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CONTENT

INTORDUCTION

This year, 2020, started with the vast spread of the virus SARAS-CoV-2 that quickly evolved to a global COVID-19 pandemic. The pandemic induced changes in both our everyday and professional life which inevitably led to an economic crisis on a global scale.

As the world was adapting to this situation, many factories and warehouses were forced to close, the production in many industries declined, state borders were closed. As other branches of the industry were slowing down, the production and manufacturing of food and drugs continued to work with full force. Social distancing was instigated, and thus, became the new normal. This social distancing era did not affect science and education, as they continued with the help of online platforms and the commodities that today's technology has to offer.

In these times of global economic crisis and a viral pandemic, the key role of science, education and food production in the sustainability and evolution of humans was once again proven. The agricultural industry did not go unaffected by COVID-19. Restrictions of movement, as well as basic aversion behavior by workers, may impede farmers from farming and food processors - who handle the vast majority of agricultural products - from processing. Shortage of fertilizers, veterinary medicines and other input could affect agricultural production. Closures of restaurants and less frequent grocery shopping diminish demand for fresh produce and fisheries products, affecting producers and suppliers. However, besides all the hardships that were brought along with the crisis did not halt the agricultural industry and food production kept on developing and functioning in order to meet the demands.

Journal of Agriculture and Plant Sciences Vol. 18. No 1 is published in the midst of the SARS-CoV-2 pandemic. This volume proves that science and scientific research are of profound importance to agriculture and food processing and production, and must not be held down, even when the world faces a pandemic.

JAPS Editorial board were given a great responsibility, to sustain the continuity of published volumes of the journal in these difficult times of a pandemic and an economic crisis. Therefore, with great honor and respect, we would like to present the latest scientific achievements in agriculture.

Editorial Board, **Editor in chief,**

July, 2020 **Prof. Liljana Koleva Gudeva, PhD**

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Original scientific paper

AGRO-BIOLOGICAL AND TECHNOLOGIACAL CHARACTERISTICS OF 'RKATSITELI WINE GRAPE VARIETY, GROWING IN TIKVEŠ VINEYARDS

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Abstract

Research is being performed to the 'Rkatsiteli wine grape variety. The vineyards are sited on the Smilica-Tikveš vineyards. Plantation was started in 1996 with standard plantation material, 2.4 m planting distance between rows, and 1.2 m distance between vines in a row. The training system is Guyot two arms, with 20 winter buds per vine. Some optimal agro-technical and ampelo-technical measures are applied. During the research period the following items were included: phenophases on development, fruiting of buds, weight of cluster (g), chemical composition of grape must (sugar, total acid, pH), and chemical analysis of wine. On the basis of obtained results, we can conclude that the variety 'Rkatsiteli belongs to the group of native varieties (coefficient of fertility 1.10) with a 235 g average weight of cluster. The must grape contains 223 g/L sugar and 6.4 g/L total acids. The wine has a medium content of alcohol (12.33% vol) and low content of reducing sugar (1.7 g/L), which is due to selective grape harvesting and the vinificitation technology (winemaking).

Key words: *'Rkatsiteli, grape, wine*

INTRODUCTION

The territory of the Republic of Macedonia is located between 40° 51' and 42° 21' north latitude. According to its climate features and EU classification, Republic of North Macedonia is considered as III-C-b zone for growing vineyards. In Republic of North Macedonia, all agricultural land under vineyards is consider as one vine region. The name of the geographic area of the region is Macedonia. The basic feature of this zone is that wines may have acidification, not to be enriched, which corresponds with the law and current practice of the production of wine in the country.

White wines are characterized by mild sourness, and they do not have the features of "terroir", while red wines are mainly of dark-rubine-red colour, with a smell of red and dark fruits and full structure too.

In the region Macedonia, there are 16 sub-regions which are characterized by different production conditions and different intensity of production. Namely, the Tikvesh region is an area to which the greatest part of grapes and wine production belongs (around 40%).

The Tikveš vineyard region with continental and partially Mediterranean climates, the agro-ecological conditions are favorable for successful cultivation of wine grape varieties of all epochs of maturity. The Tikveš vineyard is characterized by daily mean air temperatures of 12.4 – 14.5°C with annual temperature of 4500 – 5300°C and vegetation temperature sum ranging from 3950°C to 4764°C. Total annual precipitation ranges from 440 – 740 mm, and from 250 – 310 during the vegetative growth cycle (Table 1).

Table 1. Climatic factors in Tikvesh vineyard.

Vegetative period	days	229-239
Period of active vegetation	days	207-229
Average annual air temperature	C°	12.4 – 14.5
Sum of annual temperature	C°	4500-5300
Sum of temperature in the vegetative period	C°	3950-4767
Annual precipitation	mm	440-740
Precipitation in the vegetative period	mm	250-310

The 'Rkatsiteli wine group is an old Georgian type. According to the national wine group list, it belongs to the group of highly recommended vine varieties for the Tikvesh region. According to David Maghradze at. al., 2012, 'Rkatsiteli does not support wet climate and it is quite resistant to cryptogrammic diseases, and it is resistant to winter low temperatures (-18° C to–21° C). It provides wine production, grape brandy and grape juice.

MATERIAL AND METHODS

The research was conducted in the vineyards in the production areas of SOZSU Gjorche Petrov – Kavadarci, locality Smilica. The vineyard is 23 years old, founded in 1996, and it is grown on trellis with the implementation of regular agro-technical and ampelotechnical measures.

MATERIAL

The 'Rkatsiteli is an old Georgian vine variety and it belongs to an ecological geographic group convarietas pontica, subconvarietas georgica. The leaf is of medium dimension with slightly curved ends, in 5 or 3 parts. The cluster is of medium size with tubular or tubular-conical shape, and it has a separated medium wing which is set to a quite long top. It grows in the epoch, a medium late variety and it's highly vigorous. The vine is quite wild with high vine sprouts. It belongs to the group of medium profitable varieties. The grape is 200 - 235 g (Bozinovikjc Z, 2010). 'Rkatsiteli is a vine variety with high biological potential and a great area of prevalence. It provides white wines of high quality. It is best in warm places due to its high content of acids. In our region, it can survive in places of warmer climate, too.

METHODS

The research was conducted on a total number of 30 vines, i.e. 3 repetitions of 10 vines. The pruning system was double Guyot load with twenty buds, two canes with eight buds. After blossoming, we counted how many of the flowers in the inflorescences were fertilized, and how many were not, and mathematically we got the coefficient of fertility, i.e. the number of grapes per buds.

The dynamics of the growth of the grapes was being monitored through the sugar content and the total acids, so that every 5 days samples removed were taken and a chemical analysis of the unfermented wine was done in the laboratory. The chemical content was defined by measuring the sugar content and level of total acid content. The sugar content is defined with Exlos' device (a measuring device). This defines the Oechsle unfermented wine's density by the help of the Salernon table, which also defines the percentage of sugar. Oechsle degree defined the density of the water and the unfermented wine density. The total acids content is defined by titration with 0.1 NaOH and brom thymol blue as an indicator. The following parameters have been analyzed from the chemical content: alcohol (vol%), sugar (g/L), total acids (g/L), pH and volatile acids content (g/L).

Picture 1. 'Rkatsiteli grape variety

RESULTS AND DISCUSSION

The goal of the phenological research is to define the beginning and the duration of certain phenophases of the development of the vine, which affects the quantity and quality of the grapes (Mirošević N., at al., 2008). The beginning and duration of the phenophases is pre-conditioned by the genetic features of the type and ecological conditions in the environment. (Nendel C., 2010).

Table 2. Phenophases of development during vegetation

Bleeding	Bud break and short of canes	Flowering		Veraison	Full ripeness (harvest)
		beginning	and		
20.03	5.04	15.05	25.05	5.08	10.09

In Table 2, the phenological research of 'Rkatsiteli is shown. The bleeding starts as a result of the activity of the root at a temperature of around 8-10°C. It usually lasts for 14-20 days. In the conditions of the Tikvesh region, this phenophase starts on the 20.03. The bud breaks and the growth of the vine begins when the daily temperature is 10°C. In the research period, the phenophase bud breaks and short of canes starts on 5.04. The blossom begins by removing the flower cap and removing the pollen of the filament. The temperature should be over 20oC with humidity of 40%. The growth of the grapes starts with the filament and lasts until complete ripeness. The phenophase veraison starts on 5.08, and the harvest remove is on 10.09. At this period, crucial morphological and physiological changes: it changes its tenderness – it becomes softer, the colour changes, the skin becomes soft, and less elastic, and there is a change in the sugar content and total acids.

Table 3. Elements of fertility and yield of grape.

Repetition	Shoots (%)	Coefficient of fertility	Grape weight (g)	Yield (kg/vine)
1	62.73	1.04	213	4.4
2	65.94	1.15	253	5.8
3	66.29	1.1	239	5.2
1-3	64.50	1.10	235	5.1
CV%	3.02	5.02	8.64	13.68

In Table 3, the results of fertility of buds and the average weight of the grapes of the 'Rkatsiteli are given. The percentage of shoots of buds is from 62.73% to 66.29% or averagely 64.50%.

According to Sivčev et al. (2004), when shoots of variety Rkaciteli, in agroecological conditions of Radmilavac, were cut on 30 buds, leaves and coefficient of fertility were 51.48% and 0.54, respectively.

In our research, the highest coefficient of fertility is given in the second repetition from 1.15, and the lowest 1.04 in the first or 1.10 on the average. The 'Rkatsiteli belongs to the group of wines with a very high coefficient of fertility (Bozinovikjč, 2010). The removing weight of grapes is from 213 g to 239 g or 235 g on average which belongs to the group of middle grapes. The benefit is from 4.4 kg to 5.8 kg or 5.1 kg on average, where a statistically important variation of 13.68 is given.

Table 4. Dynamics of the ripeness of grapes.

Test	Sugar (brix)	pH	Total acids (g/L)
I 14.08	18.4	2.29	9.96
II 19.08	20.4	3.07	7.71
III 24.08	21.3	3.03	7.84
IV 29.08	21.4	3.15	6.79
V 03.09	22.0	3.21	6.56
VI 10.09	22.3	3.23	6.40

In Table 4, the results of the dynamic of the growth of the grapes are given. The growth dynamics was monitored through the sugar content, total acids and pH, starting from the berry softening to the harvest, i.e. reaching technological ripeness. We concluded that every 5 days the sugar contents increased averagely for 2 g/L, and the content of total acids among the first and second check is considerably decreased about 2 g/L and then for only 1 g/L. In the technological phase of ripeness, the sugar content is 22.3 brix, and the total acids 6.4 g/L, which is in the boundaries of the features and these are optimal for the production of quality wine. In the conditions of Nish (Sivčev et al., 2004), it consists of lower amount of sugar (19.4 brix) but more total acids (8.2 g/l).

The chemical analysis results of the wine are given in Table 5. The 'Rkatsiteli wine consists of 12.33 vol% alcohol and total acids 5.56 g/L. According to the content of non-fermented sugar (1.7 g/L), the wine is in the group of dry wines. The wine has low content of volatile acids of 0.32 g/L, which shows that the wine is healthy and well kept.

Table 5. Chemical analysis of the wine.

Parameters	'Rkatsiteli wine
Alcohol vol%	12.33
Reducing of sugar g/L	1.7
Total acids g/L	5.66
pH	3.33
Volatile acids g/L	0.32

CONCLUSION

According to the results given, the 'Rkatsiteli grown in the Tikvesh region belongs to the group of fertile wine groups. The coefficient of fertility of the buds is 1.1, and the average weight of the grapes is 235 g and 5.1 kg/ vine average yield.

The sugar content and the total acids is in the boundaries of the features for production of high-quality white wines.

The wine consists of 12.33 vol% alcohol, 5.56 g/L total acids, 1.7 g/L non-fermented sugar and it belongs to the group of dry wines.

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АГРОБИОЛОШКИ И ТЕХНОЛОШКИ КАРАКТЕРИСТИКИ НА СОРТАТА 'РКАЦИТЕЛИ ОДГЛЕДУВАНА ВО ТИКВЕШКОТО ВИНОГОРЈЕ

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Резиме

Истражувањата беа вршени кај винската сорта 'ркацители. Лозовиот насад се наоѓа во локалитетот Смилица - Тиквешко виногорје. Насадот е подигнат во 1996 година со стандарден саден материјал, со растојание 2,4 m помеѓу редови и 1,2 m лозите во редот. Системот за одгледување е двокрак Гиов, а секоја лоза беше оптоварена со по 20 окца. Применувани се оптимални агротехнички и ампелотехнички мерки. За време на периодот на истражување беа вклучени следниве елементи: фенофази на развој, родност на окцата, маса на гроздот (g), хемиски состав на ширата (содржина на шеќер, вкупна киселина и pH) и хемиска анализа на вино. Врз основа на добиените резултати можеме да заклучиме дека сортата 'ркацители припаѓа на групата сорти со висока родност (коефициент на плодност 1,10) со просечна маса на гроздот од 235 g. Ширата содржи 223 g/L шеќер и 6,4 g/L вкупно киселини, виното е со оптимална содржина на алкохол (12,33 vol%) и остаток на неферментиран шеќер од 1,7 g/L, а е резултат на селективно собирање на грозје и начинот на винификација.

Клучни зборови: *'ркацители, грозје, вино*

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Professional paper

EFECTS OF HERBICIDES ON BARLEY SEEDS GERMINATION DEPENDING ON GROWTH STAGE

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Abstract

An experiment was carried out including three barley varieties (Reks, NS 293 and Egej), treated with six herbicides (2,4-D, MCPP+dicamba, triasulfuron+dicamba, 2,4-D+florasulam, amidosulfuron+iodosulfuron and florasulam+flumetsulam) in the three different growth stages (tillering, first node and second node). The aim of this experiment was to consider the influence of herbicides (applied in different growth stages) on germination of barley seeds.

In all experimental years herbicides did not significantly influence barley seed germination. The growth stage during herbicide application did not affect germination of barley. If conditions do not allow herbicides to be used in the optimal period, it can be used until second node stage, without having a negative impact on the barley seeds germinations.

Key words: *barley, varieties, herbicides, growth stages, seeds germination*

INTRODUCTION

Barley (*Hordeum sativum* Jessen) in the Republic North Macedonia is grown on about 47 500 ha with average yield of 3 440 kg/ha Anonymous (2009). It is the second cultivated crop right after the wheat. It is grown as continuous crop two to three years. Because of that, barley production is disturbed by weed infestation. In barley losses due to competitive effects of weeds estimated at 15-25% of potential production. Contemporary chemical weed control in barley and wheat begins after Second World War. Since then over 50 a. i. are synthesized for selective weed control in barley and wheat. Most of the herbicides which are used in barley and wheat is foliar and tillering is optimum growth stage for application. According Folley (1985), barley is more sensitive to herbicides than wheat. Various herbicides has various influence on barley, dependent on barley varieties and the growing stages during the application. Tottman (1976), emphasizes that the knowing of growth stages during the herbicides application is of the high importance. By using the herbicides in advance growth stages barley sensitivity can be increased and barley yield elements can be reduced Allien (1966), Markovich (1978), (Rinella et al, 2001). (Friesen et al.,1964, 1968) found that in cereals the negative influence of dicamba was increased if it is applicated in later growth stages. Barley seed production in North Macedonia is present on 1019 ha with average yield of 6 000 kg/ha. Seed germination of barley is very important for quality seed production and also, for determining correct seeding rate.

There is little data about the impact of herbicides on wheat and barley seed germination. Randy (1986), emphases that wheat seed germination was not affected by metribuzine and chlorsulfuron and their combination, but metribuzine when applied alone reduced coleoptile growth of Vona variety seed.

According to this an experiment was carried out to consider the influence of herbicides on barley seeds germination depend on growth stage.

MATERIAL AND METHODS

Field trial was conducted at the Agriculture institute in Skopje. The experimental design was randomized complete block with four replicates, and harvest plot size of 16 m^2. The trial was three factorial (Factor 1-herbicides, Factor 2–varieties and Factor 3-barley growth stages during herbicides application). The studies were carried out with three barley varieties Reks, NS 293 and Egej which were seeded with seedling rate of 300 kg/ha on October 19th (1st year), November 4th(2nd year) and November 13th (3rd year). The harvest was carried out with plot combine Wintersteiger on June 22th (1st year), July 3th (2nd year) and July 18th (3rd year).

Table 1. Variants of the trial.

Variants – active ingredient (a.i.)	Rate	Time of application
Weed free control	/	/
2,4-D	1 L/ha	I, II, III*
MCPP+dicamba	4 L/ha	I, II, III*
Triasulfuron+dicamba	100 g/ha	I, II, III*
2,4-D+florasulam	0.5 L/ha	I, II, III*
Amidosulfuron+iodosulfuron	0.25 kg/ha	I, II, III*
Florasulam+flumetsulam	60m L/ha	I, II, III*

*I-tillering, II- first node, III- second node

All herbicides were applied with CO^2-pressurized backpack sprayer with 300L/ha water.
Seeds germination was measured according to ISTA methods. The data were subjected to statistical analysis applying LSD-test.

RESULTS AND DISCUSSION

In 1st year (Table 2) barley seed germination was ranged from 96,0 % at NS 293 variety treated with 2,4D+florasulam at second node stage to 97,8 % at Reks variety treated with MCPP+dicamba at tillering stage. The investigated herbicides did not significantly influence the barley seed germination.

