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APPLICATION OF MICROBIOLOGICAL FERTILIZERS IN VITICULTURE: GRAPE YIELD AND QUALITY OF WINE CV. RIESLING

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Abstract: The recommended cultivar for top quality wines Riesling in the vineyards of Grocka is in full crop. It was grafted on Kober 5 BB stock and planted on the soil type cambysoil. The content of total nitrogen is 0.1-0.15%. Supply of easily available potassium varies between 12.3-15 mg/100g a.d.s.[2], i.e. phosphorus 0.4-3.6 mg/100g a.d.s. in layer up to 40 cm.

Microbiological fertilizer was used in the study - biological preparation prepared with mixed natural populations *Azotobacter chroococcum*, *Bacillus megaterium* and *Bacillus circulans*. The space in row is idle land and the space between rows was sown each year (March-April) with a mixture of field pea and barley and ploughed in the inflorescence phase of legumes.

Grape yield varied between 8772-6804 kg/ha. Microbiological fertilizer with *Azotobacter* had the highest yield and the control treatment had the lowest yield, where only grass mixture was sown. Extremely dry climatic conditions in the trial period caused the grape yield in cv. Riesling to be extremely low. In combination of fertilizers *Bacillus megaterium* + *Bacillus circulans* wine with the most ethanol, extracts and polyphenols was obtained. The wine obtained from the control treatment had a typical taste.

Key words: microbiological fertilizer, sowing legumes and grass, yield, grape and wine quality.

I n t r o d u c t i o n

In intensive viticultural production mineral fertilizers are largely used, providing high yields with somewhat lower grape quality. The soil is maintained

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[2] a.d.s. - air dry soil

in loose condition - during vegetation it undergoes shallow treatment several times, whereby weed species are eliminated. In the phase of intensive growth of vine, weeds are competitive with regard to water and mineral matter. Some of them can be potential "hosts" to viruses (K a m a s i et al, 1999).

Legumes are sown in the space between rows in a vineyard, some of them being less aggressive in comparison to other species, e.g. from the group of grasses. The advantage is expressed in their ability to fixate the atmospheric nitrogen. In the case of delayed mineral fertilizer application, the vegetation of vine is prolonged, thereby accumulating spare nutritious matter in one-year old shoots and perennial wood. And vice versa, by ploughing in green fertilizers vine uses the optimum quantity of nitrogen for its growth and development. Erosion is decreased and the structure of soil is preserved. Along with green plant mass epiphyte microorganisms - bacteria and yeasts - enter the soil, and by ploughing in the legumes, the number of symbiotic nitrogen and carbon fixers and total biomass are increased (D r i n k w a t e r and P e t t y g r o v e, 1999).

Biofertilizers - microbiological fertilizers which contain highly efficient strains of bacteria, fungi and algae provide plants with biogenous elements: nitrogen, phosphorus and potassium (G o v e d a r i c a et al, 2002). Then there is no pollution of soil, water and atmosphere. *Azotobacter chroococcum* is present in the rhyzosphere of numerous plants, and in some plant species and genotypes it is coming close to associative nitrogen fixers (R a i č e v i ć, 1996). *Bacillus megaterium* var. *phosphaticus* participates in the process of amonification, i.e. with the effect of extracellular proteolytical and nucleolytical enzymes it dissolves nucleoproteides (Š u t i ć and R a d a n, 2001). In that way, phosphorus is transformed into inorganic form, available to the plant (D ž a m i ć and S t e v a n o v i ć, 2000). The needs of vine for potassium are great. The soil contains a silicate bacterium *Bacillus circullans* which dissolves alumosilicates from which potassium is released.

By sowing the seeds combined from legumes and grasses (2:1) between the rows and by applying the mixture of microbiological fertilizer in a row, our aim was to determine their influence on the yield and quality of grapes of the cv. Riesling.

Material and Method

The experiment was conducted on the "Radmilovac" Experimental Station of the Faculty of Agriculture in Zemun. Cultivar Riesling on the stock Kober 5 BB was planted in 1995 on the lot where there used to be a vineyard. The rested soil was dug up at the depth of 80 cm in 1993, the next year chickling was planted and ploughed at the time of its inflorescence.

