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EFFECT OF SOME PESTICIDES APPLIED IN VITICULTURE ON THE ACTIVITY OF MICROORGANISMS OF OENOLOGICAL INTEREST

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ABSTRACT

International competition in the wine sector and the demands of discerning consumers for unique wine styles are challenges with implications for the fermentation process. The basis of quality alcoholic fermentation involves knowing how yeast strains interact with the aroma, taste, consistency and color of the wine. Perfect grape health is essential for producing a wine with outstanding organoleptic qualities, but it is not enough. This study investigates the effect of pesticides applied in viticulture on yeasts present in the epiphytic microflora of grapes.

INTRODUCTION

Grapes, as a raw material, are defined mainly by their sugar and acid content, possibly by the presence or absence of the noble mold Botrytis cinerea. Following the introduction of the term terroir by OIV Resolution VITI 333 of 2010, winemakers' attention has increasingly turned to the use of indigenous flora in winemaking, particularly where the link between the wine produced and the soil from which the vines draw their sap is particularly strong. In a 2010 study conducted in New Zealand and published in The ISME Journal on December 22, 2011 (1281-1290) Grangeteau C., (2017) showed that yeast strains on grapes before harvest differed by location. This was the first worldwide investigation of the regional delimitation of yeast populations. In other words, soil composition, climate and agricultural practices are attributes that clearly define the characteristics of a wine. Although a number of studies on the impact of vine phytosanitary treatments on yeast populations in must have been carried out since the 1990s (Alice Agarbati, et al. 2019; Čadež N. et al, 2010), it is important to know that the results obtained cannot be extrapolated to grapes. The microbial ecosystem of the grape is affected by a number of factors such as: pH, temperature, humidity, ensuring accessibility to nutrients, mainly sugar (Băducă Câmpeanu C., 201, Popa A. et all, 2004, Renouf V. et all, 2005, Martins G., 2012). Consequently, the diversity of the grape microbial community can be said to be influenced by the health of the berries. Degradation of the grape berry skin structure by molds and/or climatic events such as hail and rain during the harvesting period lead to changes in the microbial ecosystem (Popa A., 2019). It is known that yeast populations in the vineyard are low and face fierce competition from moulds, most of which are oxidative species that do not convert, or convert only slightly, the sugars contained in the grapes into alcohol. Fermentative yeasts of the *Saccharomyces cerevisiae* type are found on the grape cuticle in extremely small quantities. In addition, the microflora varies significantly from plot to plot. However, producers of natural wines (such as those produced organically) do not use dry active yeasts produced by large laboratories specialized in this field, but seek to give personality to their wines, preferring to use indigenous yeast populations, despite all the risks this choice entails. In most cases, they choose to prepare a starter that makes it possible to limit fermentation difficulties. The use of a starter makes it possible to limit the lag phase, but the selection of yeasts for the preparation of this starter is a rather serious challenge. Currently there is no indicator that takes into account the complexity of the spontaneous grape flora to explain the organoleptic profile of the wine, especially given the environmental factors that, from one year to the next, can lead to the development of a favorable or unfavorable population for winemaking.