Table 2. Influence of herbicides on barley seeds germination (1st year).

Variants	Varieties								
	Reks			NS 293			Egej		
	Tillering	First node	Second node	Tillering	First node	Second node	Tillering	First node	Second node
	%	%	%	%	%	%	%	%	%
Weed free control	97.3	97.3	97.3	96.0	96.0	96.0	96.8	96.8	96.8
2,4-D	96.3	96.3	96.5	96.5	96.8	96.8	96.8	97.5	96.5
MCPP+dicamba	97.8	96.3	96.5	97.3	97.0	97.3	96.5	97.0	96.0
Triasulfuron+dicamba	96.5	97.0	96.3	96.8	96.8	96.5	96.8	96.3	96.5
2,4-D+florasulam	96.5	96.3	96.5	97.0	96.5	96.0	96.5	96.3	97.5
Amidosulfuron+iodosulfuronn	96.3	96.3	97.0	96.3	97.3	97.0	97.0	97.3	96.8
Florasulam+flumetsulam	96.5	96.5	97.0	96.8	97.3	97.0	96.8	96.3	97.3
LSD 0.05	1.7	2.2	2.0	1.4	1.3	1.6	1.9	1.9	1.9
LSD 0.01	2.3	3.0	2.7	2.0	1.8	2.1	2.5	2.5	2.6

In 2nd year (Table 3) the highest barley seed germination (98,8%) was measured at NS 293 variety treated with amidosulfuron + iodosulfuron at tillering stage. Lowest barley seed germination (97,0 %) was measured at NS 293 variety treated with 2,4-D at first and second node stage and amidosulfuron+iodosulfuron at first node stage, also at Egej variety treated with 2,4-D+florasulam and florasulam+flumetsulam at first node stage. The investigated herbicides did not significantly influence the barley seed germination.

Table 3. Influence of herbicides on barley seeds germination (2nd year).

Variants	Varieties								
	Reks			NS 293			Egej		
	Tillering	First node	Second node	Tillering	First node	Second node	Tillering	First node	Second node
	%	%	%	%	%	%	%	%	%
Weed free control	97.8	97.8	97.8	98.0	98.0	98.0	98.0	98.0	98.0
2.4-D	98.3	97.8	97.8	97.8	97.0	97.0	97.5	97.5	97.3
MCPP+dicamba	97.8	98.0	97.8	97.5	98.0	97.8	98.3	97.8	97.3
Triasulfuron+dicamba	97.5	98.0	97.5	97.8	97.5	97.0	97.3	97.3	97.8
2.4-D+florasulam	97.8	97.5	97.8	97.8	97.3	96.8	97.3	97.0	97.3
Amidosulfuron+iodosulfuronn	97.5	97.8	98.0	98.8	97.0	97.5	97.5	97.8	97.5
Florasulam+flumetsulam	97.5	97.5	97.8	97.5	98.0	97.5	97.8	97.0	97.8
LSD 0.05	1.6	1.1	1.1	1.4	1.4	1.6	1.5	1.5	1.3
LSD 0.01	2.1	1.5	1.5	1.9	1.9	2.1	2.0	2.0	1.8

In 3rd year (table 4) the highest barley seed germination (97,5%) was measured at NS 293 variety treated with 2,4-D at first node stage, also, at Reks variety treated with florasulam+flumetsulam at second node stage. Lowest barley seed germination (95,8%) was mesaured at NS 293 variety treated with florasulam+flumetsulam at second node stage. Also in this year the investigated herbicides did not significantly influence the barley seed germination.

Table 4. Influence of herbicides on barley seeds germination (3rd year.)

Variants	Varieties								
	Reks			NS 293			Egej		
	Tillering	First node	Second node	Tillering	First node	Second node	Tillering	First node	Second node
	%	%	%	%	%	%	%	%	%
Weed free control	97.0	97.0	97.0	96.8	96.8	96.8	97.5	97.5	97.5
2,4-D	96.3	96.5	96.3	96.8	97.5	96.5	96.8	96.5	96.5
MCPP+dicamba	97.0	96.3	96.5	97.0	96.8	97.0	96.5	97.0	97.0
Triasufurone +dicamba	96.8	96.8	97.0	96.5	97.0	96.8	97.0	96.5	97.0
2,4-D+florasulam	96.8	96.8	96.8	96.8	97.0	96.8	97.3	97.0	97.3
Amidosulfuron+iodosulfuronn	96.5	96.8	97.3	97.0	97.3	97.0	97.0	97.0	97.3
Florasulam+flumetsulam	96.5	97.0	97.5	97.0	97.3	95.8	97.3	96.8	96.8
LSD 0.05	1.4	1.2	1.5	1.4	1.1	1.5	1.3	1.2	1.2
LSD 0.01	1.8	1.7	2.1	1.9	1.6	2.0	1.8	1.6	1.6

There is no differences between growth stages during the herbicide applications in all three years of testing (Table 5), so growth stages have no impact on barley seed germination. Similar results were reported by (Danica et al.,1987) where examined herbicides do not have negative impact on wheat seed germination treated in tillering and shooting stage.

Table 5. Influence of growth stages on barley seeds germination.

	Average of all herbicides								
	1st year			2nd year			3rd year		
	Reks	NS 293	Egej	Reks	NS 293	Egej	Reks	NS 293	Egej
	%	%	%	%	%	%	%	%	%
Tillering	96.6	96.8	96.7	97.7	97.8	97.6	96.6	96.8	97.0
First node	96.4	96.9	96.8	97.8	97.5	97.4	96.7	97.1	96.8
Second node	96.6	96.8	96.8	97.8	97.3	97.5	96.9	96.6	97.0

According Spasic (1972), the investigated herbicides including 2,4-D, MCPA, MCPA+dicamba and terbutrin have not negative impact on wheat seed germination. Germination of galt barley variety was not affected by treating parent plants with MCPA, 2,4-D and metribuzine (Jeffery and John, 1984)

CONCLUSIONS

Based of the obtained results it can be concluded that the influence of herbicides on barley seed germination is not dependent on the growth stages during the application. If conditions do not allow herbicides to be used in the optimal period, it can be used no later than second node stage, without having a negative impact on the barley seed germination.

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ВЛИЈАНИЕ НА ХЕРБИЦИДИТЕ НА 'РТЛИВОСТА НА ЈАЧМЕНОТ ВО ЗАВИСНОСТ ОД ФАЗАТА НА ПОРАСТ

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Резиме

Во текот на три години беа изведени испитувања кај три сорти јачмен (*рекс, HC 293* и егеј) со 6 хербицидни варијанти (2,4-Д, МЦПП+дикамба, триасулфурон+дикамба, 2,4-Д+флорасулам, амидосулфурон+јодсулфурон и флорасулам+флуметсулам) во три различни фази на пораст (братење, прво коленце и второ коленце).

Целта на овој експеримент беше да се утврди влијанието на хербицидите аплицирани во три различни фази на пораст врз 'ртливоста на јачменот.

Во трите години на испитување хербицидите не влијаеа врз 'ртливоста на јачменот. Исто така, фазите на пораст на јачменот за време на третирањето немаа влијание врз 'ртливоста на семето јачмен. Ако условите не дозволуваат хербицидите да се употребат во оптималниот период, тие може да се аплицираат до фаза појава на второ коленце без да имаат негативно влијание врз 'ртливоста на јачменот.

Клучни зборови: *јачмен, сорти, хербициди, фази на пораст, 'ртливост*

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SPECIFICS OF SYMBIOTIC NITROGEN FIXATION OF CHICKPEA (*Cicer arietinum* L.)

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Abstract

Nitrogen is a limiting nutrient for plant growth and development. The ability to use nitrogen from the air by leguminous plants is their well-known feature. Nitrogen is present in the gaseous atmosphere (N_2) at about 78.1% by volume or 75.51% by weight, ie a total of 3.8x1015 t or 86.5 t / ha. However, atmospheric nitrogen, in reality, does not receive the plants directly from the air, but rather through previous adoption by Rhizobium bacteria living in the form of small tumors, called nodules on the roots of plants. These bacteria can fix nitrogen gas from the air in the soil and turn it into ammonia (NH_3), which is converted into ammonium (NH_4), which the plant can use.

Chickpea (*Cicer arietinum* L.) is a leguminous plant that has great agrotechnical significance. Characteristic of chickpeas as well as other legumes is that they enter symbiosis with nitrogen-fixing bacteria and can thus use fixed atmospheric nitrogen.

This paper presents and elaborates on the results of agrochemical analyzes of soil in the experiment with chickpea, ie differences in nitrogen content at the beginning and at the end of vegetation. The results show that the nitrogen content in the soil increased by 0.30 mg /g after the end of the vegetation in 2018 and 0.31 mg /g in 2019.

Key words: *root, inoculation, nodules, soil*

INTRODUCION

Chickpea (*Cicer arietinum* L.) - predominantly agricultural and garden culture, which is mostly cultivated in Macedonia and Dalmatian Zagora, is considered one of the earliest cultivated plants and its remains are found in the Middle East, up to 7,500 years old (Hillman, 1975). Chickpea is a leguminous plant that is used in people's diet. Seed of chickpea is similar to soybean seeds or dried beans, is used as a pot or as an addition to other vegetables when preparing food. Characteristic of the noodle as well as other legumes is that it comes into symbiosis with nitrogen-fixing bacteria and thus can receive atmospheric nitrogen. This interaction occurs when the roots of the plants secrete flavonoids that are recognized by certain bacteria that infiltrate the root and form nodules where the bacteria are equilibrated and the plants receive nutrients from the plants. These bacteria can fix nitrogen gas from the air into the soil and turn it into an ammonia (NH_3) that turns into ammonium (NH_4) that can be used by the plant. The process of fixing nitrogen (N_2-fixation) between plants and bacteria is called a symbiotic (mutually beneficial) connection. Every organism gets something from the other and gives back something in return. Rhizobium bacteria supply plants with nitrogen in the form of ammonium, and plants provide bacteria with carbohydrates as a source of energy. The rate of N_2-fixation is directly related to the plant growth rate of plants. Everything that reduces the growth of plants, such as drought, low temperature, limited plant nutrients, or diseases will also reduce the N_2 fixation. Maintaining a sufficient leaf surface is also critical to maintaining a high growth rate to support the fixation of N_2.

MECHANISM OF INFECTION

Rhizobia are free, soil saprophytes, living in symbiosis with plants from Fabaceae family. Rhizobia, once inoculated in the soil, may remain low in the absence of an appropriate host (Howieson, 1995). Plant begins a symbiosis with secretion of flavonoids, which are detected by bacteria. Flavonoids vary among different plant species and are recognized only by specific, but specific, bacterial species (Hassan & Mathesius, 2012). Flavonoids with diffusion penetrate the membrane of bacteria and induce the synthesis of NodD protein to activate the transcription of other genes involved in nodulation, including the production of Nod factor. Node factor is the primary molecule of the signal produced by the bacteria and is detected by the plant to induce nodular organogenesis. Structured Nod factor are lipohytoligosaccharides (LCOs) with a chitin oligomer pillar (Oldroyd & Downey, 2008). The nodABC genes encode the proteins needed to make the basic structure of the nod factor and are conserved in all types of rhizobia, with the exception of two species of Aeschynomene (Perret et al., 2000, Giraud et al., 2007). The kernel of the nod factor is then modified with species-specific proteins, resulting in various substitutions including glycosylation and sulphation (Long, 1996). These substitutions are specific to each host and offer another level of specificity of symbiosis (Dénarié et al., 1996; Long, 1996). Many surface polysaccharides are also involved in the specificity of the symbiosis, including lipopolysaccharides (LPSs), extracellular polysaccharides (EPSs), and capsular saccharides. The specific structure of lipohytoligosaccharides is known to be important for recognition by the Nodin host factor (NFRs) receptors that contain lysine (Endre et al., 2002).

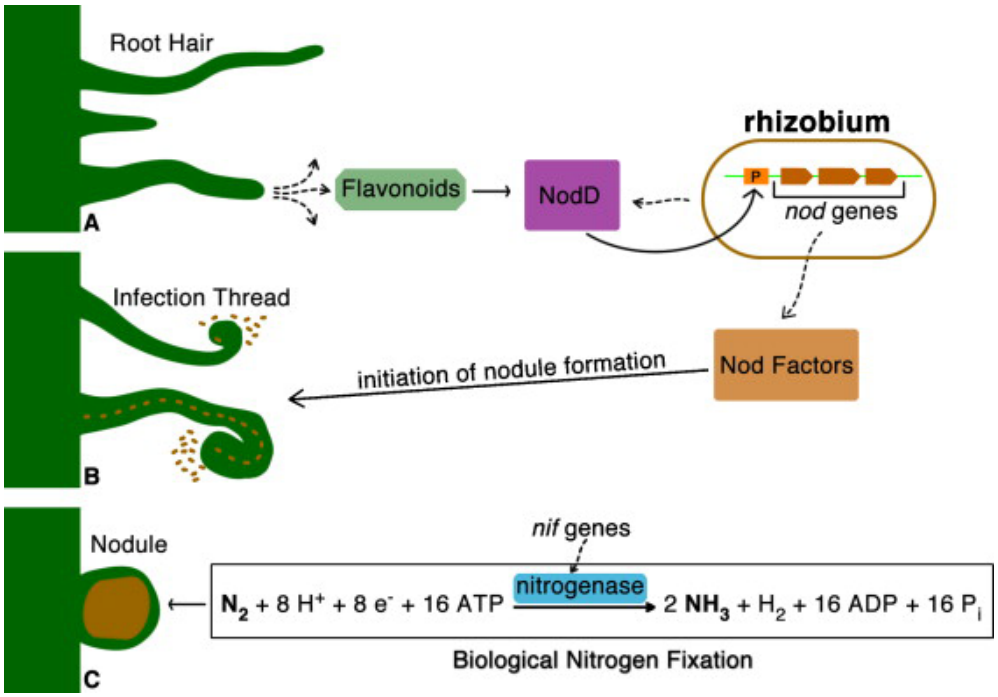

Figure 1. Mechanism of infection

(Resource: https://www.sciencedirect.com/science/article/pii/S0944501313001651)

Bending of root fibers and damage are the two mechanisms of infection that bacteria use. The entrance to the crack includes a rhizobium that enters through cracks on the lateral roots or stems (Goormachtig et al., 2004). Bending the root fibers implies recognition of the nod factor, resulting in bending of the root fibers. This is thought to involve a change in the polarity of plant cells, resulting in a new direction of growth at the root of the root (Gage, 2004). Next, the growth of the root peak changes from radial to polar elongation at the top. The continued growth of infection depends on the specificity of the nod factor, as well as the

extracellular polysaccharides (Jones et al., 2007). Both the epidermis and the cortex recognize the nod factor, the epidermis regulates bacterial infection, and the root cortex is responsible for the formation of nodules. Cortical cells develop into a nodulus duodenum. When the end of the infection reaches the primordium of the nodules, the rhizobium will enter the internal cells and become encapsulated within the peri-bacteroid membrane (Oldroys & Downey, 2008).

MATERIAL AND METHODS

For this work on the experimental field of the Goce Delcev University in the village of Pishirovo, Sveti Nikole were placed 8 varieties of chickpeas, of which 7 were Turkish (Gulumser, Cevdetbey, Azizyye, Yasa, Inci, Galatay and Azkan) and 1 domestic Macedonian variety with small grains. The trial was established on 04.04.2018 and the harvest took place on August 6, 2018, the length of the vegetation was 120 days. The soil was halomorphic, alkaline and poor with humus.

The varieties were placed in 3 repetitions per random block system and each variety was placed on an area of 10 m^2. The interval distance was 50 cm and in the order of 5cm. Seeds were not previously inoculated. The purpose of this study was to make a comparison of the amount of nitrogen that nitrogen fixing bacteria fixated in this case Rhizobium ciceri, turned unavailable nitrogen from the atmosphere to the available ammonia NH_3.

Before starting the experiment, we measured the moisture and hectoliter mass of the seeds. Table 1 shows the percentage of moisture and hectoliter mass of the seeds.

Table 1. Moisture percentage and hexahedral mass of the seeds

Variety	Moisture (%)	Hectoliter mass* (g/hl)
1. Gulumser	10.8	810
2. Cevdetbey	11.2	813
3. Aziziye	10.8	814
4. Yasa	11.0	797
5. Inci	11.1	815
6. Galatay	10.7	802
7. Azkan	11.2	801
8. Domestic	11.7	802

*The mass of grain that fits into a specified volume and it is reported in kilograms per hectolitre (g.hL-1)

We also examined seed germination percentage under controlled conditions. Table 2 shows the germination percentage of the seeds.

Table 2. Seed germination percentage

Variety	Germination percentage* (%)
1. Gulumser	96
2. Cevdetbey	99
3. Aziziye	93
4. Yasa	99
5. Inci	100
6. Galatay	94
7. Azkan	95
8. Domestic	99

* GP = seeds germinated/total seeds x 100

RESULTS AND DISCUSSION

In order to compare the amount of nitrogen in the soil, agrochemical analysis were made at the beginning and at the end of the vegetation. The Tables 3 and 4 show the results from the agrochemical analysis.

Table 3. Result from the agrochemical analysis at the beginning of vegetation (2018).

