. The length of stigmas and anther was in both varieties on the same level.

Fruit

We followed the surface of tomato fruits, the basic colour of the immature fruits, the size of fruits and the colour of the skin. Rosembergerová (2013) stated that the weight of 'Uno Rosso F1' variety fruit is about 55–60 g and at 'Brixol

Table 5 Multiple Range Tests for Number of fruits per plant by varieties

Varieties	Count	LS Mean	LS Sigma	Homogeneous Groups		
Uno Rosso	8	87.25	4.23029	X		
Brixol	8	104.125	4.23029		X	
Years	**Count**	**LS Mean**	**LS Sigma**	**Homogeneous Groups**		
2016	8	85.5	4.23029	X		
2017	8	105.875	4.23029		X	
Variants	**Count**	**LS Mean**	**LS Sigma**	**Homogeneous Groups**		
1. V	4	60.75	5.98253	X		
2. V	4	91.25	5.98253		X	
4. V	4	113.5	5.98253			X
3. V	4	117.25	5.98253			X

Method: 95.0% LSD

F1' variety it is about 68–70 g. The weight was also affected by fertilization in variants of our experiments. The highest weight of fruits was found in the 4th variant, which was fertilized in both liquid and loose form of the Rokosan fertilizer. The shape of the fruits from the side view of 'Uno Rosso F1' was slightly elongated. From the top view, the shape was rounded triangular. The 'Brixol F1' variety has a rather spherical shape and an irregular shape from the top view. Both varieties have smooth surface of fruits. The top of the fruits has the 'Uno Rosso F1' variety without the tip and the fruit has a slightly developed green ring. On the contrary, the 'Brixol F1' variety has a peak of the fruits with few pronounced spikes and the fruit has no ring. The variety 'Uno Rosso F1' has a large stem hole (5–10 mm), red flesh, and berries have four chambers. The 'Brixol F1' variety has a shallower stem hole, up to 5 mm, the red flesh and the fruits have from 5 to 10 chambers.

Phenological features

Sowing the seeds was realized on March 14, 2016. Both varieties belong to field tomatoes, which meet the requirements for industrial processing, reported by Mareček et al. (2001). After five days, the seeds germinated. Eight days after sowing, on March 22, 2016, the first primary leaves began to form. On March 30, 2016, seedlings were transplanted. The first right leaves were created 25 days after sowing. Hypocotyl colour was dark green, anthocyanin discoloration occurred. After 72 days from sowing, on May 25, 2016, we planted the seedlings on the outdoor site. The seedlings height was around 0.30–0.35 m. 'Brixol F1' variety began to blossom first, 4 days after planting, and 'Uno Rosso F1' variety after 8 days from planting (June 2, 2016). The date of the first fruit creation for the 'Uno Rosso F1' variety was June 20, 2016 (26 days from planting), for 'Brixol F1' it was June 14, 2016 (20 days from planting). In the 'Brixol F1' variety, the first fruits began to mature 53 days after planting and in 'Uno Rosso F1' 56 days after planting. The harvests were carried out manually. The first harvest took place on August 8, 2016 (75 days from planting), the second one on August 18, 2016 (85 days from planting) and the third one on August 30, 2016 (97 days from planting).

Conclusions

As for the years of experiments, the more favourable one for the cultivation of field tomatoes was the year 2017. In the 'Uno Rosso F1' variety, yield variations ranged from 2.19 to 8.96 kg per plant. The 'Brixol F1' variety showed yield from 3.19 to 9.24 kg per plant. The yield and the number of tomato fruits were best suited by variety with the Rokohumin Z fertilizer. It is also evidenced by the highest total yield in the variant 3 out of the three harvests in 2016 and in 2017. The variant fertilized only with the bulk fertilizer Rokosan P took third place in the crop level. In terms of yields and number of fruits, the second place was taken by the variety with combined fertilization of bulk (Rokosan P) and liquid (Rokohumin Z) fertilizers. Out of the two varieties in question, 'Brixol F1' was more productive in both years and in all variants. Even when evaluating the number of fruits per plant, the 'Brixol F1' was more successful in both years.