MATERIAL AND METHODS

The research took place in the vine plantation of the Banu Mărăcine Didactic Station. Located in the southernmost extremity of the Getic Plateau, between the coordinates 44°19ˈnorth latitude and 23°48ˈeast longitude, at an average altitude above sea level of 176-190 m, the Banu Mărăcine Viticultural Center is part of the Dealurile Craiovei Vineyard. In 2015 at Banu Mărăcine 10 hectares were planted with vines of the varieties: *Cabernet Sauvignon, Merlot, Fetească neagră, Tămâioasă românească* and *Chardonnay*. The first treatment for which research was done was given against mange, mealy and gray rot, its application in the plantation starting on July 22, 2020 with the variety *Fetească neagră*. The products *Mikal Flash* and *Topsin 70WDG* were applied. *Mikal Flash* is a fungicide with systemic and contact action for the control of mildew in grapevines with approval certificate no. 2153/11.10.2002. *Topsin 70WDG* is a systemic fungicide with a preventive and curative action, with a broad spectrum of action and provides control of mealy, rot and rapeseed, with registration number 22034. The second treatment researched for was applied in the platation beginning August 2, 2020 and sought to control mange, mildew, gray rot, moth and wasps. The following products were used: *Melody Compact 49WG, Talendo, Teldor* and *Decis Expert 100 EC*. *Melody Compact 49WG* is a systemic and contact fungicide for the control of mange in grapevines, approval certificate no. 2658/19.12.2006. *Talendo* is a fungicide used for the control of mealy blotch in vines, approval certificate No 2582/14.12.2005. *Decis Expert 100 EC* is a foliar insecticide for the control of pests in field and horticultural crops, approval certificate no. 123PC/22.07.2015. For each variety a row was randomly selected from those in the middle of the plantation, two vine stumps at a distance from each other were chosen on each row, and on these two stumps a bunch of vines was selected from which samples were taken. All these were marked so that the samples were taken from the same place each time. Samples were taken not more than 24 hours before and not more than 10 hours after treatment. The biological samples were collected using sterile sanitary swabs. Immediately after the biological samples were collected from the grapes, they were taken to the Microbiology Laboratory of the Faculty of Horticulture where they were inoculated on a sterile solid medium, "Yeast Malt Agar" - YMA (yeast extract, malt extract, agar), a medium favorable for the growth of microorganisms (Dragomir Tuțulescu Felicia, 2010). Each inoculated plate was scored using the same rule as for the harvested samples. After sowing, the plates were placed in a thermostat set at a temperature of 25°C. In the case of the plates sown before the application of the treatments, the following were checked: the presence of spontaneous yeast flora on the grape skin; the percentage (%) of colonies per square centimeter of plate. For the plates sown with samples collected after the application of phytosanitary treatments, the following were studied: persistence of viable spontaneous yeast flora after treatment; percentage (%) of colonies per surface area of the plate; persistence of treatment until the appearance of the undesirable flora. ImageJ, a specialized software developed by Wayne Rasband (wayne@codon.nih.gov) at the National Institute of Mental Health in Bethesda, Maryland, USA, was used to determine the ratio of yeast colony surface area to total plaque surface area. ImageJ is a public domain Java image processing program inspired by NIH Image for the Macintosh. It can display, edit, analyze, process, save, and print 8-bit, 16-bit, and 32-bit images. Can read multiple image formats, including TIFF, GIF, JPEG, JPEG, BMP, DICOM, FITS, and raw. Can calculate area statistics and pixel values of user-defined selections. Measure distances and angles. Can create density histograms and line profile graphs. Custom acquisition, analysis, and processing plugins can be developed using the ImageJ editor and a Java compiler. User-written plugins allow solving almost any image processing or analysis problem (Fig. 1).

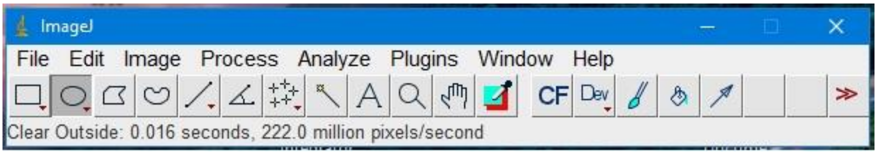


Fig.1. ImageJ – bară meniu

Starting from the fact that each sown plate was photographed every 24 hours, the way of working with ImageJ was as follows: the analyzed area of each plate was delimited and calculated; with the help of the program functions each yeast colony was delimited; the calculation of the total area occupied by the yeast colonies (Fig.2); ImageJ software uses pixels as the unit of measurement for the area and therefore, with the help of the mathematical apparatus we calculated in percentages (%) how much is the area occupied by yeast colonies of the total area of the analyzed plate using the following formula:

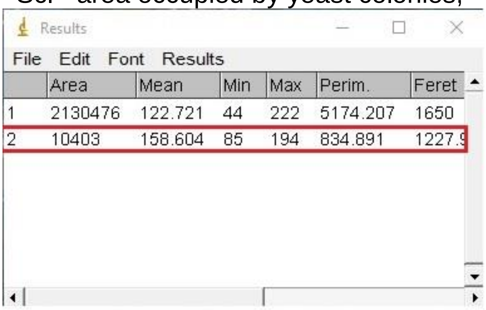
x= Scl/Sp×100

Where:

x - amount of yeasts expressed in percent;

Sp - surface area of the plate;

Scl - area occupied by yeast colonies;



	Area	Mean	Min	Max	Perim.	Feret
1	2130476	122.721	44	222	5174.207	1650
2	10403	158.604	85	194	834.891	1227.9

Depending on the resolution of the photographs the number of pixels may be different, for this reason percentage values were used.