Parameter		Value	Measuring unit	Soil classification	Method
pH	KCl	7.53	/	very low alkaline	ISO- 10390:2005 (E)*
	H_2O	8.34	/	alkaline	
EC (1:2)		0.32	mS/cm	optimal	Electrical Conductivity: A County Extension Soil Laboratory Manual, E. A. Hanlon, Jr.
total N		1.57	mg/g	good nitrogen supply	ISO 11261:1995 (E)
available P_2O_5		71.52	mg/100g	good phosphorus supply	Modified method for the determination of readily available phosphorus and potassium soil samples with AL method according to Egnér, Riehm, Domingo
available K_2O		87.53	mg/100g	good potassium supply	Modified method for the determination of readily available phosphorus and potassium soil samples with AL method according to Egnér, Riehm, Domingo
humus		2.42	%	poorly supplied with humus	Chemical methods of soil testing , Stojanović 1966

* accredited methods

Table 4. Results from the agrochemical analysis at the end of vegetation (2018).

Parameter		Value	Measuring unit	Soil classification	Method
pH	KCl	7.62	/		ISO- 10390:2005 (E)
	H_2O	8.31	/	alkaline	
EC (1:2)		0.43	mS/cm	optimal	Electrical Conductivity: A County Extension Soil Laboratory Manual, E. A. Hanlon, Jr.
total N		1.80	mg/g	good nitrogen supply	ISO 11261:1995 (E)
available P_2O_5		65.60	mg/100g	good phosphorus supply	Modified method for the determination of readily available phosphorus and potassium soil samples with AL method according to Egnér, Riehm, Domingo
available K_2O		83.35	mg/100g	good potassium supply	Modified method for the determination of readily available phosphorus and potassium soil samples with AL method according to Egnér, Riehm, Domingo
humus		2.80	%	poorly supplied with humus	Chemical methods of soil testing , Stojanović 1966

* accredited methods

Table 5. Result from the agrochemical analysis at the beginning of vegetation (2019)

Parameter		Value	Measuring unit	Soil classification	Method
pH	KCl	7.54	/	very low alkaline	ISO- 10390:2005 (E)
	H_2O	8.23	/	alkaline	
EC (1:2)		0.71	mS/cm	optimal	Electrical Conductivity: A County Extension Soil Laboratory Manual, E. A. Hanlon, Jr.
total N		1.09	mg/g	good nitrogen supply	ISO 11261:1995 (E)
available P_2O_5		77.36	mg/100g	good phosphorus supply	Modified method for the determination of readily available phosphorus and potassium soil samples with AL method according to Egnér, Riehm, Domingo
available K_2O		125.67	mg/100g	good potassium supply	Modified method for the determination of readily available phosphorus and potassium soil samples with AL method according to Egnér, Riehm, Domingo
humus		2.51	%	poorly supplied with humus	Chemical methods of soil testing , Stojanović 1966

Table 6. Results from the agrochemical analysis at the end of vegetation (2019)

Parameter		Value	Measuring unit	Soil classification	Method
pH	KCl	7.71	/	very low alkaline	ISO- 10390:2005 (E)
	H_2O	8.50	/	alkaline	
EC (1:2)		0.65	mS/cm	optimal	Electrical Conductivity: A County Extension Soil Laboratory Manual, E. A. Hanlon, Jr.
total N		1.40	mg/g	good nitrogen supply	ISO 11261:1995 (E)
available P_2O_5		58.36	mg/100g	good phosphorus supply	Modified method for the determination of readily available phosphorus and potassium soil samples with AL method according to Egnér, Riehm, Domingo
available K_2O		174.04	mg/100g	good potassium supply	Modified method for the determination of readily available phosphorus and potassium soil samples with AL method according to Egnér, Riehm, Domingo
humus		2.73	%	poorly supplied with humus	Chemical methods of soil testing , Stojanović 1966

From the results (Table 3, 4, 5 and 6), we can see the difference in the available nitrogen from 1.57 mg/g to 1.80 mg/g in year 2018 and from 1.09 mg/g to 1.40 mg/g and to conclude that the chickpea fixed 0.23 mg/g of nitrogen (2018) and 0.31 mg/g (2019). Regarding the varieties we had a difference regarding the formation of nodules, in certain varieties were more effectively involved in symbiosis with bacteria. Most nodules were formed in the Cevdetbey variety. Apart from more nodules, plants of this variety have formed the largest seeds. Apart from differences in nitrogen, we also have differences in the humus content of the soil. Before the vegetation the content of humus was 2.42% and at the end of the vegetation 2.80% in 2018 and in 2019 the content of humus at the beginning of the vegetation was 2.50% and at the end was 2.73% . This is due to the increased activity of microorganisms that break down organic matter and make it available to plants, thereby increasing fertility of the soil.

Figure 2. Nodule on the root of chickpea.
(Resource: http://eagri.org/eagri50/AMBE101/lec18.html)

CONCLUDING REMARKS

Symbiotic nitrogenation (SNF) is an important biological feature that allows leguminous plants to grow efficiently in conditions of nitrogen restriction and also have significant agronomic and environmental benefits. Due to their unique ability to form a symbiotic relationship with a group of nitrogen fixating bacteria called "Rhizobia", the beans represent an important and diverse plant group.

The bacteria fixes the atmospheric N_2 to ammonia for direct plant use.

In our experiments we proved that the chickpea enriches the soil with nitrogen for 0.30 mg/g in 2018 and 0.31 mg/g in 2019.

Also we can conclude that apart from the increased nitrogen content, we have an increased quantity of humus-specific complex of nitrogen compounds that occur due to the mineralization of plant residues under the influence of enzymes released from microorganisms living in the soil.

That significantly affects the bulk density of soil and contributes to its retention of moisture and nutrients.

Therefore, the cultivation of leguminous plants is recommended, in this case, the chickpea in crop rotation.

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http://eagri.org/eagri50/AMBE101/lec18.html

СПЕЦИФИЧНОСТИ НА СИМБИОТСКА АЗОТОФИКСАЦИЈА КАЈ НАУТ (*Cicer arietinum* L.)

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Резиме

Азотот е ограничувачки хранлив елемент за растот и развитокот на растенијата. Способноста да се користи азотот од воздухот од страна на легуминозните растенија е нивна добро позната карактеристика. Азотот се наоѓа во атмосферата во гасовита форма (N_2) околу 78,1% волуменски или 75,51% тежински, односно вкупно 3,8x1015 t или 86,5 t/ha. Сепак, атмосферскиот азот, во реалноста, растенијата не го примаат директно од воздухот, туку преку претходно усвојување од страна на Rhizobium бактериите кои живеат во вид на мали тумори, наречени нодули на корените на растенијата. Овие бактерии можат да го фиксираат азотниот гас од воздухот во почвата и да го претворат во амонијак (NH_3), кој се претвора во амониумова форма (NH_4), што може да ја користи растението.

Наутот (*Cicer arietinum* L.) е легуминозно растение, кое има големо агротехничко значење. Карактеристично за наутот како и за другите легуминози е тоа што стапува во симбиоза со азотофиксирачки бактерии и на тој начин може да го користи фиксираниот атмосферски азот.

Во овој труд се прикажани и образложени резултатите од агрохемиските анализи на почвата во опитот со наут, односно разликите во количината на азот на почетокот и на крајот од вегетацијата. Од резултатите може да се види дека количините на азот во почвата се зголемиле за 0.30 mg/g по завршување на вегетацијата на наутот во 2018 година и 0.31 mg/g во 2019 година.

Клучни зборови: *симбиоза, инокулација, нодули, почва*

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OBSERVATION OF GRAPEVINE PHYTOPLASMAS STATUS *(CANDIDATUS PHYTOPLASMA SOLANI)* IN THE REPUBLIC OF NORTH MACEDONIA

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Abstract

Phytoplasmas ("*Candidatus Phytoplasma*") are non-cultivable wall-less bacteria found in plant phloem and insect vector cells. Economically important Grapevine yellows (GY) diseases all over the world are caused by phytoplasmas belonging to the ribosomal subgroups 16SrV-C and -D (etiological agents of Flavescence dorée, FD) and 16SrXII-A ("*Candidatus Phytoplasma solani*", etiological agents of *Bois noir,* BN) which cause similar symptoms but in epidemiology considerably different.

The aim of this study was to identify and characterize the phytoplasmas associated with GY in vineyards in our country, through the molecular analysis of the genes *stamp* and *map*. The obtained results showed the presence of BN *Bois noir*, *stolbur*, economic important phytoplasma that cause serious losses on grapes. In detail, molecular characterization evidenced that BN phytoplasma strains belong to three distinct *stamp* genotypes (St1, St30, St37), while FD phytoplasma is not present in our country yet.

This data will be helpful for further analysis focused on investigating the epidemiology of BN and FD.

Key words: *grapevine yellows, Flavescence doree, Bois Noir, stamp and map genes*

INTRODUCTION

Grapevine yellows (GY), a group of diseases that were originally thought to be caused by viruses, are now known to have a phytoplasma etiology. The first disorder reported from Vitis vinifera, and the most widely known of the GY group, is certainly *Flavescence dorée* (FD), which appeared in South-West France in the 1950's, where from it spread to other viticultural districts of France, Northern Italy and neighbouring European countries. *Bois noir* (BN), which symptoms are indistinguishable from those of FD, was also firstly reported from France, then from the most important viticultural areas of Europe, including Italy (Belli *et al.,* 2010).

Phytoplasmas are plant pathogenic bacteria in the class *Mollicutes* and were formerly called "*mycoplasma - like organisms*" (MLOs) (Doi *et al.*, 1967). They are transmitted by insect vectors (leafhoppers and psyllids) and infect hundreds of plant species worldwide, including many economically important crops, fruit trees, and ornamental plants (Hogenhout et al., 2008; Oshima *et al.,* 2013). More than 700 plant species are affected by phytoplasma diseases and many of them show symptoms such as yellowing, witches broom (proliferating shoots), phyllody (leaf-like petals and sepals), virescence (greening of floral organs), and sometimes withering of plants (Lee *et al.,* 2000).

Phytoplasmal diseases are the primary factors limiting production of many important crops all over the world: since the risk to introduce these diseases by the movement of phytoplasma infected plants (mainly propagating material) precise and strict quarantine regulations are applied in all over the world.

The presence of BN phytoplasmas in North

Macedonia was reported for the first time in 2003, in a survey limited to a small viticultural region, i.e. Veles and Skopje areas (Šeruga *et al*., 2003).

The affected vines show downward rolling of the leaves accompanied by yellow or bright red discoloration of veins and blades, berry withering and uneven or total lack of cane lignification. GY, however, have different phytoplasma species as causal agent, as well as different insect vectors, which are either leafhoppers or planthoppers (Homoptera: Auchenorrhyncha) that feed either specifically or occasionally on the vines. (Belli *et al*., 2010). It seems, that the relationship between diseases and insect vectors is subject that needs to be more deeply investigated for a better understanding of GY epidemiology and for the hopeful development of new sustainable means for their containment. Damages caused by GY may be extensive and economically relevant, since the most of the diseased vines are lost.

The cycle of transmission of phytoplasmas depends on the life cycle (monovoltine, multivoltine) and feeding habit (monophagous, polyphagous) of their insects vectors, polyphagous vectors have the potential to inoculate a wide range of plant species, depending on the resistance to infection of each host plant. (Bosco *et al.*, 1997).

The transmission process consists of three steps, acquisition, latent period, and inoculation. Vectors acquire phytoplasmas by feeding on infected plants for some hours/ days and become inoculative after a latent period of two or more weeks during which the microorganisms multiply in their organs and hemolymph. A method to reduce alternative vector host plants of phytoplasma-infected crop plants and weeds is by roguing. The most effective means of insect vector control is through physical prevention – either by use of screening or by use of a mineral coating on the plant itself.

The primary means of controlling phytoplasma vectors is by insecticides; however, increasing pressure to find less toxic and more biologically based techniques to control, or at least manage, insect vectors necessitates an even greater reliance on solid understandings of the biology of insect vectors from the cellular to the ecological level. (Conti & Alma, 2002).

The activities carried out in this paper were laboratory analyses conducted on Vranec variety in Peshirovo locality (N. Macedonia).

MATERIAL AND METHODS

GY monitoring and sample collection in vineyards

In order to determine the GY associated phytoplasmas (check status of BN and FD phytoplasmas) in the examined vine regions, leaf samples of symptomatic grapevines (*Vitis vinifera* L.) were collected in September and October 2018 in N. Macedonia (Figure 1 a. and b.). In details, leaf samples were collected from several symptomatic plants from the variety Vranec in Peshirovo (Ovce Pole, N. Macedonia) (Table 1).

Figure 1. Collected plant samples from vineyards in Peshirovo, Ovce Pole, N. Macedonia

- Typical symptoms on leaves (triangle form, reddening);
- Mix infection of symptomatology (red spots on leaves and reddening – virus and phytoplasmas symptoms)

Table 1. Selected grapevine leaves samples from Peshirovo, North Macedonia for laboratory analyses.

Location	Variety	Lab code of plant samples
Pesirovo, Sv. Nikole	Vranec, local red variety	BN34
		BN35
		BN36
		BN37
		BN38

Laboratory analysis

Leaf samples collected in vineyards were stored at –20°C, for the following molecular analyses: (i) total nucleic acid extraction; (ii) identification of phytoplasmas by amplification of the genes *stamp* (BN) and *map* (FD); (iii) molecular characterization of phytoplasmas through analysis of nucleotide sequences of the genes stamp and map.

Extraction of total nucleic acids

Total nucleic acids (TNA) were extracted from 1g of plant material using a modification of the cethyltrimethyl-ammonium bromide (CTAB) procedure described by Angelini et al. (2001). Nucleic acids were diluted in sterile deionised water to a final concentration of 20 ng/µl.

Analysis of Nanodrop-Spectrophotometry

The NanoDrop is a spectrophotometer that allows us to quantify nucleic acids from different samples, using micro-volumes and freeing itself from the use of the classic cuvettes. It is composed of an instrumental part and software installed in a computer (Figure 2).

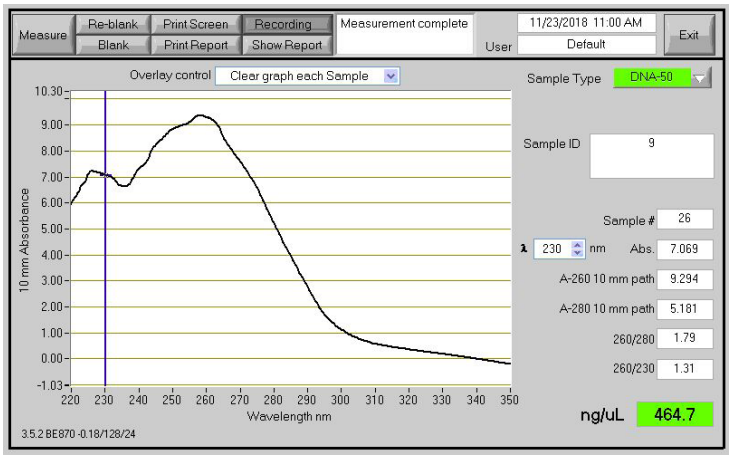

Figure 2. Example of DNA analysis by Nanodrop-spectrophotometer.

Molecular Identification - Polimerase chain reaction PCR

The molecular detection of BN phytoplasma was carried out on the stamp gene (coding the antigenic protein of 'Ca. P. solani' membrane). *Stamp* gene was amplified by nested-PCR using primer pairs: Stamp-F (5'-GTAGGTTTTGGATGTTTTAAG-3') / Stamp-R0 (5'-AAATAAAAGAACAAGTATAGACGA-3'), followed by primers Stamp-F1 (5'-TTCTTTAAACACACCAAGAC-3') / Stamp-R1 (5'- AAGCCAGAATTTAATCTAGC-3') (Fabre et al., 2011). The reaction conditions were as follows: (i) dPCR: 94°C x 4 min; 35 cycles consisting in: 94°C x 30 s, 56°C x 30 s and 72°C x 1 min 30 s; final extension at 72 °C x 7 min; (ii) nested-PCR: 94°C x 4 min; 35 cycles consisting in: 94°C x 30 s, 52°C x 30 s and 72°C x 30 s; final extension at 72 °C x 7 min.

The PCR mix was as follows: Taq 0.125 U / µl; Buffer 1 X; $MgCl_2$ 1.5 mM, Forward example 0.4 µM, Reverse example 0.4 µM, dNTPs 0.2 µM.

All PCR products were analysed by electrophoresis in 1% agarose gel and stained with Midori Green. Electrophoresis was performed using 100 V for 30 minutes. DNA was visualized under UV light on a transilluminator.

The molecular detection of FD phytoplasma was carried out on the *map* gene (coding methionine aminopeptidase). Plant total nucleic acids were employed as templates in nested PCR

assays performed using primer pair: FD9f5 (5'-CAAAAAATTACTTTTGGCGGGAC-3') and MAPr1 (5'-TGCTCAAAATGAGCGCTTAAAC-3'), followed by FD9f6 (5'-GTCGCTTTAGAATCGACACA-3') and MAPr2 (5'-TCGGAAGTAACAGCAGTCCA-3'). Direct and nested PCR conditions were: 1 min at 92°C and 35 cycles, with 1 cycle consisting of 30 s at 92°C, 30 s at 52°C, and 1 min 30 s at 66°C (Arnaud et al., 2007). The PCR mix was as follows: Taq 0.125 U/ml; Buffer 1 X; $MgCl_2$ 1.5 mM, Forward primer 0.4 mM, Reverse primer 0.4 mM, dNTPs 0.2 mM. All PCR products were analyzed by electrophoresis in 1% agarose gel and stained with Midori Green.