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MONITORING OF MOTH PESTS IN APPLE TREE ORCHARD

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The work suggests importance of monitoring apple tree pests from moth group in growing conditions of Nitra, Slovakia. In 2014 there was observed occurrence of moths e.g. Codling moth (*Cydia pomonella* L.), Appleseed moth (*Grapholita lobarzewskii* Now.), Hawthorn berry moth (*G. janthinana* Dup.), and Summer fruit tortrix moth (*Adoxophyes orana* Fish. v. Roesl.) in the apple tree orchard located in the Botanical Garden of SUA in Nitra with help of pheromone traps. The date of first generation occurrence of Codling moth, Appleseed moth and Summer fruit tortrix moth was recorded on April 23. All the pests showed two peaks of flight activity, but with Hawthorn berry moth three periods of higher occurrence were recorded. The course of temperatures influenced number of pests trapped in traps remarkably. The number of pest individuals was highly influenced by rainy weather and lower temperatures in months when there was expected their highest harmfulness. The recorded values might be influenced by plant species diversity of the experimental orchard as well as that of the surrounding area.

Keywords: moths, apple tree, integrated pest management, automated meteorological station, pheromone traps

There is a wide range of insect pests in the orchards in the Slovak territory which cause remarkable damages from the aspect of fruit yield as well as fruit quality every year. Besides that they influence vitality of fruit trees and life period of orchards negatively. Apple trees produce one of the most important fruit species grown in Slovakia. The productive area of apple tree in Slovakia was 3,074.1 ha in 2016, which was 48.2% of all fruit orchards in the Slovak territory (Meravá, 2017). Moths are the most dangerous pests of apple trees. These are lepidopterans with harmful caterpillar stage. The larvae cause damages by feeding on leaves and fruits. Their development starts from mid-May and their damaging activity continues until fruit harvest (Tancik, 2013). During the last decade of 20th century, appearing and spreading of insect species was observed that had not presented serious or any problem to fruit production previously, while in others, increase of number of generations within a season was recorded. These phenomena are attributed to climatic change generally. Global warming contributed to successful spreading of new pest species from warmer regions and enabled their acclimatization in the Slovak territory. This situation calls for changes in the system of plant pest control, increases its complexity; period of pesticide application is changing, importance of pest signalization is increasing as well as the use of non-chemical pest control methods.

Several monitoring techniques have been developed and applied to monitoring moth pests in fruit orchards. The most effective approach involves sex-pheromone-baited traps (Davis, French and Venette, 2005).

Temperature is among decisive environmental factors influencing the rate of development and activity of pests. Graf et al. (1999) established thermal thresholds for *Grapholita lobarzewskii* and found them more or less the same as for the codling moth *Cydia pomonella* and *Adoxophyes orana* established previously by various authors.

Material and methods

The experiment was conducted in the fruit orchard located in the Botanical garden of the Slovak University of Agriculturein Nitra in 2014. There was observed occurrence of pests of moth group in the apple orchard with lower level of orchard management.

Climatic characteristics of the site are as follows: mean annual temperature 9.6 °C, sum of active daily temperatures 2,880 °C, annual sum of precipitation 595 mm, altitude 130 m, sunshine duration 1550 hours a year, average number of days with snow cover 50, maximum snow cover depth 70 cm (Year-book of Slovak Hydrometeorological institute, 1991) – values for 1951–1980 period.

The orchard is a part of demonstrational, educational and pomological collections of different fruit species, and only a small portion of yield is used commercially. The total area of the orchard is approximately 4 ha. Chemical pest and disease control corresponds with the lower production intensity (2 to 6 chemical treatments within a season depending on fruit species) and pesticides are used only in a part of the orchard area. Age of apple trees within the orchard is diverse, with youngest trees being 8 years old, and the oldest ones even 25 years old. Apple trees are planted in blocks alternated with blocks planted with other fruit

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species. Soil management system is natural sod, and soil surface is mulched with moved grass (obviously 4 times a season) and shoot grinding on spot after pruning. Prevailing portion of young orchard is planted with Braeburn cv. and few other cultivars as supplemental. In older part of the orchards Golden Delicious, Idared and Šampion are prevalent, however cultivar assortment is very broad. Various rootstocks were used, from dwarfing (mostly M9) for trees formed as slender spindle on wire support to medium vigorous and vigorous for bush trees and semi-standards with natural crown.