Fig.2. Calculation of the area occupied by yeast colonies

RESULTS AND DISCUSSIONS

In order to capture the effect of pesticides on yeasts, photographs were taken at regular time intervals and the size of the yeast colony was measured in relation to the surface area of the Petri plates on which the sowing was done, and then monitoring graphs were drawn to monitor the evolution of the yeast colonies for each grape variety and after each phytosanitary treatment applied.

Treatment 1 - Mikal Flash și Topsin 70WDG
Soiul Fetească neagră

a)

Results

File Edit Font Results

	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAngle
1	2137808	127.984	69	247	5183.902	1674	891	1755	90
2	812	123.236	101	158	100.531	32	1175	291	0

b)

Fig.3 a) Fetească neagră – sample 1 - before treatment;

b) Results obtained

Results

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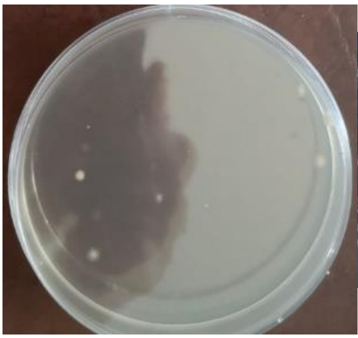
	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAngle
1	996486	122.267	52	196	3539.194	1143	623	1204	90
2	2842	155.522	104	181	564.784	904.554	566	1126	86.641

;

Fig.4 a) Fetească neagră – sample 1 - after treatment; b) Results obtained



Results

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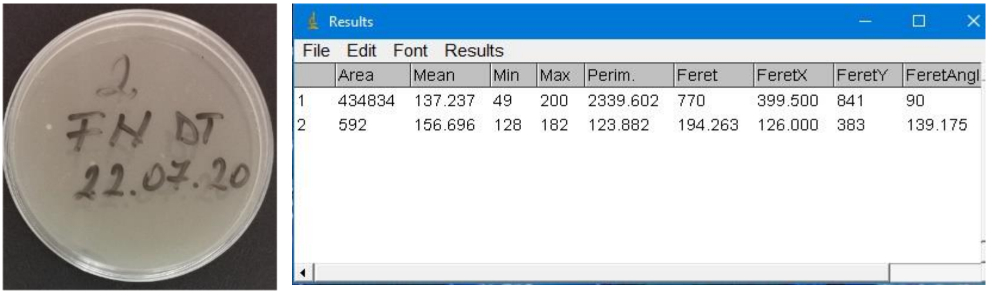
	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAn
1	2136846	135.690	68	249	5182.091	1658	901.500	1697	90
2	402	138.010	76	202	104.569	752.559	433.000	622	145.339

b)

a)

Fig.5 a) Fetească neagră – sample 1 - before treatment; b) Results obtained



a) b)

Fig.6 a) Fetească neagră – sample 1 - after treatment; b) Results achieved

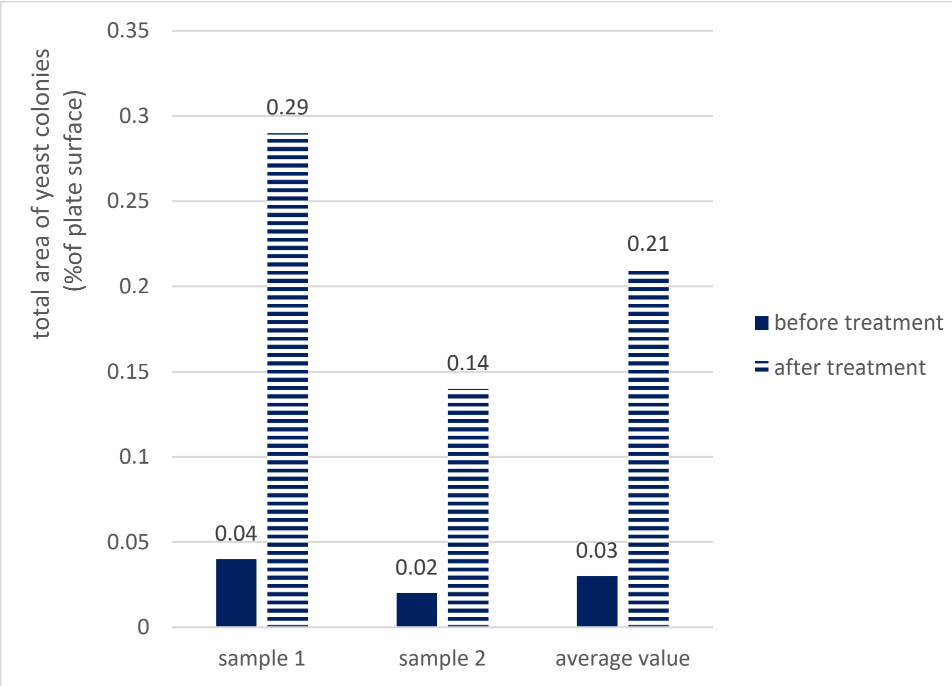
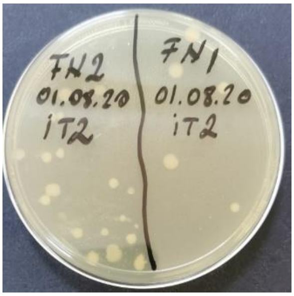
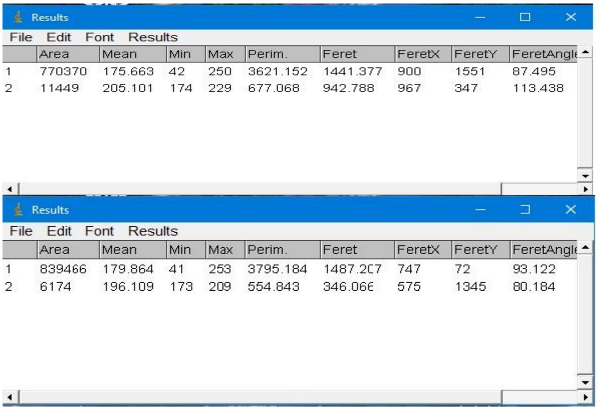