Nucleotide sequence analysis

The PCR products of *stamp* and *map* genes, amplified by vine plants, were sequenced at a commercial service (Eurofins Genomics, Germany) with a minimum coverage of 3X. The obtained nucleotide sequences, whose quality has been evaluated through electropherogram analysis, have been assembled with the CAP (Contig Assembly Program) function of the BioEdit software, version 7.2.5 (Hall, 1999). In order to characterize the phytoplasma strains, the nucleotide sequences of the *stamp* and *map* genes were inserted into a database containing the sequences of the representative strains of the stamp gene variants of *'Ca. P. solani'* and the map gene variants of phytoplasmas associated with FD, available in GenBank (Quaglino et al., 2016; Casati et al., 2017)

The sequences have been aligned through the "ClustalW Multiple Alignment" function of the Bioedit software. The alignments obtained were used to calculate the sequence identity with the "Sequence identity Matrix" function. Based on the sequence identity of the stamp and map genes the phytoplasmas were inserted into gene variants already known or proposed for the first time in this work. In addition, the alignments were used for the subsequent phylogenetic analysis conducted with the MEGA6 software (Tamura et al., 2013), using the 'neighbour-joining' algorithm (bootstrap 1000).

RESULTS AND DISCUSSION

Identification of GY phytoplasmas

PCR amplification showed that: (i) three (BN34, BN36, BN38) out of five Vranec plants from Macedonia were infected by BN phytoplasma, and FD phytoplasma was not detected in tested samples (Figure 3).

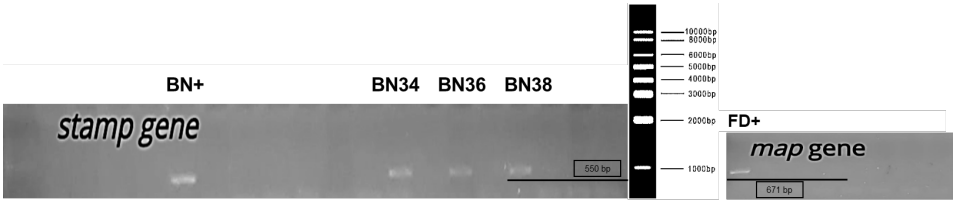

Figure 3. Visualization of PCR products obtained through the amplification of *stamp* and *map* genes.

Table 3. BN and FD phytoplasma identification in grapevines.

Locality	Variety	Sample	Date of sampling	PCR	
				stamp (BN)	map (FD)
Peshirovo, Ovce Pole	Vranec	BN34	IX.2018	+	-
		BN35	IX.2018	-	-
		BN36	IX.2018	+	-
		BN37	IX.2018	-	-
		BN38	IX.2018	+	-

Molecular characterization

Analysis of the gene *stamp*

Sequence identity analysis of *stamp* gene nucleotide sequences showed that BN phytoplasma strains identified in grapevine BN34, BN36 and BN38 are characterized by distinct *stamp* gene variants. In detail, strains identified in plants BN34 and BN38 have stamp variants undistinguishable from the variants St1 and St30, respectively. The strain infecting the plant BN36 showed best identity with sequence variants St4, St15 and St37 (Table 4, 5).

Table 4. Sequence identity matrix of the *stamp* gene. Comparison between the gene variants was found. The identical gene variants are indicated by the same colour.

	Seq->	1	2	3	4	5	6	7	8	18	19	32	33	34	39	40
1	BN34	ID	0.964	0.997	1	0.987	0.952	0.962	0.979	0.962	0.956	0.927	0.997	0.985	0.952	0.964
2	BN36	0.964	ID	0.966	0.964	0.976	0.987	0.995	0.968	0.995	0.978	0.946	0.966	0.978	0.985	0.995
3	BN38	0.997	0.966	ID	0.997	0.985	0.954	0.964	0.977	0.964	0.954	0.925	1	0.983	0.954	0.966
4	Rqg50_(KC703019)_St1	1	0.964	0.997	ID	0.987	0.952	0.962	0.979	0.962	0.956	0.927	0.997	0.985	0.952	0.964
5	Rqg31_(KC703017)_St2	0.987	0.976	0.985	0.987	ID	0.964	0.974	0.991	0.974	0.968	0.938	0.985	0.997	0.964	0.976
6	Rpm35_(KC703015)_St3	0.952	0.987	0.954	0.952	0.964	ID	0.983	0.955	0.983	0.974	0.938	0.954	0.966	0.985	0.983
7	STOL_(FN813261)_St4	0.962	0.995	0.964	0.962	0.974	0.983	ID	0.966	0.995	0.978	0.944	0.964	0.976	0.981	0.991
8	GGY_(FN813256)_St5	0.979	0.968	0.977	0.979	0.991	0.955	0.966	ID	0.97	0.964	0.942	0.977	0.989	0.955	0.972
18	P7_(FN813258)_St15	0.962	0.995	0.964	0.962	0.974	0.983	0.995	0.97	ID	0.974	0.948	0.964	0.976	0.981	0.995
19	L973_(FN813255)_St16	0.956	0.978	0.954	0.956	0.968	0.974	0.978	0.964	0.974	ID	0.942	0.954	0.968	0.976	0.974
32	Vv12_274_(KJ469717)_St29	0.927	0.946	0.925	0.927	0.938	0.938	0.944	0.942	0.948	0.942	ID	0.925	0.936	0.934	0.946
33	Vv24_(KC703022)_St30	0.997	0.966	1	0.997	0.985	0.954	0.964	0.977	0.964	0.954	0.925	ID	0.983	0.954	0.966
34	Rqg42_(KC703016)_St31	0.985	0.978	0.983	0.985	0.997	0.966	0.976	0.989	0.976	0.968	0.936	0.983	ID	0.964	0.978
39	Carv2_(KT184880)_St36	0.952	0.985	0.954	0.952	0.964	0.985	0.981	0.955	0.981	0.976	0.934	0.954	0.964	ID	0.981
40	Char7_(KT184881)_St37	0.964	0.995	0.966	0.964	0.976	0.983	0.991	0.972	0.995	0.974	0.946	0.966	0.978	0.981	ID

Table 5. *Stamp* and *map* sequence variants identified in BN and FD phytoplasma strains identified in this study.

		PCR		Variety of sequence	
	samples	*stamp*	*map*	*stamp*	*map*
North Macedonia Pesirovo	BN34	+	-	St1	-
	BN35	-	-	-	-
	BN36	+	-	St4, St15, St37	-
	BN37	-	-	-	-
	BN38	+	-	St30	-

Phylogenetic analysis

Phylogenetic analysis of *stamp* gene alignment showed the presence of four main clusters: cluster a, related to nettle epidemiology (nettle-related), and clusters b-I, b-II and b-III, related to bindweed epidemiology (bindweed-related). The BN phytoplasma strains identified in Vranec variety from North Macedonia grouped in the clusters b-II and b-III (Figure 4).

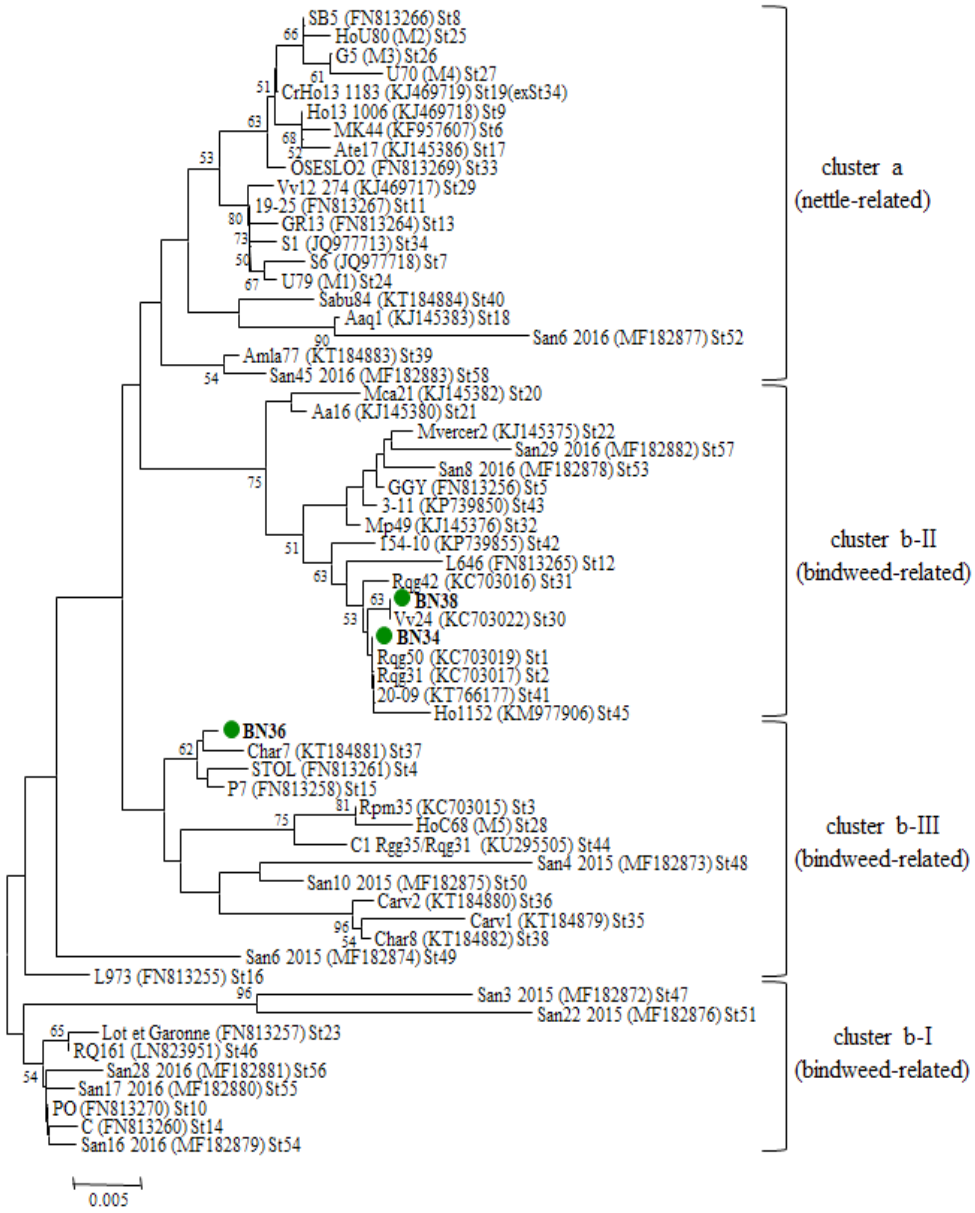

Figure 4. Phylogenetic tree built on the basis of the nucleotide sequences of the stamp gene.

CONCLUSION

The results obtained in this thesis have confirmed the prevalence of *'Ca. P. solani'*, the etiological agent of BN, in N. Macedonia (Kostadinovska et al., 2014), and the absence of FD. This evidence could suggest that such varieties could be less susceptible to phytoplasmas associated with GY.

Molecular characterization by the analysis of *stamp* gene nucleotide sequences showed the presence of distinct variants (St1, St30, St37) among BN phytoplasmas identified in North Macedonia. These variants were largely identified, in previous studies, in Macedonia, Serbia, Croatia and Georgia (Mitrev et al., 2008, Cvrkovic et al., 2014; Kostadinovska et al., 2014; Quaglino et al., 2016). BN epidemiology involves a broad range of plant hosts and insect vectors (Marcone et al., 1997; Schneider et al., 1997; Mori et al., 2015).

This study, for the first time observed a complete laboratory analyzes for BN and FD phytoplasmas, including molecular identification, nucleotide sequence analysis and phylogenetic analyzes which group BN phytoplasma in clusters b-II and b-III.

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ИСПИТУВАЊЕ НА ФИТОПЛАЗМАТСКИОТ СТАТУС КАЈ ВИНОВАТА ЛОЗА (*CANDIDATUS PHYTOPLASMA SOLANI*) ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА

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Резиме

Фитоплазмите (*Candidatus Phytoplasma*) се безклеточни форми на бактерии кои не се изолираат на хранлива подлога, и кои се наоѓаат во растителниот флоем и во клетките на инсектите вектори. Економското значење на болестите предизвикани од „жолтилата кај виновата лоза" *Grapevine yellows* (GY) е големо насекаде во светот и најчесто распространетите фитоплазми припаѓаат на рибозоналните подгрупи 16SrV-C и -D (етиолошки причинител *Flavescence dorée*, FD фитоплазмата) и 16SrXII-A (*Candidatus Phytoplasma solani,* етиолошки причинител *Bois noir*, BN фитоплазмата). Овие две групи на фитоплазми предизвикуваат слични симптоми, но разликите се забележуваат во епидемиологијата.

Целта на ова истражување беше идентификација и карактеризација на фитоплазмите кои припаѓаат на GY групата („жолтила") во лозовите насади во нашата земја, со помош на молекуларна анализа на геновите *stamp* и *map*. Добиените резултати од истражувањето го докажаа присуството на BN *Bois noir*, *stolbur*, економски значајна фитоплазма која предизвикува значителни економски загуби кај грозјето. Од деталните истражувања, преку молекуларна карактеризација на видовите од BN фитоплазмата го потврдивме присуството на три различни *stamp* генотипови (St1, St30, St37), додека FD фитоплазмата сè уште не е потврдена во нашата земја.

Оваа дата база на податоци добиени од нашето истражување може да биде корисна за идни анализи фокусирани на испитување на епидемиологијата на BN и FD.

Клучни зборови: *„жолтила кај виновата лоза", Flavescence doree, Bois Noir, stamp и map гени*

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INVESTIGATION OF THE CORRELATIONS BETWEEN QUANTITATIVE TRAITS WHICH DETERMINE YIELD IN THE VINE CULTIVAR BOLGAR AND THE HYBRID COMBINATION BOLGAR X RUSSALKA 1

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Abstract

The correlations between quantitative traits, which determine yield in the vine cultivar Bolgar and F_1 progeny of the hybrid combination Bolgar x Russalka 1 have been investigated through Path analysis. It has been found that there are no highly significant traits for the formation of yield from this cultivar, for which the correlation coefficients, direct and total indirect influences have positive values. Positive correlations have been reported between the two parent cultivars and F1 progeny for the traits: shoot and fruiting shoot fertility coefficient, cluster width, total number of shoots, fruiting shoots and clusters. All studied fertility coefficients in the cultivar Bolgar exert positive direct influences, determined by moderate correlations, on the seedlings from F1 progeny. The correlations of the other traits and the influence of separate parent cultivars have positive or negative values, which can be used in the selection of valuable elite forms.

Key words: *quantitative traits, yield; seeded and seedless vine cultivar; F1 progeny; correlations; direct and indirect influences; Path analysis*

INTRODUCTION

It is known that, by means of Path analysis it is possible to determine the degree, to which the variability of a certain quantitative trait influences the change of a single trait or a group of other traits. The total correlation and the relative participation of each trait in yield formation are established through this method, and it allows their comparing with results from other experiments. The advantages of Path analysis combined with the analytical potential of the production function are a model, which can be efficiently applied in the experimental procedures in viticulture (Mokreva 2004, 2007). The type of the effect from the influence of a certain significant agrobiological factor, combined with the remaining ones, on the phenotypic manifestation of the variable value – the cumulative trait (yield), is also of interest. Their application is especially important and valuable in the complicated development of new table seeded and seedless vine cultivars (Smirnov 1977; Golodriga et al., 1985; Pospisilova, Palenik 1988; Valchev 1990; Troshin 1990, 1997). The purpose of the current investigation is to determine the degree of correlations between quantitative traits and their relative participation in yield formation for the vine cultivar Bolgar and F_1 progeny of the hybrid combination Bolgar x Russalka 1.

MATERIAL AND METHODS

During seven consecutive years, in 30 plants from F_1 progeny of the hybrid combination Bolgar (P_1 – seeded) x Russalka 1 (P_2 – seedless), 21 quantitative traits were determined, related to the phenology, fertility, quality and grape yield (Bulgarian Ampelography, 1990; Roychev 2012). The experimental results were processed by means of Path analysis (Rokitskii 1973; Lidanski 1988). The studied traits were conditionally divided into six groups. The direct and indirect

influences of the indicated traits of parent cultivars on yield formation in seedlings and the degree of correlations between them were analyzed. The presented results are a part of a larger-scale research related to the application of Path analysis in selection for the obtaining of new seeded and seedless vine cultivars.

RESULTS AND DISCUSSION

In the cultivar Bolgar (P_1) few traits from different groups manifest high, significant and moderate correlations with yield (Table 1). Most often, the high direct positive effect is eliminated by almost the same in size total indirect influence, deriving from the interdependence of the other traits or vice versa. This is typical not only for the traits with higher correlation coefficients (0,625) – total number of clusters, (0,530) - total number of fruiting shoots, (0,476) - total number of shoots, (0,357) – berry length, (0,383) – berry width, (0,357) – shoot fertility coefficient, (0,288) – main shoot fertility coefficient, but also for all remaining ones. There are no traits significant for the yield, in which the correlation coefficients, direct and total indirect influences have positive values, which means that the yield in this cultivar depends on numerous different factors and their interaction.

Table 1.