In the experiment there were monitored various moth species e.g. *Cydia pomonella* L., *Grapholita lobarzewskii* Now., *G. janthinana* Dup., *Adoxophyes orana* Fish. v. Roesl., via number of individuals caught in pheromone traps placed in the apple orchard. The experiment was conducted during the 2014 season.

Prior to placing pheromone traps in the orchard the bionomies of individual pest species were studied thoroughly as well as the principles of proper use of pheromone traps. Special pen-register was created afterwards for recording the number of pest individuals captured in pheromone traps. To gather meteorological data – temperature, precipitation – an automated meteorological station (AMS) installed in the orchard was used. Temperatures were used to determine the sums of effective temperatures (SET) necessary for peaks of pest flight activity and periods between them.

The pheromone traps were distributed in the orchard during green leaf tip stage (BBCH 09) which was on April 4, 2014. Placing of individual pheromone traps respected the producer installation guides. Traps were hanged on central leader or scaffold branches or on wire support sparsely, at the height of minimum 1.5 m above soil surface in inner parts of the apple tree block. Due to a relatively small orchard area, 1 pheromone trap with specific pheromone was installed for each the monitored moth pest.

Number of individuals caught in pheromone traps was observed and recorded regularly 3 times a week (Monday, Tuesday, Friday) starting from their placing into the orchard until the end of the season. Numbers of captured individuals were recorded in a special pen register. After the captured pest counting sticky boards of traps were cleaned to remove any rests of pest individuals which could distort results in the next checking term. After finishing of the observation period the data were processed, and expressed via flight curves to identify peaks of pest flight activity.