Fig. 7. Processing of the results obtained from the analysis of the samples 24 hours after sowing for the variety Fetească neagră

Treatment 2 - Melody Compact 49WG, Talendo, Teldor și Decis Expert 100 EC

Treatment 2 was applied on August 2, 2020, and samples were collected on August 1 and 2, 2020, with the first determinations being made one day after sowing, Fig. 8, Fig. 9.



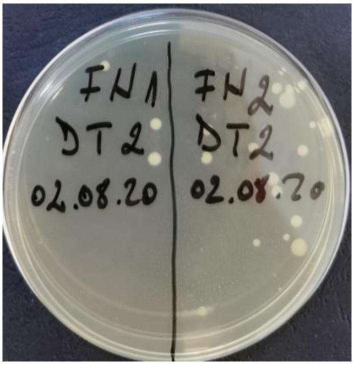


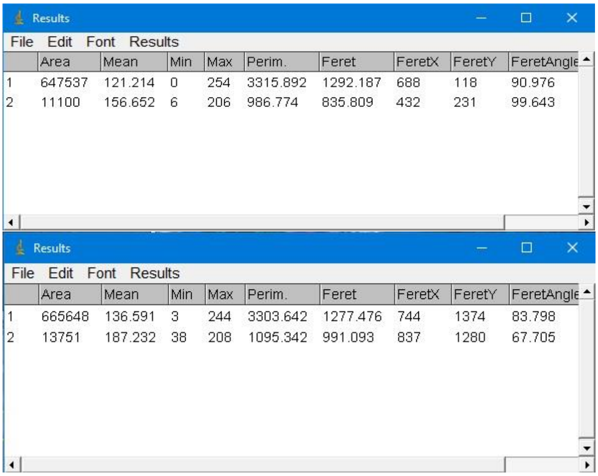
	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAngle
1	770370	175.663	42	250	3621.152	1441.377	900	1551	87.495
2	11449	205.101	174	229	677.068	942.788	967	347	113.438

	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAngle
1	839466	179.864	41	253	3795.184	1487.207	747	72	93.122
2	6174	196.109	173	209	554.843	346.066	575	1345	80.184

a) b)

Fig.8.a) Feteasca neagră – samples 1 and 2 - before treatment; b)Results obtained





	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAngle
1	647537	121.214	0	254	3315.892	1292.187	688	118	90.976
2	11100	156.652	6	206	986.774	835.809	432	231	99.643

	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAngle
1	665648	136.591	3	244	3303.642	1277.476	744	1374	83.798
2	13751	187.232	38	208	1095.342	991.093	837	1280	67.705

a) b)

Fig.9.a) Feteascǎ neagră – samples 1 and 2 - after treatment; b) Results obtained

Observation results for the variety Feteascǎ neagrǎ:

- in this case, we did not see a decrease in the yeast population following the phytosanitary treatments applied either;
- similarly, a more rapid increase in the yeast concentration was observed after each treatment, as follows: after the first treatment, we determined a 7-fold increase in the yeast concentration (admittedly, in this case starting from a much lower initial yeast concentration), and after the second treatment, the increase was 70%;
- starting from an average yeast concentration of 0.03% on the day before the first treatment and reaching an average yeast concentration of 1.89% a few hours after the second treatment, the average yeast concentration increased by about 63 times during the research period.