The traits which exert positive influence on yield formation in the cultivar Bolgar (P1) are in the first, second, third and sixth group, with total relative participation 95,6% (Table 2). The most significant presence belongs to all traits connected with the actual fertility of vines (59,2%). Out of them, the larger share belongs to total number of clusters (24,8%) and fruiting shoots (17,9%), as well as the three fertility coefficients – totally (16,5%). Comparatively more traits from a larger number of groups participate in yield formation for this cultivar.

Table 2. Relative participation of traits in the formation of grape yield from the seeded cultivar Bolgar (P_1).

Groups	№	Total yield variation	100,0
		Total relative participation of the most important traits 95,6%, from which:	%
I	x_1	Shoot fertility coefficient	8.1
	x_2	Main shoot fertility coefficient	5.3
	x_3	Fruiting shoot fertility coefficient	3.1
II	x_6	Cluster length (cm)	2.5
III	x_9	Berry length (mm)	8.1
	x_{10}	Berry width (mm)	9.3
VI	x_{18}	Total number of buds	2.1
	x_{19}	Total number of shoots	14.4
	x_{20}	Total number of fruiting shoots	17.9
	x_{21}	Total number of clusters	24.8
Other traits			4.4

Table 1. Direct and indirect influences of the studied traits on grape yield per vine for Bolgar (P_1).

Groups	Traits	[b]	Direct and indirect influences																				Total indirect influences	r
			x1	x2	x3	x4	x5	x6	x7	x8	x9	x10	x11	x12	x13	x14	x15	x16	x17	x18	x19	x20		
I	Shoot fertility coefficient	x_1	-49,488	24,798	-20,283	0,693	-0,382	-0,100	-2,845	5,532	-3,574	8,940	-4,154	6,118	-1,154	-4,639	0,158	0,769	1,587	7,821	-1,361	-10,225	38,267	48,943	0,357
	Main shoot fertility coefficient	x_2	-27,568	38,618	-40,611	-1,850	-0,374	-0,365	-6,662	5,529	-7,485	5,660	7,668	-2,858	9,195	-3,388	-0,734	-1,232	-2,287	6,046	-0,467	-1,662	29,235	-35,230	0,288
	Fruiting shoot fertility coefficient	x_3	-14,573	26,254	-48,488	0,298	-0,191	-0,328	8,560	-0,295	-10,944	8,415	12,044	-18,851	6,928	12,254	-0,228	1,729	-1,713	6,214	8,686	5,108	17,041	56,789	0,221
II	Bilberry-shape berries (%)	x_4	4,371	5,956	2,613	-8,486	8,863	0,141	5,548	0,137	3,747	-2,116	-5,571	1,832	14,201	-14,780	-1,322	1,953	-0,957	-1,588	1,421	4,726	-12,549	6,365	-0,078
	Average cluster weight (g)	x_5	5,395	-4,747	3,755	-1,509	2,877	0,257	3,433	1,444	-4,135	3,985	2,879	1,532	9,594	-8,963	-0,791	1,034	-5,361	-10,847	13,595	9,325	-28,512	-2,712	0,165
	Cluster length (cm)	x_6	-3,427	18,597	-15,618	0,767	-0,721	-1,197	-6,804	5,418	-3,422	-7,783	16,748	8,327	7,319	1,494	-0,213	-4,595	1,958	1,575	-4,879	-3,688	15,252	1,384	0,197
	Cluster width (cm)	x_7	-6,348	1,328	1,738	1,598	-0,543	-0,448	-18,782	-0,378	4,018	-14,151	9,071	-6,963	17,443	13,442	-0,435	-5,851	4,188	-6,813	-0,852	-7,829	19,514	18,354	0,152
III	Average weight of 100 berries (g)	x_8	6,398	-6,163	-8,475	0,825	-0,118	0,183	-8,195	-36,188	10,852	-12,852	-7,125	-11,375	10,923	4,947	-0,868	13,344	-0,089	8,254	5,531	4,368	-18,287	34,595	-0,137
	Berry length (cm)	x_9	-5,632	18,612	-24,002	0,936	0,462	-0,158	2,838	13,752	-35,787	30,738	14,391	6,852	-19,278	7,329	1,556	-6,863	-6,878	9,189	-5,418	2,091	6,225	26,114	0,357
	Berry width (cm)	x_{10}	-8,488	4,785	-11,005	0,315	0,264	0,214	5,960	9,836	-18,330	48,182	-16,542	13,849	-30,024	4,918	2,288	-4,376	-1,877	7,205	-6,158	-3,974	17,689	-42,889	0,383
	Berry shape index	x_{11}	4,895	6,795	-16,527	0,886	0,291	-0,483	-4,888	6,089	-9,804	-17,355	41,147	-6,738	14,701	3,380	-0,958	-3,168	-6,447	2,320	2,995	7,768	-14,568	-41,218	-0,061
IV	Budding-flowering (days)	x_{12}	6,859	2,547	-13,854	0,152	-0,108	0,008	-3,891	-9,752	3,829	-13,753	8,768	-48,882	9,758	31,134	-1,148	5,400	1,153	8,244	-4,683	0,118	7,543	48,844	-0,138
	Flowering-softening (days)	x_{13}	0,542	6,757	-7,870	-1,839	0,555	-0,175	-6,385	-7,722	9,988	-35,885	12,178	-6,048	48,486	-11,225	-3,167	3,223	-3,453	-15,705	8,571	5,641	-11,157	-49,885	-0,158
	Softening-fruit maturity (days)	x_{14}	-4,057	2,886	14,915	-2,849	0,555	0,038	5,269	3,745	4,057	-4,577	-2,938	27,494	12,028	-48,488	-1,368	-8,632	-3,154	1,416	5,385	1,057	-18,858	45,489	0,081
	Budding-fruit maturity (days)	x_{15}	1,795	7,514	-3,628	-2,385	0,638	-0,071	-2,218	-8,478	11,295	-27,589	10,965	-13,089	44,116	-17,795	-3,687	5,054	-3,441	-8,153	5,717	4,141	-6,928	3,361	-0,206
V	Sugars (%)	x_{16}	-1,788	-2,538	-5,511	-0,789	0,168	0,308	5,801	-26,455	9,584	-18,886	-7,360	-12,487	9,037	1,854	-1,017	17,722	-2,688	5,046	3,612	5,017	-11,712	-17,587	-0,225
	Acids (g/dm³)	x_{17}	-4,157	-5,445	6,308	0,405	-1,885	-0,154	-4,862	0,285	11,534	-5,286	-17,308	-3,188	-11,186	5,652	0,888	-3,812	16,941	1,822	-3,488	-9,714	22,234	-15,258	0,091
VI	Tufet number of buds	x_{18}	-10,272	7,145	-11,359	0,414	-1,818	-0,151	4,011	-0,289	-7,593	18,870	3,091	-0,323	-25,257	-2,125	0,542	2,894	0,585	38,982	-15,802	-6,914	23,734	-38,719	0,183
	Tufet number of shoots	x_{19}	-1,782	8,551	1,257	0,295	-1,262	-0,187	-8,580	6,268	-4,495	11,423	-3,978	-6,085	-13,738	8,077	0,858	-2,865	1,683	14,973	-31,880	-16,094	41,784	31,479	0,476
	Tufet number of fruiting shoots	x_{20}	-18,342	2,678	12,888	1,337	-1,188	-0,192	-6,264	6,748	2,368	7,549	-14,067	8,214	-12,329	2,158	0,858	-3,911	6,555	5,397	-21,946	-22,728	54,157	23,265	0,538
	Tufet number of shoulders	x_{21}	-24,758	17,845	-15,369	1,289	-1,318	-0,288	-5,868	5,771	-2,550	12,158	-9,836	-4,535	-8,884	7,897	0,508	-3,314	5,445	11,710	-28,543	-19,678	62,882	-62,887	0,625

The structure of correlations between separate parents and F_1 progeny by groups of traits is different (Table 3). In the first group, the correlations are positive at shoot fertility coefficient (0,176)-P_1, (0,235)-P_2 and fruiting shoot fertility coefficient (0,165) -P_1, (0,382) -P_2; in the second group – cluster width (0,138)-P_1, (0,608)-P_2, in the sixth group – total number of shoots (0,305)-P_1, (0,009)-P_2, total number of fruiting shoots (0,155)-P_1, (0,057)-P_2 and total number of clusters (0,291)-P_1, (0,027) -P_2. In all traits in the fourth group – phenophases and periods, fifth group – chemical composition, and sixth group – actual fertility of vine, the positive correlations prevail of the cultivar Bolgar (P_1) with F_1 progeny, and in the first group - of the cultivar Russalka 1 (P_2). Except for berry shape index for Bolgar (P_1), both parent cultivars have negative correlations for the berry traits. We should also point out the higher positive correlations of Bolgar (P_1) with F_1 progeny regarding the traits total number of buds (0,385), acids (0,578), budding-flowering (0,399) and cluster length (0,525), and of Russalka 1 (P_2) – main shoot fertility coefficient (0,313), millerandage berries (0,358) and average cluster weight (0,278).

Table 3. Correlation coefficients between the traits of the studied vine cultivars Bolgar (P_1), Russalka 1 (P_2) and the plants from the hybrid combination - F_1 progeny.

Groups	№	Traits	Cultivars		F_1	P_1	P_2
I	1	Shoot fertility coefficient	F_1 progeny	F_1	1	0,176	0,235
			Bolgar	P_1		1	-0,133
			Ryssalka 1	P_2			1
	2	Main shoot fertility coefficient	F_1 progeny	F_1	1	-0,098	0,313
			Bolgar	P_1		1	0,124
			Ryssalka 1	P_2			1
	3	Fruiting shoot fertility coefficient	F_1 progeny	F_1	1	0,165	0,382
			Bolgar	P_1		1	-0,236
			Ryssalka 1	P_2			1
II	4	Millerandage berries (%)	F_1 progeny	F_1	1	-0,042	0,358
			Bolgar	P_1		1	0,218
			Ryssalka 1	P_2			1
	5	Average cluster weight (g)	F_1 progeny	F_1	1	-0,112	0,278
			Bolgar	P_1		1	0,135
			Ryssalka 1	P_2			1
	6	Cluster length (cm)	F_1 progeny	F_1	1	0,525	-0,350
			Bolgar	P_1		1	-0,270
			Ryssalka 1	P_2			1
	7	Cluster width (cm)	F_1 progeny	F_1	1	0,138	0,608
			Bolgar	P_1		1	0,117
			Ryssalka 1	P_2			1
III	8	Average weight of 100 berries (g)	F_1 progeny	F_1	1	-0,294	-0,190
			Bolgar	P_1		1	-0,044
			Ryssalka 1	P_2			1
	9	Berry length (mm)	F_1 progeny	F_1	1	-0,048	-0,251
			Bolgar	P_1		1	0,174
			Ryssalka 1	P_2			1
	10	Berry width (mm)	F_1 progeny	F_1	1	-0,016	-0,075
			Bolgar	P_1		1	-0,327
			Ryssalka 1	P_2			1
	11	Berry shape index	F_1 progeny	F_1	1	0,035	-0,294
			Bolgar	P_1		1	0,295
			Ryssalka 1	P_2			1

IV	12	Budding-flowering (days)	F_1 progeny	F_1	1	0.399	-0.106
			Bolgar	P_1		1	-0.220
			Ryssalka 1	P_2			1
	13	Flowering-softening (days)	F_1 progeny	F_1	1	0.088	-0.042
			Bolgar	P_1		1	0.317
			Ryssalka 1	P_2			1
	14	Softening–technological maturity (days)	F_1 progeny	F_1	1	0.089	-0.440
			Bolgar	P_1		1	0.255
			Ryssalka 1	P_2			1
	15	Budding-technological maturity (days)	F_1 progeny	F_1	1	0.135	-0.228
			Bolgar	P_1		1	0.305
			Ryssalka 1	P_2			1
V	16	Sugars (%)	F_1 progeny	F_1	1	0.169	-0.208
			Bolgar	P_1		1	0.186
			Ryssalka 1	P_2			1
	17	Acids (g/dm^3)	F_1 progeny	F_1	1	0.578	-0.019
			Bolgar	P_1		1	0.159
			Ryssalka 1	P_2			1
VI	18	Total number of buds	F_1 progeny	F_1	1	0.385	-0.096
			Bolgar	P_1		1	0.099
			Ryssalka 1	P_2			1
	19	Total number of shoots	F_1 progeny	F_1	1	0.305	0.009
			Bolgar	P_1		1	0.260
			Ryssalka 1	P_2			1
	20	Total number of fruiting shoots	F_1 progeny	F_1	1	0.155	0.057
			Bolgar	P_1		1	0.065
			Ryssalka 1	P_2			1
	21	Total number of clusters	F_1 progeny	F_1	1	0.291	0.027
			Bolgar	P_1		1	0.053
			Ryssalka 1	P_2			1

All fertility coefficients in the cultivar Bolgar (P_1) exert positive direct influence determined by a moderate correlation, on the seedlings from F_1 progeny – shoot fertility coefficient (0,267; 0,235), main shoot fertility coefficient (0,328; 0,313), fruiting shoot fertility coefficient (0,433; 0,382) (Table 4). Both parent cultivars have direct positive influence with a significant correlation coefficient on the trait millerandage berries. The direct influences of all traits in the sixth group of the cultivar Russalka 1 (P_2) are positive, and of Bolgar (P_1) – only in total number of fruiting shoots and clusters. Positive influences and correlations in the two cultivars were reported for cluster width, and in average cluster weight only their direct effects are positive. In sugars (0,227), acids (0,255), the phenological traits (except for budding-flowering), berry shape index (0,306) and berry length (0,162), the direct influence of Russalka 1 (P_2) is positive, and of Bolgar (P_1) – negative. In cluster length all influences and correlations are negative in both parent cultivars.

Table 4. Direct and indirect influences of the studied parent vine cultivars Bolgar (P_1) and Russalka 1 (P_2) on the plants from the hybrid combination - F_1 progeny.

Groups	№	Traits	Cultivars		Direct and indirect influences		r
I	1	Shoot fertility coefficient	Bolgar	P_1	*0,267*	-0,032	0,235
			Ryssalka 1	P_2	0,047	*-0,180*	-0,133
	2	Main shoot fertility coefficient	Bolgar	P_1	*0,328*	-0,015	0,313
			Ryssalka 1	P_2	-0,032	*0,156*	0,124
	3	Fruiting shoot fertility coefficient	Bolgar	P_1	*0,433*	-0,051	0,382
			Ryssalka 1	P_2	0,071	*-0,307*	-0,236
II	4	Millerandage berries (%)	Bolgar	P_1	*0,368*	-0,010	0,358
			Ryssalka 1	P_2	-0,015	*0,233*	0,218
	5	Average cluster weight (g)	Bolgar	P_1	*0,297*	-0,019	0,278
			Ryssalka 1	P_2	-0,033	*0,168*	0,135
	6	Cluster length (cm)	Bolgar	P_1	*-0,288*	-0,062	-0,350
			Ryssalka 1	P_2	-0,151	*-0,119*	-0,270
	7	Cluster width (cm)	Bolgar	P_1	*0,603*	0,005	0,608
			Ryssalka 1	P_2	0,083	*0,034*	0,117
III	8	Average weight of 100 berries (g)	Bolgar	P_1	*-0,222*	0,032	-0,190
			Ryssalka 1	P_2	0,065	*-0,109*	-0,044
	9	Berry length (mm)	Bolgar	P_1	*-0,243*	-0,008	-0,251
			Ryssalka 1	P_2	0,012	*0,162*	0,174
	10	Berry width (mm)	Bolgar	P_1	*-0,080*	0,005	-0,075
			Ryssalka 1	P_2	0,001	*-0,328*	-0,327
	11	Berry shape index	Bolgar	P_1	*-0,305*	0,011	-0,294
			Ryssalka 1	P_2	-0,011	*0,306*	0,295
IV	12	Budding-flowering (days)	Bolgar	P_1	*-0,021*	-0,085	-0,106
			Ryssalka 1	P_2	-0,008	*-0,212*	-0,220
	13	Flowering-softening (days)	Bolgar	P_1	*-0,070*	0,028	-0,042
			Ryssalka 1	P_2	-0,006	*0,323*	0,317
	14	Softening–technological maturity (days))	Bolgar	P_1	*-0,467*	0,027	-0,440
			Ryssalka 1	P_2	-0,042	*0,297*	0,255
	15	Budding-technological maturity (days)	Bolgar	P_1	*-0,274*	0,046	-0,228
			Ryssalka 1	P_2	-0,037	*0,342*	0,305
V	16	Sugars (%)	Bolgar	P_1	*-0,246*	0,038	-0,208
			Ryssalka 1	P_2	-0,041	*0,227*	0,186
	17	Acids (g/dm^3)	Bolgar	P_1	*-0,166*	0,147	-0,019
			Ryssalka 1	P_2	-0,096	*0,255*	0,159
VI	18	Total number of shoots	Bolgar	P_1	*-0,158*	0,062	-0,096
			Ryssalka 1	P_2	-0,061	*0,160*	0,099
	19	Total number of shoots	Bolgar	P_1	*-0,078*	0,087	0,009
			Ryssalka 1	P_2	-0,024	*0,284*	0,260
	20	Total number of fruiting shoots	Bolgar	P_1	*0,048*	0,009	0,057
			Ryssalka 1	P_2	0,007	*0,058*	0,065
	21	Total number of clusters	Bolgar	P_1	*0,013*	0,014	0,027
			Ryssalka 1	P_2	0,004	*0,049*	0,053

CONCLUDING REMARKS

There are no highly significant traits for yield formation from the cultivar Bolgar, in which the correlation coefficients, direct and total indirect influences have positive values. With a total relative participation 95,6%, the productivity of this cultivar is determined by traits, which are predominantly connected with the actual fertility of vines – total number of clusters (24,8%) and fruiting shoots (17,9%), fertility coefficients – totally (16,5%).