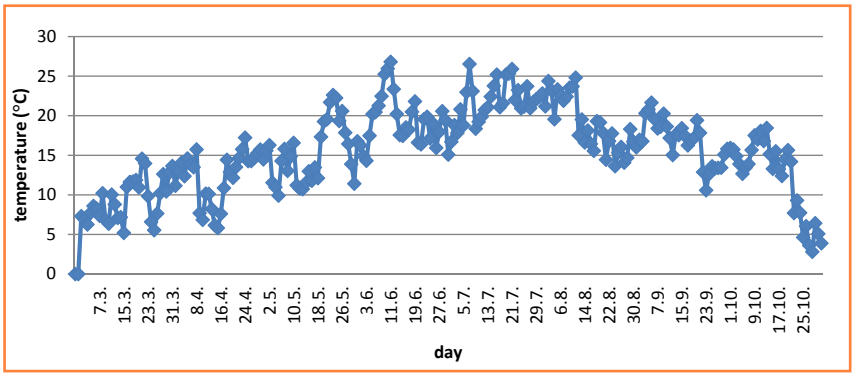

Figure 1 Mean daily air temperatures in March – October 2014

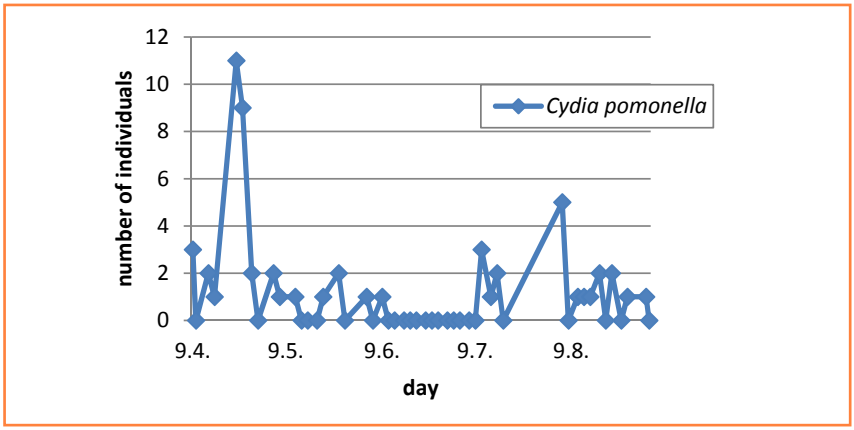

Figure 2 Flight curve of Codling moth (*Cydia pomonella* L.) in 2014

Results and discussion

There are presented the processed data obtained in the locality of the Botanical garden of SUA in Nitra in the 2014 season. The figure 1 shows the course of mean daily temperatures prior to and during the observation period of pest flight activity in the 2014 season. Mean daily air temperature of the observed period (March – October 2014) was 14.9 °C.

The observed period was typical with frequent precipitation. Within March – October period there were 52 days with precipitation above 1 mm and 22 days with precipitation above 5 mm. This fact remarkably influenced pest flight activity.

Figures 2–5 present numbers of pest individuals captured in traps within the observed period. For better clarity the graphs are customized – only periods with nonzero values of captured pests are presented; however, the observation period was longer (from April 9 to October 31, 2014).

First adults of Codling moth were recorded on April 4 (3 adults). Peak of the first generation flight was on April 23. The flight activity during the following period was low. The second generation of the pest was recorded with the peak on August 6.

The greatest number of Hawthorn berry moth adults was recorded on May 26. The course of flight activity was remarkably influenced by weather conditions. Based on the numbers of individuals captured in traps we assume that

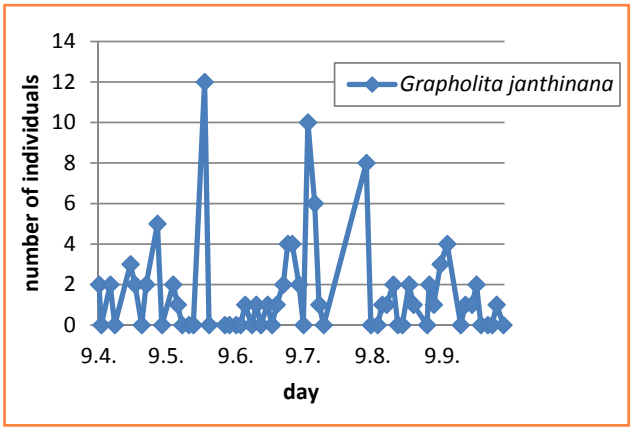

Figure 3 Flight curve of Hawthorn berry moth (*G. janthinana* Dup.) in 2014

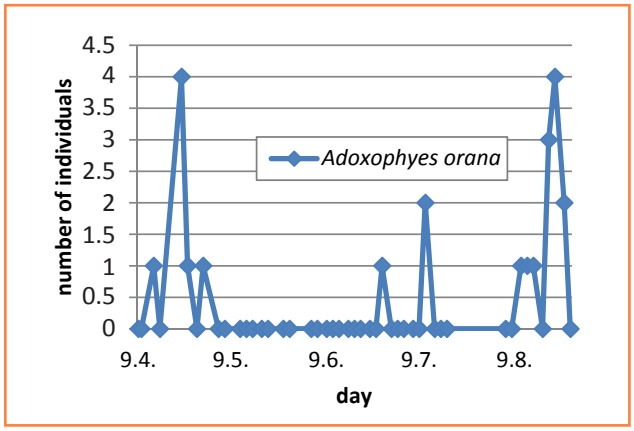

Figure 5 Flight curve of Summer fruit tortrix moth (*Adoxophyes orana* Fish. v. Roesl.) in 2014