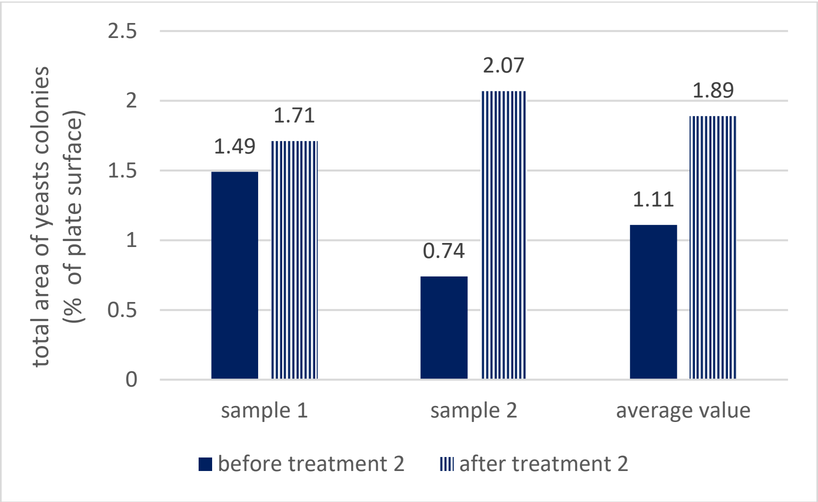


Fig.10. Processing of the results obtained by analyzing the samples 24 hours after sowing for the variety Fetească neagră

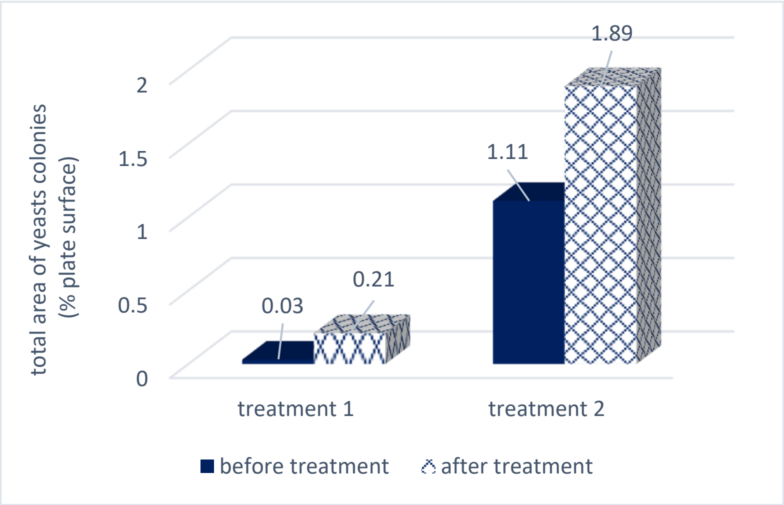
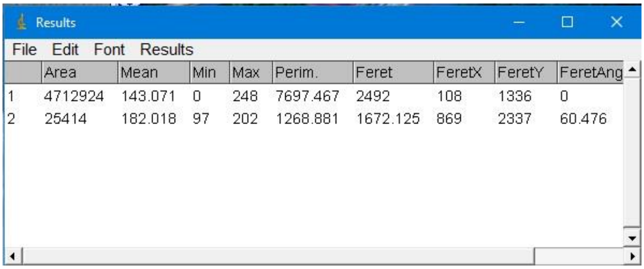


Fig. 11. Comparative results after treatments

Treatment 1 - Mikal Flash și Topsin 70WDG

Merlot variety

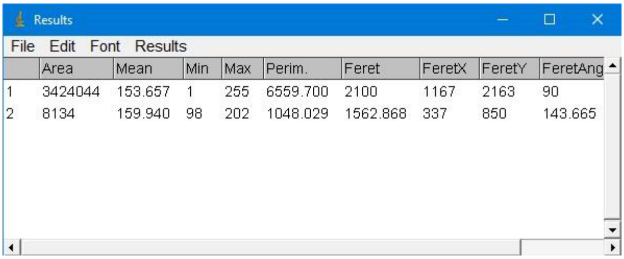
In this case it is a slightly different situation, treatment 1 was planned for July 25, 2020, the first samples were collected on July 24, 2020, but on July 25 - 26 it rained and the treatment was postponed to July 28, 2020, determinations were made according to the method, one day after sowing, Fig.12, Fig.13, Fig.14 and Fig.15.