Positive correlations have been established between the two parent cultivars and F1 progeny in the traits shoot and fruiting shoot fertility coefficient, cluster width, total number of shoots, fruiting shoots and clusters. For Bolgar they are higher regarding total number of buds, acids, budding-flowering and cluster length, and for Russalka 1 – main shoot fertility coefficient, millerandage berries and average cluster weight.

Positive direct influence determined by a moderate correlation, on the seedlings from F_1 progeny, is exerted by all studied fertility coefficients in the cultivar Bolgar. The correlations in the other traits and the influence of the separate parent cultivars have positive or negative value, which can be used in the selection of valuable elite forms.

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ИСТРАЖУВАЊЕ НА КОРЕЛАЦИЈАТА ПОМЕЃУ КВАНТИТАТИВНИТЕ КАРАКТЕРИСТИКИ КОИШТО ГО ОДРЕДУВААТ ПРИНОСОТ КАЈ СОРТАТА БЛГАР И ХИБРИДОТ БЛГАР Х РУСАЛКА 1

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Резиме

Корелациите помеѓу квантитативните карактеристики, коишто го одредуваат приносот кај сортата *блгар* и $Ф_1$ потомок на хибридна комбинација блгар х русалка 1, беа испитани преку Path анализа. Откриено е дека нема карактеристики коишто се многу значајни за формирање на принос од оваа сорта за кои коефициентите на корелација, директните и вкупните индиректни влијанија имаат позитивни вредности. Позитивни корелации се утврдени помеѓу двете родителски сорти и F_1 потомството за својствата: развиени и родни ластари, коефициент на родност, ширина на гроздот, вкупен број на ластари, родни ластари и гроздови. Сите анализирани коефициенти на родност кај сортата блгар имаат позитивни директни влијанија, утврдени со умерени корелации, врз семениците од F_1 потомство. Корелациите на другите особини и влијанието на одделните родителски сорти имаат позитивни или негативни вредности кои можат да се користат при изборот на вредни елитни форми.

Клучни зборови: *квантитативни карактеристики, родност, семена и бессемена сорта, F1, потомок*

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HEALTHCARE OF THE IMPORTED POTATO SAMPLES IN THE REPUBLIC OF NORTH MACEDONIA

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Abstract

This study relates to performing healthcare of seed and mercantile potatoes on imports, as well as sampling at cross border entry points in the Republic of North Macedonia, for the period 2014-2017. Plant pathogens control is in accordance with the monitoring programme for seed and mercantile potatoes in our country, and refer to the presence or absence of the following most important harmful organisms such as: bacterial diseases - wilt and brown potato rot - *Ralstonia solanacearum* and potato ring rot - *Clavibacter michiganensis* subsp. *sepedonicum*; fungal pathogens - potato cancer - *Synchitrium endobioticum* perc.; and cyst nematodes - *Globodera pallida* (Stone) Behrens and *Globodera rostochiensis* (Wollenweber) Behrens on potatoes (Islam. and Chowdhury, 1998). All samples, were laboratory tested at State Phytosanitary Laboratory, and a health status with absence of harmful organism was confirmed.

Individually, both, by years, and total for all years, from the period of investigation 2014 until 2017, a survey at „Tabanovce", cross-border point has the highest number of both performed, visual health examinations and laboratory analyzed samples. The largest import of mercantile potatoes was in 2014 at 3.044 t, and at least in 2017 110 t. The largest import of seed material was imported in 2017 at 7.386 t, while the least imported in 2016 was 842 t. For the period 2014 to 2017, annual import averaged about 2.680 t of seed potatoes and 1.368 t of mercantile potatoes.

This research explains, for the first time, the importance of mercantile and seed potatoes import in the Republic of North Macedonia from the abroad in the three cross border places, and the quantity and quality of imported material. This study confirmed that the presence of the following harmful organisms (*Ralstonia solacearum, Clavibacvter michiganensis ssp. sepedonicus; Synchitrium endobioticum; Globodera pallida* and *Globodera rostochiensis*, has not been established (no introduction, occurrence and further spread).

Key words: *potatoes, harmful organisms, imports, cross-border*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a very important crop as a food source in the diet of humans, livestock, in the food industry for the production of alcohol and starch, as it contains a large amount of starch, a protein high in vitamins such as: A, B1, B2, B6, C, mineral substances and trace elements. By its economic importance it is measured with wheat, maize, but in areas where there are no optimal conditions for its cultivation, it comes first. In Europe, it is important because it has contributed to the eradication of hunger, and today it occupies a major place in human nutrition (FAO STAT, 2012).

The focus of our research includes sampling properly at the points of entry into the Republic of North Macedonia, the manner, procedures and procedures of the sampling itself, in order to obtain relevant data to prove the presence or absence of certain harmful organisms on the territory of the Republic of North Macedonia (Official Gazette of the Republic of Northern Macedonia" No. 29/05, 81/08, 20/09, 57/10, 17/11, 148/11, 69/13, 43/14 and 158/14).

The main aim was the analysis of the annual reports on visual health examinations and analyzed laboratory samples, as well as

the cross border in the investigated period. The results of the annual data were processed by arithmetic mean, percentage difference between visual examinations and laboratory analyzes, sum of samples taken, error of mean for confirmation of arithmetic mean, median as a number indicating the mean value of samples taken, standard deviation which determines the variability in the data set itself.

In this research, health conditions and laboratory analyzed samples, where the smallest deviation confirms the data are close to the mean, and the larger that the obtained samples for laboratory analysis are in a wider range of values which determines the dispensability of the performed visual health examinations and samples.

MATERIAL AND METHODS

Our research has processed all available data resulting from the implementation of phytosanitary monitoring in the field of health of plants, which is a regular annual activity that includes systematic collection, processing and data on the presence of certain harmful organisms in the Republic of North Macedonia.

Phytosanitary monitoring includes conducting a health examination, sampling for laboratory analysis, monitoring the state of health of the plants and systematic control of the infected, endangered and non-infected areas, was first implemented in 2013, and its implementation has continued to establish the presence of daily absence of quarantine and certain economically harmful organisms.

The importance and uniqueness of this research is derived from obtaining a relevant and complete picture of the import of seed and mercantile potatoes throughout our country, with particular emphasis on the Tabanovce cross-border point, considering other cross-borders.

Completeness refers to the processing of data over a four years period from 2014 to 2017, both annual analyzes for each year individually and for total data for all years. The implementation of the prescribed procedures and procedures through this research provides a complete picture of the health phytosanitary control performed by the state phytosanitary inspectors, which are in accordance with EU standards in order to obtain relevant data on the presence or absence of harmful phytosanitary organisms in seed and mercantile potatoes (Official Gazette of the Republic of Northern Macedonia", no.151/14; Official Gazette of the Republic of Northern Macedonia" No. 65/10; Official Gazette of the Republic of Northern Macedonia" No. 88/00).

Our research begins at the cross-boarding as they are the first barrier to prevent the introduction of harmful organisms, and thus prevent the emergence and further spread of harmful organisms on the territory of our country, which are imported by importing seed material of potatoes. The Republic of North Macedonia, unfortunately due to a series of disadvantages, all the seed potato material used for production comes from abroad, as there is no selection of seed planting material. Small exceptions are the small individual producers that produce for their own purposes and are of negligible character. Particular emphasis is placed on phytosanitary control at the Tabanovce border crossing point, as imports of almost all potato seed material come from the Netherlands, followed by Germany. The place of entry is Tabanovce cross-border point by road, truck traffic.

Visual health examinations and samples taken for further analysis in seed and mercantile potatoes to prove presence or absence refer to the following harmful organisms:

- Bacterial wilt and brown potato rot - *Ralstonia solanacearum*
- Potato cancer - *Synchitrium endobioticum* perc.
- Potato ring rot - *Clavibacter michiganensis* subsp. *sepedonicum*
- nematodes *Globodera pallida* (Stone) Behrens and *Globodera rostochiensis* (Wollenweber) Behrens in potatoes (Official Gazette of the Republic of North Macedonia" no. 62/17; Official Journal of the Republic of North Macedonia" no. 34/14; 'Official Journal of the European Communities Republic of North Macedonia" no. 62/1).

The samples are submitted to the State

Phytosanitary Laboratory, Department of Diagnostics. On a recent supervisory visit by the Accreditation Institute of the Republic of North Macedonia (IARNM), State Phytosanitary Laboratory was assessed as a laboratory in an ever-evolving process of improving the quality management system and implementing new advanced methods in all areas of diagnostics, bringing the number of accredited methods are constantly increasing. For our research, the subject is potato tubers and soil from border crossings. Laboratory analyzes of the examined organisms in mercantile and seed potatoes are carried out by the following methods:

*EPPO standard CD 2006/63 / EC, 2006 Immunofluorescence test (IC test) for the detection and identification of *Ralstonia solanacearum* in potato tubers. Plant material: (200 tubers) * EPPO standard CD 2006/56 / EC, 2006 Immunofluorescence test (IC test) to prove and identify the bacterium *Clavibacter michiganensis* ssp. sepedonicus in Potato Tubers Plant Material: (200 Tubers) * EPPO PM 7/28 (1), 2003 Evidence and identification of *Synchytrium endobioticum* by macroscopic visualization and microscopy in potato tubers. Plant material: (200 tubers)

*Methods for soil nematode extraction, 2b Cotton Filter Method (Oostenbrink, 1960 & Townshend, 1963) Procedure for proving the presence of *Globodera pallida* and *Globodera rostochiensis* in soil. Material: soil

RESULTS AND DISCUSSION

After the laboratory analysis, State phytosanitary laboratory submits a Report confirming the presence or absence of the harmful organism.

Table 1 and Figure 1. 2014 show that almost all of the seed material in terms of the number of visual health examinations and analyzed samples was performed at Tabanovce (24).

Table 1. Number of healthcare performed (visual and laboratory analysis) of mercantile and seed material imported in 2014 at three cross-borders.

Cross-boarder	Material	Number of visual healthcare	Total number of samples for analyses	Difference / Number / samples	% analyses/ Check number
Tabanovce	Mercantile	24	12	12	-50%
	Seeds	24	23	1	-4%
Kafasan	Mercantile	9	2	7	-78%
	Seeds	0	0	0	0%
Blace	Mercantile	11	11	0	0%
	Seeds	1	1	0	0%
Total number		69	49	20	-29%
Average		11.5	8.2	3.3	-29%
Medium value error		4.3	3.6		
Mediana		10.0	6.5		
Modus		24.0	0.0		
Standard deviation		10.6	8.9		
Minimum		0.0	0.0		
Maximum		24.0	23.0		

The percentage of visual health cares, compared to total samples taken for laboratory analysis differs in terms of mercantile and seed material. Thus, at the Tabanovce BCP a total of 24 visual health examination of mercantile material was performed, while laboratory analyzes were performed on 12 samples. In the field of seed material both in terms of the number of laboratories analyzes performed 23 and in the field of visual health examinations 24 is much higher.

Whereas the difference between total samples taken for laboratory analysis in terms of the number of performed health examinations is 20 samples or 29% more than the number of visual health examinations performed. The

average rehearsals for all three border crossings for this year amounted to 11.5 number of visual health examinations performed, 8.2 total samples taken for laboratory analysis. 5. The deviation is not very high and without much variability with respect to the visual (10.6) and analyzed samples (8.9) shown in Table 1.

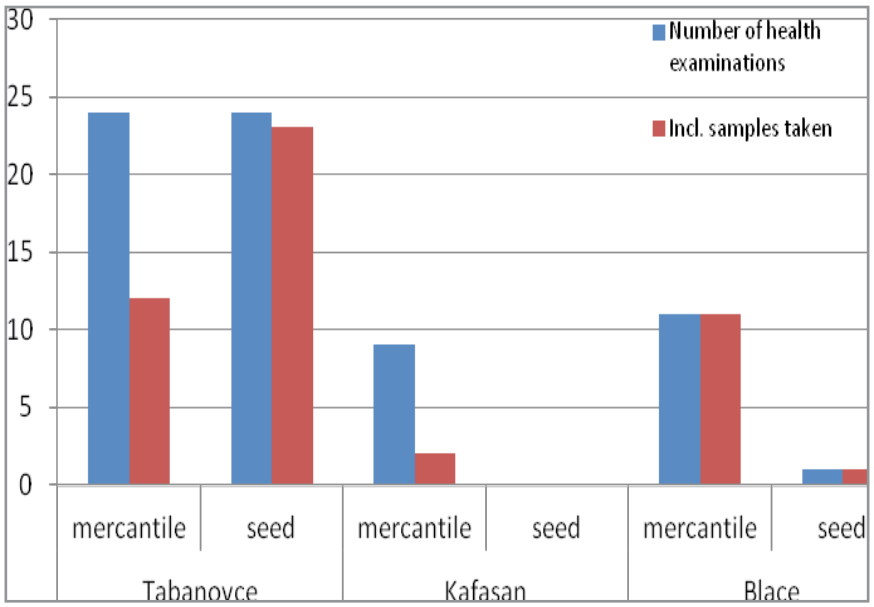

Figure 1. Number of visual health examinations performed, and laboratory samples taken analysis of three border crossings in 2014.

It should be noted that the control of imported material, whether it is seed or mercantile, does not end the work of state phytosanitary inspectors, their work continues on the field during the production process and storage. Phytosanitary control continues throughout the territory or country by region. In 2015, it can be seen that almost all seed material, in terms of the number of visual health examinations and analyzed samples, is at cross border Tabanovce (32), which can be seen from Table 2 and Figure 2).

The percentage of visual health examinations performed in relation to total samples taken for laboratory analysis differs in terms of mercantile and seed material. In 2015, at the Tabanovce border crossing, 32 visual health examinations of potato seed material were performed, while only 5 were taken for further laboratory analysis.

The difference between the total samples taken for laboratory analysis in terms of the number of visual health examinations performed was 34, the laboratory analyzes were 7. The average rehearsals for all three border crossings for 2015 amounted to 5.7 number of visual health examinations performed and 1.2 total samples taken for laboratory analysis. The deviations between the two types of materials (mercantile and seed) in terms of visual health examinations and laboratory samples were greater than 2014 and were significantly different between groups and accounted for 12.9 and 1.9, respectively.

It should be noted that all data subject to this research are in accordance with the monitoring programmes developed by the Republic of North Macedonia, which monitoring programs are developed each year. They are prepared at the end of the year for the following year. Monitoring programmes vary each year not only by cultures, but also by specific cultures as well as this research to see the number of visual health examinations and laboratory analyzes performed.

They are based on the assessment of the danger of introduction, and then the emergence and further spread of certain harmful organisms by the introduction of seed and seed material from the crops, considering the available data from the past period for the presence or absence of harmful organisms in our country.

Table 2. Number of healthcare performed (visual and laboratory analysis) of mercantile and seed material imported in 2015 at three cross-borders.

Cross-boarder	Material	Number of visual healthcare	Total number of samples for analyses	Difference / Number / samples	% analyses/ Check number
Tabanovce	Mercantile	0	0	0	/
	Seeds	32	5	-27	-84%
Kafasan	Mercantile	0	0	0	/
	Seeds	1	1	0	0%
Blace	Mercantile	0	0	0	/
	Seeds	1	1	0	0%
Total number		34	7	-27	-0.84
Average		5.7	1.2	-4.5	-0.3
Medium value error		5.3	0.8		
Mediana		0.5	0.5		
Modus		0.0	0.0		
Standard deviation		12.9	1.9		
Minimum		0.0	0.0		
Maximum		32.0	5.0		

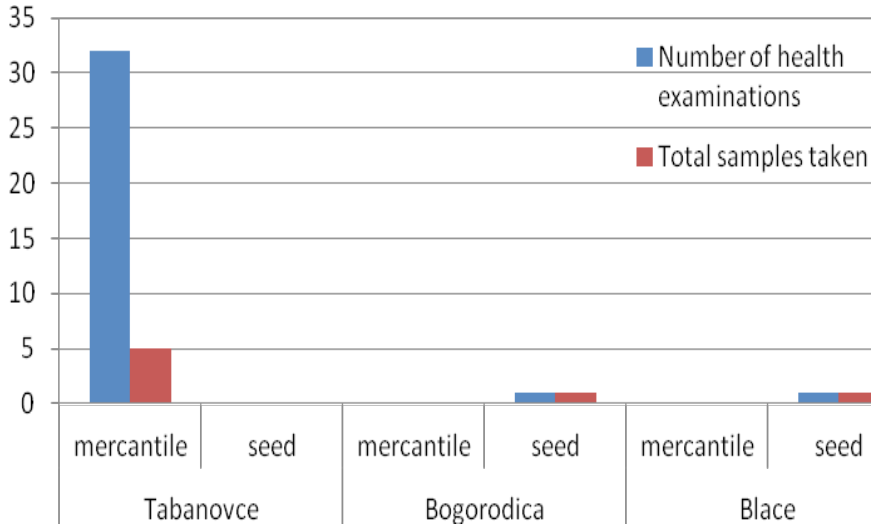

Figure 2. Number of visual health examinations performed and laboratory samples taken analysis of three border crossings in 2015.