The type of soil is normal cambysoil on a carbonate geological stock. A_1 horizon is of crumbly structure, reaching 34 cm. B horizon is textural, yellow ore

nuance reaches the hydroxide of iron, at the depth of 34-97 cm; according to the mechanical content it is clay to sandy clay, of good drainage quality, heated well and moderately wet without longer water retention. C horizon is of clay composition at the depth of up to 1 m. The process of humus weakening is pronounced when crops are grown. Chemical reaction in ploughing layer is mildly acid to neutral. The contents of total nitrogen is small and it is present only in the ploughing layer 0.1-0.15 %. Supply of easily available potassium varies between 12.3-15 mg/100g a.d.s., with the depth the interval of varying being widened, which corresponds to medium availability. The content of easily available phosphorus is low 0.4-3.6 mg/100g a.d.s. in the layer of up to 40 cm.

In the study, microbiological fertilizer - biological preparation prepared from the mixed populations of *Azotobacter chroococcum*, *Bacillus megaterium* and *Bacillus circulans* was used. The strains of microorganisms used for this research derive from the collection of microoganisms of the Microbiology Department, Faculty of Agriculture of Zemun. Microorganisms from the natural population in soil were multiplied on adequate media and put on sterile peat. Microbiological fertilizer had been dissolved in water immediately before use, placed into the soil at the depth of 20 cm, at the beginning of vegetation. The experiment was designed according to the block system and the strain *Azotobacter chroococcum* - variant A, mixture of populations *Azotobacter chroococcum* and *Bacillus megaterium* - variant AB, mixture of populations *Azotobacter chroococcum*, *Bacillus megaterium* and *Bacillus circulans* - variant ABC and control K, were used. The space inside the row is idle land, and the space between rows was sown each year (March - April) with the mixture of seeds of field pea and barley and it was ploughed in the legume`s inflorescence phase. Grape was picked in the phase of full maturity, grape yield, number and mass of grapes, sugar contents and total acids were determined for the period 2000-2002. Microvinification according to variants was performed in 2000 and 2001. It includes the contents of ethanol, extracts, polyphenol, ashes, free and fixed sulphodioxide, evaporable and total acids in vine.

Results and Discussion

Heat conditions, height and distribution of precipitation largely differ in the study period in comparison with perennial average. Mean annual temperature of air is approximately 10.9ºC compared to 13.6ºC in 2000. In the study period, August was the warmest month (24.5ºC) and by far the highest temperature was recorded in July (40.5ºC) of the same year. Precipitation was approximately Rh=665.1 mm, of which Rh=410.5mm in the vegetation period. Total amount of precipitation in the first study year was Rh=277.5 mm. During the vegetation period there was only Rh=137.9 mm. The locality of "Radmilovac" Experimental

Station is insufficiently humid area, since the value of hydrothermic coefficient is HTC=1.24 (S i v č e v, 1996).

T a b. 1. - Average values of grape yield and quality of the cv. Riesling (Radmilovac, 2000-2002)

Variant	Number of clusters per vine	Grape mass g	Grape yield per vine kg	Grape yield per 1 ha	Veg. potential Vp	Sugar contents in must %	Total acids contents in must g/l	Maturity index
A	20.49	128	2.63	8772	4.96	20.04	6.1	3.34
AB	22.03	105	2.32	7747	3.64	21.1	5.8	3.64
ABC	19.09	109	2.10	6998	3.96	19.6	6.3	3.11
Control	19.60	104	2.04	6804	3.44	20.6	5.9	3.49

Differences between the applied microbiological fertilizers were greater in the number of clusters per vine in comparison with average grape mass. Greater effect was achieved in the treatments with *Azotobacter chroococcum* - A and *Azotobacter chroococcum* + *Bacillus megaterium* - AB. Grape yield per area unit was low both in the control variant and in the application of all three microbiological fertilizers. With the use of pure nitrogen fixers the grape yield was highest, typical of cv. Riesling. Vegetative potential, comparing grape yield per vine and mass of discarted vine during mature cutting, in this variant is between medium and low value. The results of C o r i n a et al. (1999) point that the vegetative and production potential depends primarily on the method of soil maintenance, and then on the choice of vine stock. When the soil is treated constantly, vine is characterized by greater vegetative potential, sometimes even by expressed, high yield. However, vine is much more sensitive to being infected with *Botrutis cinerea* Pers.Ex Fr. according to the same author.