	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAng
1	4712924	143.071	0	248	7697.467	2492	108	1336	0
2	25414	182.018	97	202	1268.881	1672.125	869	2337	60.476

a) b)

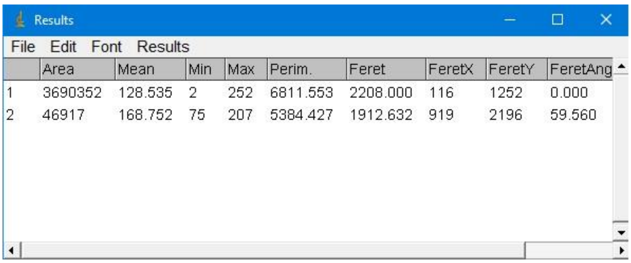
Fig.12. a) Merlot – sample 1 - before treatment; b) Results obtained



	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAng
1	3424044	153.657	1	255	6559.700	2100	1167	2163	90
2	8134	159.940	98	202	1048.029	1562.868	337	850	143.665

a) b)

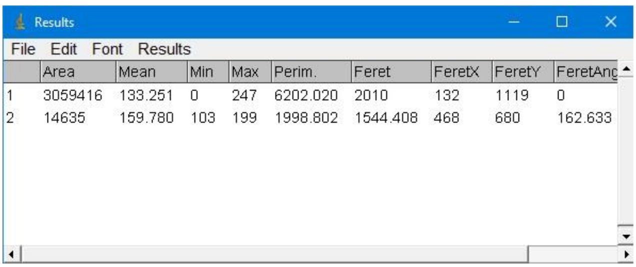
Fig.13. a) Merlot - sample 2 - before treatment; b) Results obtained



	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAng
1	3690352	128.535	2	252	6811.553	2208.000	116	1252	0.000
2	46917	168.752	75	207	5384.427	1912.632	919	2196	59.560

a) b)

Fig.14. a) Merlot - sample 1 - after treatment; b) Results obtained



	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAng
1	3059416	133.251	0	247	6202.020	2010	132	1119	0
2	14635	159.780	103	199	1998.802	1544.408	468	680	162.633

a) b)

Fig.15.a) Merlot - sample 2 - after treatment; b) Results obtained

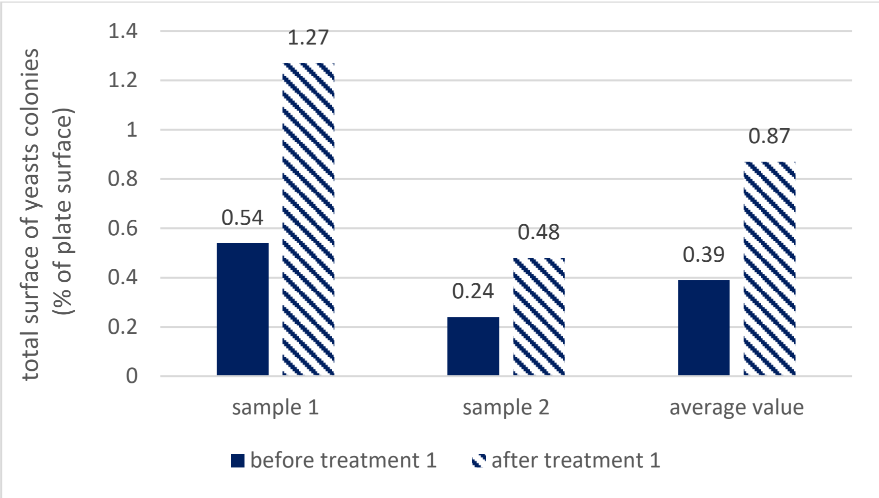
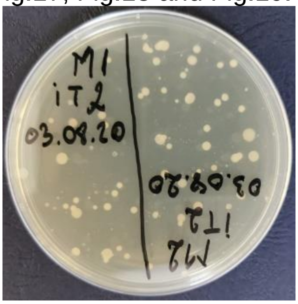