In 2016, the state of visual health examinations of mercantile and seed material as well as the total samples taken for laboratory analysis did not differ with respect to the border crossings. Thus, the Tabanovce cross-border has the highest number of both visual and laboratory analyzed samples 19 and 17, in relation to Bogorodica and Novo Selo (Table 3). The difference between the visual health examinations and the laboratory analyzed samples of Tabanovce is minimal and it is 11%. Of the three border crossings on average for 2016, samples of mercantile and seed material were visualized 7, while laboratory analyzes were 6.

Table 3. Number of healthcare performed (visual and laboratory analysis) of mercantile and seed material imported in 2016 at three cross-borders.

Cross-boarder	Material	Number of visual healthcare	Total number of samples for analyses	Difference / Number / samples	% analyses/ Check number
Tabanovce	Mercantile	19	17	-2	-11%
	Seeds	0	0	0	/
Kafasan	Mercantile	1	1	0	0%
	Seeds	0	0	0	/
Blace	Mercantile	1	1	0	0%
	Seeds	0	0	0	/
Total number			19	-2	-0.11
Average			3.2	-0.3	0.0
Medium value error		3.1	2.8		
Mediana		0.5	0.5		
Standard deviation		7.6	6.8		
Minimum		0.0	0.0		
Maximum		19.0	17.0		

The median of both types of samples of visual health examinations is 0.5, which is the same for the two groups, so that the difference of mean values obtained from all samples is close, and it is observed that most samples are analyzed from one place. Also, the deviation is not high and is small between groups or little variability in terms of visual (7.6) and analyzed samples (6.8) Table 3.

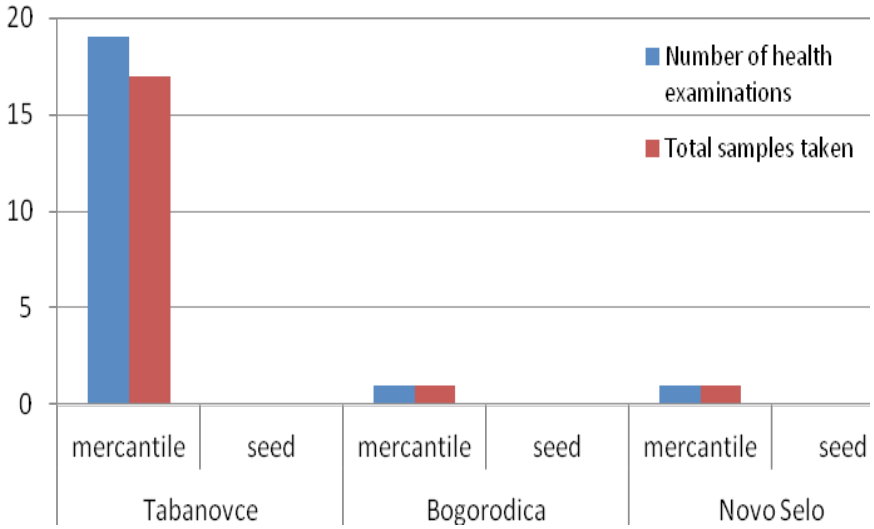

Figure 3. Number of visual health examinations performed and laboratory samples takenanalysis of three border crossings in 2016.

The medical examinations and samples taken for analysis at the Tabanovce cross border points as well as Bogorodica and Novo Selo are of mercantile material (Figure 3).

Compared to all years, visits and percentages of visual health examinations and laboratory analyzes, compared to total visits, for 2014, 2015 and 2016, potato varied by more than 20%. Thus, the number of visual health examinations performed for the three years is 507, and the total number of border checkpoints is 1,511 or 34% difference compared to the examinations related to seed and mercantile potatoes and total examinations.

The total samples taken for laboratory analysis of potato (seed and mercantile) in

these three years is 251, while the total analyzed laboratory samples are 1,161 or 22% difference. The difference between the visual health examinations and the analyzed laboratory samples for this period is 256 examinations, while the total border crossing examinations compared to the visual and analyzed laboratory samples is 350 analyzed samples or 27% more.

According to the 2017 Phytosanitary Policy Program for health examinations carried out in production regions and border crossings to determine the presence or absence of harmful organisms, due to the probability of possible risks of harmful organisms to seed and mercantile potatoes, garden crops, fruit species, vines and tobacco, a total of 764 samples and 1234 harmful organisms were examined.

Out of the total number of seed and mercantile potatoes 349 health examinations were performed, and 323 harmful organisms were examined. As of 2017, the Phytosanitary Directorate, on the recommendation of experts from the European Commission, does not develop monitoring programs on the basis of the number of performed health or laboratory analyzes, but on the basis of examinations or laboratory analyzes of harmful organisms.

Table 4. Number of healthcare performed (visual and laboratory analysis) of mercantile and seed material imported in 2017 at three cross-borders.

Region and cross-borders	Number of predicted harmful organisms	Number of harmful tested organisams	Number provided / examined	Percentage analysis / views
Polog	18	18	0	0%
Southwest	14	13	-1	-7%
Northeast	72	59	-13	-18%
Skopje region	30	40	10	33%
Southeast	27	27	0	0%
Pelagonia	13	8	-5	-38%
Eastern	68	53	-15	-22%
Vardar region	9	7	-2	-22%
Cross-borders	98	98	0	0%
Total	349.0	323.0		
Average	38.8	35.9		
Total number of samples	251	225		
Average of samples per region	31.4	28.1		
Average value	8.8	7.2		
Mediana	22.5	22.5		
Standard deviation	24.88	20.34		
Minimum	9.0	7.0		
Maximum	72.0	59.0		

The total number of predicted harmful organisms for 2017 is 349 or 251 from the regions and 98 from the border crossings. The average number of predicted harmful organisms injects 38.8. The number of examined (laboratory analyzed) harmful organisms per region is 225 and 98 from the border crossings. The difference between the regions in the visual samples and the laboratory analyzed amounts to a minimum of 26 trials, with no differences at the border crossings.

This leads to the conclusion that in 2017 these differences between the two predicted and investigated parameters are unified. This mediation also indicates the median elapsed, i.e. the mean values of the set of values equal to 22.5 for both parameters.

Table 5. Number of visual health examinations and laboratory analyzes of mercantile and seed material imported from 2014 to 2016 carried out at all border crossings.

Cross-border	Material	Number of healthcare control	Laboratory analyses
Tabanovce	Mercantile	91	50
	Seeds	29	28
Blace	Mercantile	14	14
	Seeds	2	2
Bogorodica	Mercantile	1	1
	Seeds	0	1
Kafasan	Mercantile	9	2
	Seeds	0	0
Novo Selo	Mercantile	1	1
	Seeds	0	0
Average mercantile		23.2	13.6
Average seeds		6,2	6.2
Average mercantile		116	68
Average seeds		31	31

The number of visual health examinations of mercantile and seed material for the period 2014 to 2016 averaged 23.2 in all border crossing mercantile potatoes and 6.2 seed potato material in terms of the number of visual health examinations performed, and samples for laboratory analysis 13.6 mercantile and 6.2 potato seed material (Table 5).

The total number of mercantile potato material for visual health examinations in this period was 116, and laboratory analyzed samples 68. For seed material, the number of health examinations was 31, as well as laboratory analyzed samples.

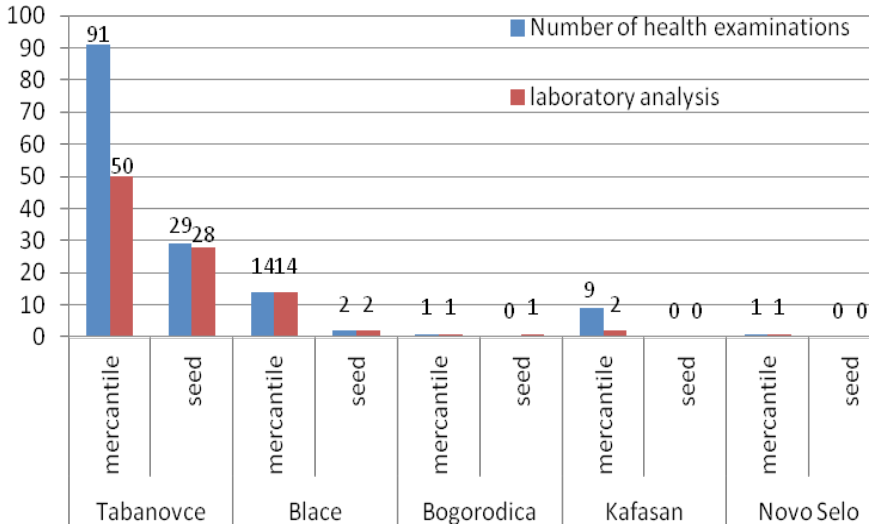

Figure 4. Number of visual health examinations performed and samples taken for laboratory analysis of all border crossings 2014-2016.

As individually by years and total for the three years 2014, 2015 and 2016, Tabanovce cross border point has the highest number of performed visual health examinations as well as laboratory analyzed samples 75 and 34 which can be clearly seen from Figure 4.

Imports of mercantile and potato seed material vary throughout the period of this research. The largest import of mercantile potatoes made in 2014 (3,044t), which is the largest material ever imported in the years taken as the subject of this research. The least mercantile potato was imported in 2017 (110t).

Table 6. Import of seed and mercantile potatoes in the Republic of North Macedonia in the period 2014 to 2017 in tons.

Year	Material	Import od seed and mercantile potato in t
2014	Mercantile	3.044
	Seeds	1.434
2015	Mercantile	1.210
	Seeds	1.061
2016	Mercantile	1.110
	Seeds	842
2017	Mercantile	7.386
	Seeds	1.010
Total (2014-2017)	Mercantile	12.750
	Seeds	4.347
Average (2014-2017)	Mercantile	3.188
	Seeds	1.087

The largest import of seed material was imported in 2014 at 1.434 t, while the least imported in 2016 at 842 t (Table 6 and Figure 5). The average annual mercantile potato imports are around 3.188 t and the import of seed potatoes is 1.087 t.

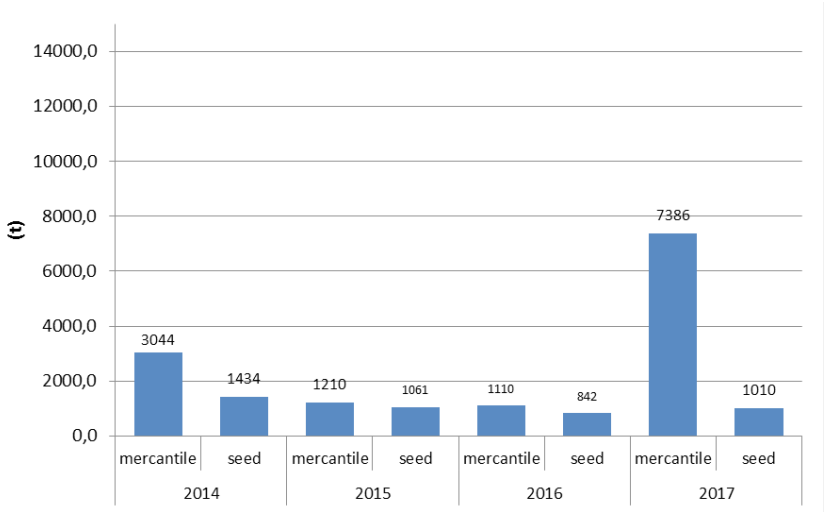

Figure 5. Import of mercantile and seed potatoes in the period from 2014 to 2017 in tons.

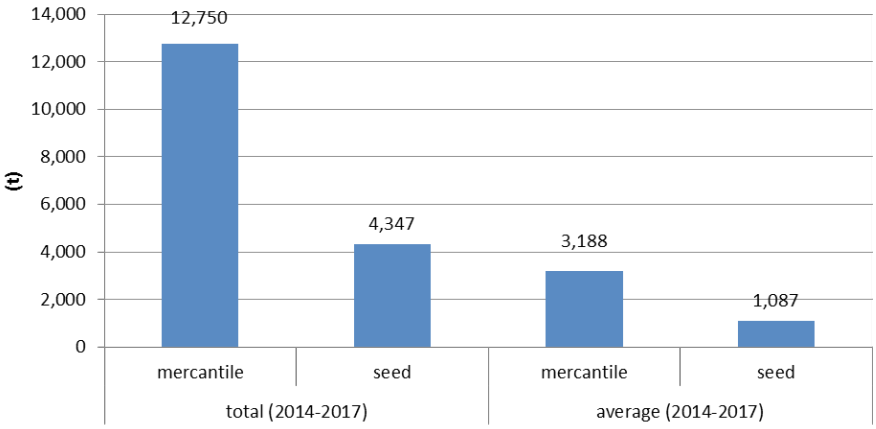

Figure 6. Total and average quantities of mercantile and seed potatoes in the period 2014 to 2017.

CONCLUSION

Individually, both by years and total for all years of research 2014, 2015, 2016 and 2017 at the Tabanovce cross-border has the highest number of both performed visual health examinations and laboratory analyzed samples. The largest import of seed material was imported in 2014 of 1.434 t, while the least was imported in 2016 of 842 t. For the period 2014 to 2017, annual imports averaged about 4.347 t of seed potatoes and 12.750 t of mercantile potatoes. The average annual mercantile potato imports are around 3.188 t, and the import of seed potatoes is 1.087 t.

This study confirmed that the presence of the following harmful organisms (Ralstonia solacearum, *Clavibacvter michiganensis* ssp. *sepedonicus*; *Synchitrium endobioticum*; *Globodera pallida* and *Globodera rostochiensis*, has not been established (no introduction, occurrence and further spread).

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"Official Gazette of the Republic of Northern Macedonia" No. 65/10 Lists of harmful organisms, plants, plant products and more objects and objects

"Official Gazette of the Republic of Northern Macedonia" No. 88/00 Order on Measures for Import of Potatoes into the Republic of Macedonia.

"Official Journal of the European Communities Republic of Northern Macedonia" no. 62/17: Order to carry out special supervision for the determination of the presence of the bacterium Ralstonia solanacearum (Smith) Yabuuchi et al. and laboratory test procedures

"Official Journal of the Republic of Northern Macedonia" no. 34/14: Order to conduct special surveillance to establish presence of the fungus Synchytrium endobioticum (Schilb.) Perc. and procedures for laboratory examination.

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Rulebook on Seed Potato Trade (Official Gazette of the Republic of Northern Macedonia No. 62/09)

Rulebook on the Manner of Conducting a Health Check and the Manner of sampling the garden crops outdoors and in the indoors over which phytosanitary monitoring is carried out ("Official Journal of the Republic of Northern Macedonia" no. 113/13)

Rulebook on the manner of conducting a health check and the manner of sampling of seed and mercantile potatoes over which they are performs phytosanitary monitoring ("Official Gazette of the Republic North Macedonia" no. 113/13)

Rulebook on the Manner of Performing Phytosanitary Inspection at import of consignments from list V, section B, section 1 (*) ('Official Journal of the European Union Republic of Northern Macedonia" no. 131/10)

ЗДРАВСТВЕНАТА СОСТОЈБА НА КОМПИРОТ ПРИ УВОЗ ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА

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Резиме

Идејата, целта и резултатите од ова истражување се однесуваат на здравствената контрола при увоз, како и земањето на мостри на граничните премини (места) на влез во Република Северна Македонија, за временскиот период 2014-2017 год., кај примероци од семенски и меркантилен компир. Испитувањата беа направени согласно со мониторинг програмата за семенски и меркантилен компир, а се однесуваат за докажување на присуство односно отсуство на следниве штетни организми: бактериско венење и кафеаво гниење на компирот - *Ralstonia solanacearum*, рак на компирот - *Synchitrium endobioticum* perc., прстенесто гниење на компирот - *Clavibacter michiganensis* subsp. *sepedonicum*, цистолики нематоди кај компирот *Globodera pallida* (Stone) Behrens и *Globodera rostochiensis* (Wollenweber) Behrens.

Поединечно, како по години така и вкупно за сите години од истражувањето (за периодот од 2014 год. заклучно со 2017 год.), на граничниот премин Табановце имаше најголем број на извршени здравствени визуелни прегледи, како и лабораториски анализи.

Најголем увоз на меркантилен компир е извршен во 2017 година - 7.386 t, а најмалку во 2016 година - 1.110 t. Најголем увоз на семенски материјал има во 2014 година - 1.434 t, додека најмалку е увезен во 2016 година - 842 t. Во период од 2014 до 2017 година е увезено повеќе меркантилен компир (12.750 t). За период од 2014 до 2017 година годишниот увоз во просек се движи околу 1.087 t на семенски компир и 3.188 t меркантилен компир. Програмата за мониторинг има значајна улога во детекција на штетници и болести пред истите да бидат внесени во земјата, што е случај и тука кај увозот на меркантилен и семенски компир. Доколку се појави одреден штетен организам, токму со раната детекција преку мониторингот ќе се овозможува поефикасно искоренување и нивно понатамошно ширење. Со ова истражување потврдивме дека штетните организми (Ralstonia solacearum, *Clavibacvter michiganensis* ssp. *sepedonicus*; *Synchitrium endobioticum*; Globodera pallida и *Globodera rostochiensis*) досега не беа потврдени (на гранични премини при увоз или понатамошно ширење).