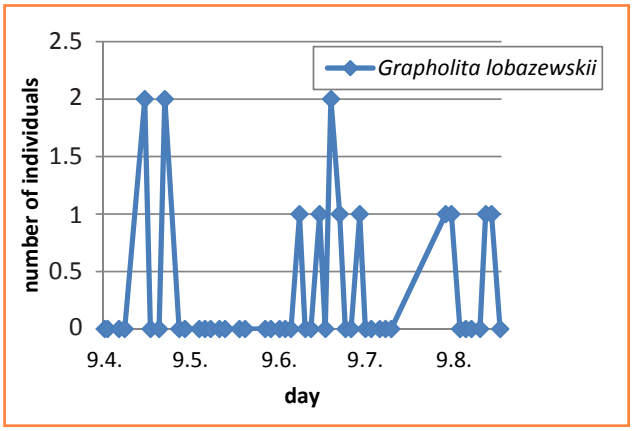

Figure 4 Flight curve of Appleseed moth (*Grapholita lobarzewskii* Now.) in 2014

the peak of the second generation of the pest was on July 11.

There was low occurrence of Appleseed moth in the 2014 season in the experimental locality as shown in the figure 4. Greater number of adults captured in the traps was found on April 23, April 30 and June 27 (2 adults per observation).

Two generations of Summer fruit tortrix moth were observed within the 2014 season, first with the peak on April 23, second on August 22, both with 4 adults captured in the traps.

The obtained data are related to the locality of the Botanical garden of SUA in Nitra and as we suppose they may be influenced with species diversity and distribution of plots planted with various fruit species within the area of the orchard, and long term low level of pest management programme in part of the orchard which is in coincidence with assertions of Joshi et al. (2016) about differences of Codling moth populations and flight patterns in abandoned and commercially managed orchards.

According to Praslička et al. (1997), the flight activity of Codling moth starts in the end of May and the peak of flight activity is in June. The maximum oviposition is reported in the second half of June. Our records set the beginning of flight activity of the pest on April 23, which is in concidence with the results of Fadamiro (2004) of experiments conducted at different locations in Minnesota (USA) where he found the first capture of *C. pomonella* males at ≈110 DD base 10°C, which corresponded to apple bloom at the experimental locations. In our experiment, the maximum oviposition was in the second half of May due to the fact that 2014 was the year with an early onset of spring. The same date of the first flight was found with Hawthorn berry moth in 2014 by Juračková (2014) in the region of Louny, while with Codling moth on April 22, 2014 in the region of Litoměřice (Northeastern Bohemia). Cigániková (2011) reported the beginning of Appleseed moth flight activity in 2008 on June 18 at SET 424 °C and the peak of the first generation June 25. Our observations showed the greatest number of adults caught in pheromone traps on April 23, April 30 and June 27, however, their number was lower. Different peaks of second generations of the pests indicate various thermal requirements.

Out of the observed moth pests, the most numerous ones were Codling moth and Hawthorn berry moth followed by Summer fruit tortrix moth and Appleseed moth, though the flight activity patterns were different in individual pests. Belo (2011) obtained similar results in commercial apple tree orchard in Ostratice (approximately 40 km from our experimental site).

Conclusions

The aim of the work was to gather information on flight activity of selected significant pests of moth group in the apple tree orchard which was used for modelling of activity of the observed pests and predicting their occurrence regarding the meteorological factors in the specific production region. At the experimental site there were observed meteorological data (temperature, precipitation) with the use of an automated meteorological station installed in the experimental orchard. The occurrence of the selected moth species was monitored, e.g. Codling moth (*Cydia pomonella* L.), Appleseed moth (*G. lobarzewskii* Now.), Hawthorn berry moth (*G. janthinana* Dup.), and Summer fruit tortrix moth (*Adoxophyes orana* Fish. v. Roesl.) with the use of pheromone traps. Flight curves were created which indicated different flight activities. With some of the observed pests, the same term of the first generation was found – April 23, with Summer fruit tortrix moth, Codling moth, and Appleseed moth. With the mentioned species,

2 periods of high flight activity were recorded with exception of Appleseed moth with 3 periods of higher numbers. Dynamics of temperature indicated the influence on second generation massiveness (low temperature caused smaller numerousness of pests; however, activity was recorded during longer period). Frequent precipitation and low temperatures in June and August which corresponded with periods of pest harmful activity influenced its flight activity greatly. The results might be distorted due to fruit species diversity in orchard, and proximity of gardens with great plant diversity.

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