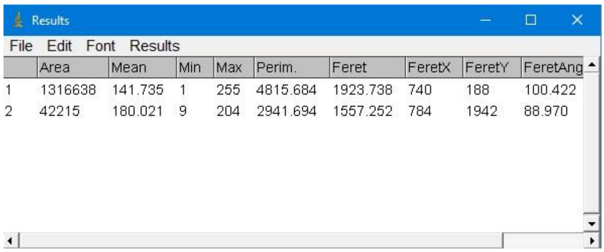
Fig.16. Processing of the results obtained after analyzing the samples 24 hours after sowing for Merlot – treatment 1

Treatment 2 - Melody Compact 49WG, Talendo, Teldor și Decis Expert 100 EC

Soiul Merlot

Treatment 2 was applied on August 4, 2020, and samples were collected on August 3 and 4, 2020, with the first determinations being made one day after sowing, Fig.17, Fig.18 and Fig.19.

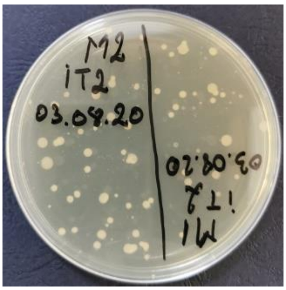


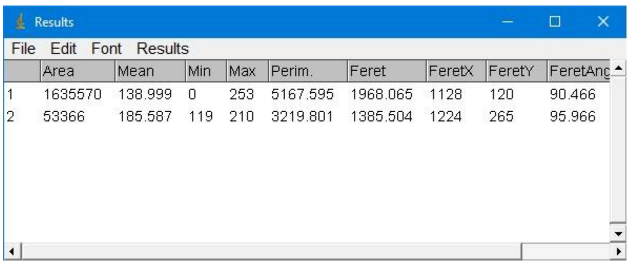


	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAng
1	1316638	141.735	1	255	4815.684	1923.738	740	188	100.422
2	42215	180.021	9	204	2941.694	1557.252	784	1942	88.970

a b

Fig.17.a) Merlot - sample 1 - before treatment; b) Results obtained



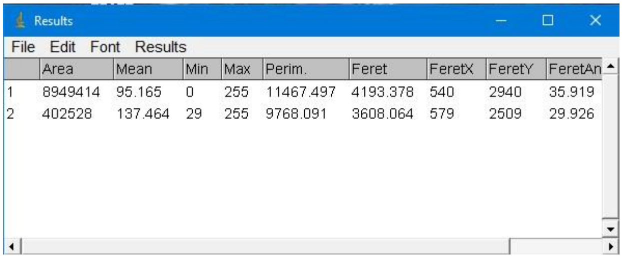


	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAng
1	1635570	138.999	0	253	5167.595	1968.065	1128	120	90.466
2	53366	185.587	119	210	3219.801	1385.504	1224	265	95.966

a) b)

Fig.18.a)Merlot - sample 2 - before treatment; b) Results obtained

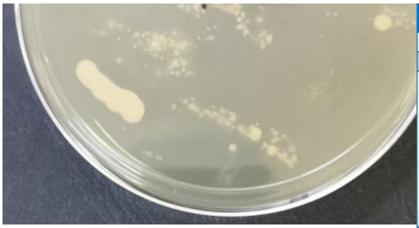
a)



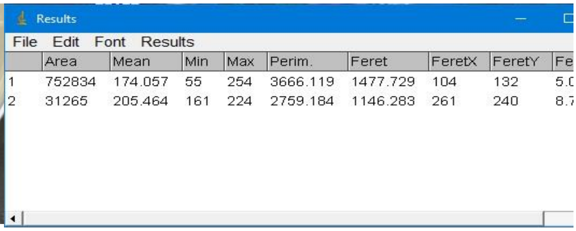
	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	FeretAn
1	8949414	95.165	0	255	11467.497	4193.378	540	2940	35.919
2	402528	137.464	29	255	9768.091	3608.064	579	2509	29.926

b)

a) Merlot - sample 1 - after treatment; b) Results obtained



a)



	Area	Mean	Min	Max	Perim.	Feret	FeretX	FeretY	Fe
1	752834	174.057	55	254	3666.119	1477.729	104	132	5.0
2	31265	205.464	161	224	2759.184	1146.283	261	240	8.7

b)

a) Merlot - sample 2 - after treatment; b) Results obtained

Observation results for Merlot:

- as before, again we did not witness a decrease in the yeast population;