Sugar contents in must varies between 19.6%, which is the characteristic of the variant ABC - *Azotobacter chroococcum* + *Bacillus megaterium* + *Bacillus circulans*, and 21.1% in the variant AB - *Azotobacter chroococcum* + *Bacillus megaterium*. C o r i n o et al. (1999) concluded that both yield and vegetative growth are decreased by maintaining grass cover between rows, while on the other hand, the wine has high contents of polyphenol and good quality.

Lastly, protection of the environment is given great significance, with the emphasis on the decrease of errosion. It is well known that every year 0.5-3.0 cm of surface layer of soil containing 2% of organic matter, 0.1% of nitrogen, 1.5% of potassium and 0.15% of phosphorus is lost through errosion in vineyards. These values are high if we know that it takes 200-300 years for the 1-2 cm of the soil lost by errosion. (P e r e t and K o b l e t, cit acc. to Branislava Sivčev, 1988).

The results given in tab. 2 show that in the treatment AB and control treatment, the wine of better quality and sensory characteristics was obtained. By

applying microbiological fertilizer ABC the wine obtained was without cultivar fragrance with pronounced acids and without refinement. The wine in which the treatment AB, the combination of *Azotobacter chroococcum* + *Bacillus megaterium,* was applied seems fuller, but "duller" in taste and with different fragrance feature. In the use of microbiological fertilizer *Azotobacter chroococcum* the wine was full, of pleasant fragrance and nice acids. In the control treatment, in which the application of multiplied bacteria was left out, and the green fertilizer was ploughed in between rows, the wine obtained was characterized by freshness, it was drinkable and of typical fragrance of cv. Riesling (J o v i ć et al. 2000). The results of M a i g r e and A e r n y (2000) on the analysis and organoleptic characteristics of wine cv. Gamet Black point to the fact that by applying green fertilizers without mineral nitrogen, total level of nitrogen in must is decreased and the contents of higher alcohols phenyl-2 ethanol and 2- and 3-methyl-1-buthanol in wine are increased. In general, these wines are less typical and tannines are of poorer quality, but the fragrance is more prominent due to the increased nitrogen in must.

T a b. 2. - Some important indices of wine quality (Radmilovac, 2000-2001)

Treatment	Ethanol vol %	Ethanol g/l	Extract g/l	S mg/l free	S mg/l total	Evapor. acids g/l	Total acids g/l	Ashes qty g/6	Gallic acid g/l
A	11.82	93.3	16.9	15.36	153.60	0.24	8.21	1.90	0.215
AB	13.74	108.5	19.3	24.32	222.72	0.28	6.32	2.10	0.210
ABC	12.25	96.7	16.6	48.64	158.72	0.18	8.06	1.92	0.100
Control	12.95	100.2	18.0	33.28	217.16	0.30	7.45	2.09	0.205

C o n c l u s i o n

In the study period the amount and distribution of precipitation during vegetation were low and uneven, and the temperature conditions were extremely high. The vineyards of Grocka, where the "Radmilovac" Experimental Station is situated, is characterized by moderately continental climate. However, in the given period it has acquired characteristics of arid climate. The year 2000 is especially prominent with the precipitation of 137.9 mm during the vegetation period and the warmest month August, average temperature = 24.5ºC.

Grape yield per unit of area was 7,580.25 kg/ha, on average, which is insufficient for cv. Riesling - clone 239.

Comparing the applied microbiological fertilizers and grape yield, the activity of *Azotobacter chroococcum* - treatment A, with ploughing in green mass of field pea and barley, had a positive influence on the quantity of grapes 8,772 kg/ha.