Клучни зборови: *компир, штетни организми, увоз, гранични премини*

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THE INFLUENCE OF DIFFERENT CONCENTRATIONS OF PLANT ESSENTIAL OILS ON GROWTH AND REPRODUCTION OF *Salmonella enteritidis*

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Abstract

Plant essential oils have been reported to possess antimicrobial properties and therefore have potential usage as natural antimicrobials of food. The aim of the study was to examined the antimicrobial effect of sweet basil and thyme essential oils against growth and reproduction of Salmonella enteritidis reference strain ATCC 13076 (*S. enteritidis* RS) and *Salmonella enteritidis* epidemical strain (*S. enteritidis* ES) cultivated on plate. Therefore, the samples were prepared as a dip application from different concentrations of sweet basil and thyme essential oils (1%; 2.5% and 5%) with initial concentration of bacteria from 109 CFU/mL and were cultivated on plate. The control samples were prepared as dip application of bacteria without added essential oils. All samples were exposed at 37°C and 46°C. The growth of *S. enteritidis* RS and *S. enteritidis* ES was observed only in the control samples without added sweet basil and thyme essential oils. There was not any growth of both Salmonella enteritidis strains in the samples dipped in the 1%; 2.5% and 5% sweet basil and thyme essential oils. The results from the ANOVA indicate that the utilized essential oils in combination with temperature regime was significantly ($p < 0.001$) reduced the CFU number of the both strains of *Salmonella enteritidis*. These results support the possibility of using sweet basil and thyme essential oil as natural preservatives in food to contribute in the reduction of *Salmonella enteritidis* at acceptable levels in view to prevent the risk for consumers.

Key words: *Salmonella, sweet basil, thyme*

INTRODUCTION

Food diseases are caused by consuming foods that have been contaminated by an infectious agent or a toxin produced by it. According to the World Health Organization (WHO), 30% of people in industrialized countries suffer from foodborne diseases, with at least two million people worldwide in the world died from diarrhea caused by *Salmonella* (Burt, 2004, Jones, 2011). Salmonellosis prevalence in USA is around 1.3 million cases of foodborne illness, with about 15.000 hospitalizations and 500 deaths per year (Sampathkumar, 2003; Isaacs et al., 2005).

Generally, many foods support the growth of bacteria of the genus *Salmonella*, but its presence is most commonly associated with raw meat, primarily poultry, eggs, milk, dairy products and non-processed foods (Bajpai *et al.*, 2012; Im et al., 2015). DeKnegt et al. (2015) investigated the appearance of *Salmonella serovarants* in animals and humans in 24 countries of the European Union in the period 2007-2009 and found out that chicken meat is the main cause of salmonellosis in humans in Europe. Additionally, *Salmonella enteritidis* in high 95.9% from foodborne outbreak was

the main etiological factor for salmonellosis in humans. Ivić-Kolevska and Kocić (2009) have established a trend of increasing the level of food contamination with *Salmonella* spp. in the Republic of North Macedonia, especially in the period 2006-2007. The most common contaminated food products were mechanically chopped chicken, milk and dairy products, sweets, etc. According to data from other researches made in Serbia, *Salmonella enteritidis* together with *Salmonella typhimurium* are considered the most important pathogenic microorganism present in foodstuffs (Karabasil *et al.*, 2013, Rašeta *et al.,* 2014).

Based on the previous researches (Yoshikawa, 1980) it was well established that bacteria from the genus *Salmonella* have grown on agar with the addition of blood products such as Mac Conkey Agar or Eosin-Methylene blue. Bismuth sulphate agar or deoxycholate agar is used as selective bases for the isolation of bacteria of the genus *Salmonella* that ferment glucose and mannose, but not lactose or sucrose.

In order to protect food from contamination with pathogens and other harmful microorganisms, including bacteria of the genus *Salmonella*, many scientists have examined the antifungal, antibacterial and antioxidative properties of plant essential oils and their application in food technology. In several studies (Lachowicz *et al.*, 1998; Shirazi *et al.,* 2014) the antimicrobial activity of the essential oils of *Ocimum* spp., including the basil was examined, and it was found that the basil essential oil had a mild antimicrobial activity over three Gram positive bacteria (*Lactobacillus plantarum, Listeria monocytogenes, Staphylococcus aureus*) and several Gram negative bacteria (*Escherichia coli*, *Pseudmonas aeruginosa, Salmonella thyphimurium, Yersinia enterolitica*), yeasts (*Rhodotorula*) and moulds. Similarly, Ela *et al.* (1996) determinate the antibacterial and antifungal effect of basil essential oil on *Staphylococcus aureus, Escherichia coli* and *Aspergillus niger.* Essential oils were effective to reduce the levels of *Salmonella* spp. in meat products derived from turkeys (Nair *et al.*, 2014). Also, the antimicrobial activity of essential oils of oregano, thyme, basil, marjoram, lemon grass, ginger and clove, were investigated *"in vitro"* with method of dilution on agar and there was determined the minimum inhibitory concentration (MIC) against Gram (+) (*Staphylococcus aureus* and *Listeria monocytogenes*) and Gram (-) strains (*Escherichia coli* and *Salmonella enteritidis*) (Barbosa *et al.*, 2009).

Thyme and orange oils were effective in reducing the concentration of *Salmonella enteritidis* and *Campylobacter coli* when culture was inoculated in broths and whole wings (Thanissery and Smith, 2014a).

The aim of the study was to determine the antimicrobial effect of basil and thyme extracts on the growth and reproduction of bacterial cells of two strains of *Salmonella* spp.: reference test strain *Salmonella enterica* subsp. *enteric serovar* Enteritidis (ATCC® 13076 ™) and *Salmonella eteritidis* (group D) - epidemic strain, in food isolates, after exposure at 37°C and 46°C (temperature which correlate with drying procedure of pasta during their production).

MATERIALS AND METHODS

The test samples were prepared as emulsions of different concentrations of sweet basil and thyme essential oils in physiological solution (PhS) up to final concentration of 1%, 2.5% and 5% (micellar solution), and have been inoculated particularly with each of the bacterial strains tested: *Salmonella enteritidis* reference strain ATCC 13076 (*S. enteritidis* RS) and *Salmonella enteritidis* epidemical strain (*S. enteritidis* ES). The control samples were prepared as dip application of bacteria in physiological solution without added essential oil. Therefore, in order to compare bacterial growth, two types of samples were used: control samples - inoculums of the tested bacterial strains in PhS and micellar solutions of sweet basil and thyme essential oil that were inoculated with each of the tested bacterial strains.

Fresh sweet basil and thyme essential oil emulsions (Fitofarm, Skopje) were prepared for each phase of the experiment at concentrations of 1%, 2.5% and 5%, which were used as "micellar solutions" for inoculation with bacteria.

All samples were prepared in duplicate. The ready suspension from *S. enteritidis* RS and *S. enteritidis* ES have been inoculated in 5 mL of

the micellar solutions of sweet basil and thyme essential oils (1%, 2.5% and 5%) as well as 5 mL of PhS (control), in initial concentration of bacteria from 109 CFU/mL. In all samples were added 90 ml Salenit F broth (Merck KGaA, Germany) and subsequently one from the duplicate sample was exposed at a temperature of 46°C (pasta drying temperature) for 9 hours, and the other one was exposed at 37°C for 18 hours (incubation temperature). Then, the samples were cultivated on plate for enumeration according ISO 6579-1 (2017).

Dilutions 1:20 and 1: 200 were prepared from all samples and from them 0.1 mL was inoculated on *Müller-Hinton agar* (Merck KGaA, Germany), for enumeration of bacterial cell count (CFU). Petri plates were incubated at 37°C (incubator - Boxun B, Shanghai Boxun Industry and Commerce Co Ltd) for 18 hours (ISO 6579-1, 2017).

Each control and target samples procedure was previously validated in three independent successive experiments, by calculating the mean values used for statistical calculations.

Using one-way analysis of variance (ANOVA) there was tested the statistically significant differences in Log10 number of bacterial cells of *S. enteritidis* RS and *S. enteritidis* ES cultured under laboratory conditions depending from the used concentration of the sweet basil and thyme essential oils. For those variables for which the F-value showed statistical significance, a post-hoc test was applied (Bonferroni test).

RESULTS AND DISCUSSION

Table 1 show the results for bacterial cell counts of *S. enteritidis* RS (Log10) and *S. enteritidis* ES (Log10) in PhS and micellar solutions with different concentrations of sweet basil and thyme essential oils in the samples previously exposed at 37°C and 46°C and incubate at 37°C for 18 hours.

Table 1. Number of bacterial cells of *S. enteritidis* RS (Log10) and *S. enteritidis* ES (Log10) inoculated in PhS as control and micellar solutions with different concentrations of sweet basil and thyme essential oils.

Strain	Exposure	Basil essential oil				Thyme essential oil			
		Control	1%	2.5%	5%	Control	1%	2.5%	5%
S. enteritidis RS	37°C	5.53	0.00	0.00	0.00	5.51	0.00	0.00	0.00
	46°C	5.30	0.00	0.00	0.00	5.45	0.00	0.00	0.00
S. enteritidis ES	37°C	5.51	0.00	0.00	0.00	5.51	0.00	0.00	0.00
	46°C	5.70	0.00	0.00	0.00	5.70	0.00	0.00	0.00
$(\overline{x} \pm S_{\overline{x}})$		5.51±0.082				5.54±0.054			

The averages Log10 CFU values for S. enteritidis RS and *S. enteritidis* ES in control samples, regardless of the exposure temperature, were similar and ranged from 5.30 to 5.70. There wasn't any growth of both Salmonella enteritidis strains in the samples dipped in the 1; 2.5 and 5% sweet basil and thyme essential oils. Thus, according to the research done by Rattanachaikunsopun & Phumkhachorn (2010), basil essential oil (*Ocimum basilicum*) has shown a high antimicrobial effect on *Salmonella enteritidis*.

Nabrdalik & Grata (2016) investigated the effect of basil essential oil added at different concentrations (0.25%, 0.5%, 1.0%, 2.0% and 4.0%) on *Salmonella enteritidis* in a nutrient broth supplemented with 0.05% (v / v) Tween 80 (polyethylene sorbitol ester). The initial number of bacterial cells was 10^8 CFU/mL (8 log CFU/mL). The samples were exposed to a temperature of 37°C for 4 h, 24 h, 48 h and 168 h. The reduction in the bacterial cell count of *Salmonella enteritidis* ranged from 3% to 26% at 4 h exposure, to 22% - 46% at one-week exposure. In absolute terms, the initial bacterial count of 10^8 CFU / mL (8 log CFU / mL) after 24-hour exposure decreased to 6.0287, 5.8783, 5.5903, 5.6037 and 5.6007 log CFU / mL corresponding to the concentrations of basil essential oil used (0.25%, 0.5%, 1.0%, 2.0 and 4.0%), which was statistically significant at $p < 0.05$.

Further, for example, Boskovic (2016) found a statistically significant decrease in the initial number of bacterial cells from more bacterial species of the genus *Salmonella* (10^6 = 6 Log cfu / g) inoculated into minced pork with added thyme essential oil in concentrations from 0.3%, 0.6% and 0.9%. The decrease in the number of

bacterial cells increased proportionally with the increasing of the essential oil concentration (0.3%, 0.6% and 0.9%).

According to research done by Millezi *et al.* (2011), the minimum inhibitory concentration of thyme essential oil (*T. vulgaris*) to *P. aeruginosa* and *S. enteritidis*, was 5% and 10%, respectively. According to Thanissery & Smith (2014b), the combination of 0.5% thyme and orange essential oils inhibit the growth of *Salmonella* and *Campylobacter bacteria*. Even when thyme essential oil was added in much smaller portions (0.1%) in chopped lamb packaged in a modified atmosphere, as tested by Karagözlü *et al.* (2011), an antimicrobial effect was found resulting in a significant extension of the shelf life.

Table 2 shows the statistically significant differences between the number of bacterial cells of *S. enteritidis* RS and *S. enteritidis* ES inoculated in PhS and in micellar solution of basil and thyme essential oil with different concentration, cultivated on nutrient media and exposed at 37°C and 46°C.

Table 2. Statistical differences in Log10 number for CFU/ml of *S. enteritidis* RS and *S. enteritidis* ES dipped in different concentration of basil and thyme essential oil and cultivated on nutrient media.

Dependent variable: concentration of basil and thyme essential oil			
Source of variation	df between groups	df in groups	F-value
Log_{10} *S. enteritidis* RS + basil essential oil	3	4	2217.181***
Log_{10} *S. enteritidis* RS + thyme essential oil	3	4	33367.111***
Log_{10} *S. enteritidis* ES + basil essential oil	3	4	3481.000***
Log_{10} *S. enteritidis* ES + thyme essential oil	3	4	3481.000***

***statistical significance at level p<0,001

The results from the ANOVA indicate that the utilized essential oils in combination with temperature regime was significantly ($p < 0.001$) reduced the CFU number of the both strains of *Salmonella enteritidis*. The Bonferroni post-hoc test showed that there was a statistically significant deference between the control samples compared to target samples in the Log10 number for CFU/ml of the both strains of *Salmonella enteritidis* cultivated on nutrient media in laboratory conditions, as is shown in Tab. 3, 4, 5 and 6.

Table 3. Bonferoni test for difference in the Log10 number for CFU/ml of *S. enteritidis* RS depending from concentration of sweet basil essential oil.

Log_{10} *S. enteritidis* RS + basil essential oil	1%	2,5%	5%
Control	5.41*	5.41*	5.41*
1%	1	0.00	0.00
2,5%		1	0.00

*statistical significance at level p<0,05

Table 4. Bonferoni test for difference in the Log10 number for CFU/ml of *S. enteritidis* RS depending from concentration of thyme essential oil.

Log_{10} *S. enteritidis* RS + thyme essential oil	1%	2,5%	5%
Control	5.48*	5.48*	5.48*
1%	1	0.00	0.00
2,5%		1	0.00

*statistical significance at level p<0,05

Table 5. Bonferoni test for difference in the Log10 number for CFU/ml of *S. enteritidis* ES depending from concentration of sweet basil essential oil.

Log_{10} *S. enteritidis* ES + basil essential oil	1%	2,5%	5%
Control	5.61*	5.61*	5.61*
1%	1	0.00	0.00
2,5%		1	0.00

*statistical significance at level p<0,05

Table 6. Bonferoni test for difference in the Log10 number for CFU/ml of *S. enteritidis* ES depending from concentration of thyme essential oil

Log_{10} *S. enteritidis* ES + thyme essential oil	1%	2,5%	5%
Control	5.61*	5.61*	5.61*
1%	1	0.00	0.00
2,5%		1	0.00

*statistical significance at level p<0,05

A unique statistically significant difference on the level p<0,05 in the Log10 number for CFU/ml for *S. enteritidis* RS and S. enteritidis ES was established between controls without addition of basil and thyme extracts and target samples containing basil and thyme essential oil, regardless from the concentration of the oils. In that context, it should be mentioned that a large number of authors were interested in testing the antimicrobial effect of basil and thyme essential oil on pathogenic bacteria in food from animal origin, such as eggs, meat, milk and milk products. All of them revealed that there was an antimicrobial effect on sweet basil and thyme essential oil. The results obtained from the research are in correlation with more literature data related to the examination of the effect of extracts of essential oils.

CONCLUDING REMARKS

The main reason for the illness of people in the world, caused by food, is the presence of foodborne *Salmonella enteritidis*. The antifungal, antibacterial and antioxidant capacity of essential oils from plants and their application in production technology reduces the risk of the presence of pathogenic microorganisms in food. The inhibitory effect of basil and thyme essential oils on the growth and reproduction of *Salmonella enteritidis* in a laboratory experiment that reproduces the conditions of preparation of the pasta, prior to the exposure at a temperature of 46°C, can be the basis for their use as natural preservatives in food like pasta.

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ВЛИЈАНИЕ НА РАЗЛИЧНИ КОНЦЕНТРАЦИИ НА ЕКСТРАКТИ НА БОСИЛЕК И ТИМИЈАН ВРЗ РАСТОТ И РАЗМНОЖУВАЊЕТО НА *Salmonella enteritidis*

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Резиме

Salmonella enteritidis е една од најчестите патогени бактерии кои предизвикуваат заболување кај луѓето преку консумирање храна. Цел на истражувањето беше да се утврди ефектот на екстракти од босилек и тимијан во концентрација од 1%, 2,5% и 5% врз растот и размножувањето во лабораториски услови на два соја *Salmonella enteric* subsp. *enetrica* serotype Enteritidis ATCC 13076 референтен сој и *Salmonella enteritidis* (група D) - епидемиски сој изолиран од храна. Мострите беа експонирани на температури 37°C и 46°C (температура на која се врши сушење на тестенините во процесот на нивното производство). Растот на двата соја на Salmonella беше утврден единствено во контролниот примерок, без додаток на екстракт од босилек и тимијан. Во примероците со додаток на екстракти од босилек и тимијан, независно од концентрацијата на екстрактот, не беше евидентиран раст на *Salmonella enteritidis*. Според добиените резултати од статистичката обработка на податоците со користење на ANOVA, екстрактите од босилек и тимијан во сите три испитувани концентрации покажаа високо статистички значајно влијание на ниво $p<0,001$ врз Log_{10} концентрациите на *Salmonella enteric* subsp. *enetrica* serotype Enteritidis ATCC 13076 референтен тест сој и *Salmonella enteritidis* (група D) - епидемиски сој.

Клучни зборови: *Salmonella, босилек, тимијан*

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