- similarly, there was a more rapid increase in yeast concentration following each treatment, as follows: after the first treatment we determined a 1.23-fold increase in yeast concentration, after the second treatment the increase was about 34%;

- in this case we started from an average yeast concentration of 0.39% on the day before the first treatment and reached an average yeast concentration of 4.33% a few hours after the second treatment, the average yeast concentration increasing by about 11 times during the research period;

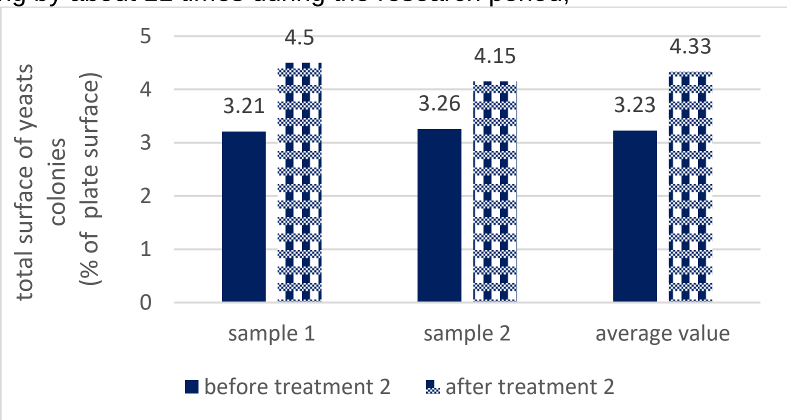
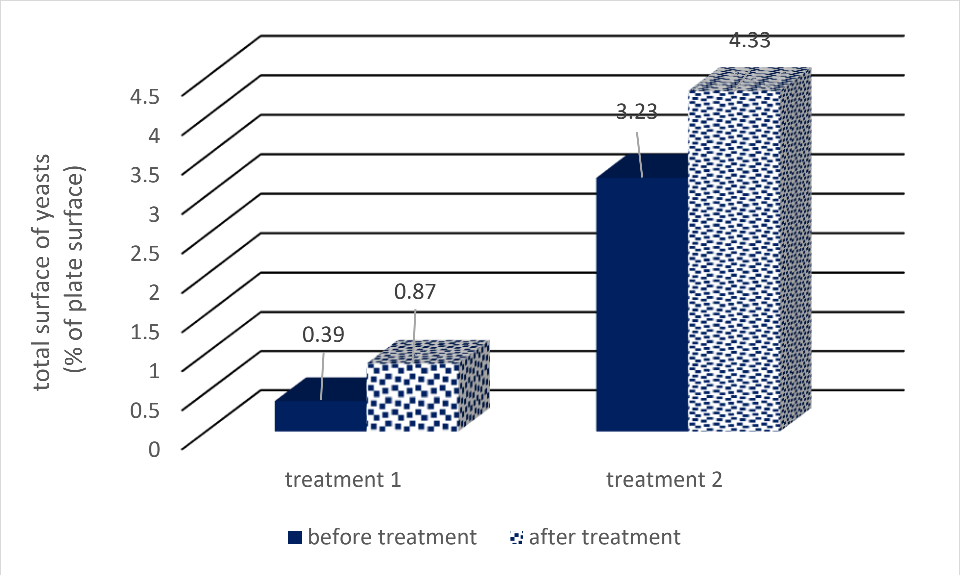


Fig.19. Processing of the results obtained after analyzing the samples 24 hours after sowing for Merlot – treatment 2



CONCLUSIONS

With the help of computerized means and statistical methods, after analyzing the Petri plates on which the samples harvested in the vineyard of the Banu Mărăcine Didactic Station were sown, the following were found:

- following the phytosanitary treatments applied, in none of the cases studied was any decrease in the yeast population recorded;
- the lowest initial concentration of yeasts was measured in the case of the variety Fetească neagră, the average value calculated on the day of the first treatment being only 0,03%;
- the highest initial concentration was found in Merlot, with an average value of 0.39% on the day of the first treatment;
- in all the cases studied, a more rapid increase in yeast concentration was observed in the first 24 hours after each treatment, due to the destruction of competing microorganisms after each phytosanitary treatment;
- the highest increases in yeast concentrations were recorded after the first treatment, after the second treatment the increases were much more moderate,
- the pesticides used showed very good selectivity;
- the phytosanitary treatments applied after the grapes had no negative effects on the yeasts of oenological interest, in all the cases studied, the increases in the concentrations of indigenous yeasts were observed following a quasi-similar trend.

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