Average mass of a cluster of 128 g with the application of microbiological fertilizer *Azotobacter chroococcum* - A points to the cv. Riesling - 239.

Vegetative potential with the application of microbiological fertilizer *Azotobacter chroococcum* - A is approaching the category of poor thickness, which is not the characteristic of Riesling in normal conditions. The lack of humidity during the entire period of vegetation influenced the poor increase of young shoots.

High contents of sugar and somewhat lower contents of total acids was achieved by the application of microbiological fertilizer *Azotobacter chroococcum* + *Bacillus megaterium* - AB. The differences between treatment A and control with respect to those two indices were the lowest.

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PRIMENA MIKROBIOLOŠKIH DJUBRIVA U VINOGRADARSTVU: PRINOS GROŽDJA I KVALITET VINA U SORTE RIZLING RAJNSKI

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R e z i m e

Ispitivanja su obavljena na sorti Rizling rajnski – klon 239 okalemljen na podlozi Kober 5 BB na Oglednom dobru "Radmilovac"Poljoprivrednog fakulteta u Zemunu. U prostoru izmedju redova svake godine u rano proleće zasejavana je smeša legumonoze: stočnog graška i jarog ječma (2:1). U fazi cvetanja leguminoze zelena masa je zaoravana. Zemljište u redu u vinogradu je ručno obradjivano 2-3 puta u toku vegetacije.

U radu je korišteno mikrobiološko djubrivo-biopreparat pripremljen od mešanih populacija *Azotobacter chroococcum, Bacillus megaterium i Bacillus circulans*. Sojevi mikroorganizama koji su korišteni za ova istraživanja su iz kolekcije mikroorganizama Katedre za mikrobiologiju Poljoprivrednog fakulteta u Zemunu. Mikroorganizmi su umnoženi u odgovarajućim hranljivim medijumima i naneti na sterilan treset. Mikrobiološko djubrivo je neposredno pre upotrebe rastvoreno u vodi, unešeno u zemljište na dubini do 20 cm, na početku vegetacije. Ogled je postavljen po blok sistemu a korišćen je soj *Azotobacter chroococcum* – varijanta A, smeša populacija *Azotobacter chroococcum i Bacillus megaterium* – varijanta AB, smeša populacija *Azotobacter chroococcum, Bacillus megaterium i Bacillus circulans* – varijanta ABC i kontrola K. Prostor u redu je jalovi ugar, a prostor izmedju redova zasejavan je svake godine (mart-april) smešom semena stočnog graška i ječma i u fazi cvetanja leguminoze zaoravan. Berba grožđja obavljena je u fazi pune zrelosti, utvrdjen je prinos grožđja, broj i masa grozda, sadržaj šećera i ukupnih kiselina za period 2000 do 2002. godine. Mikrovinifikacija po varijantama obavljena je u 2000. i 2001. godini. Obuhvata sadržaj etanola, ekstrakta, polifenola, pepela, slobodnog i vezanog sumpordioksida, isparljive i ukupne kiseline u vinu.

Ispitivanom periodu visina i raspored padavina u toku vegetacije bili su niski i neujednačeni, a temperaturni uslovi ekstremno visoki. Gročansko vinogorje, u kome se nalazi Ogledno dobro "Radmilovac", odlikuje se umereno kontinentalnom klimom, medjutim u navedenom periodu poprimilo je odlike aridne klime. Posebno se ističe 2000. godina sa visinom padavina u toku vegetacije 137,9 mm i najtoplijim mesecom avgustom t_{prosek}=24.5^{o}C.

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Prinos grožđja po jedinici površine u proseku iznosio je 7580.25 kg/ha. Uporedjujući primenjena mikrobiološka djubriva i visinu prinosa grožđja, aktivnost *Azotobacter chroococcum* – tretman A uz zaoravanje zelene mase stočnog graška i raži, pozitivno je uticala na količinu grožđja 8772 kg/ha.

Vegetativni potencijal uz primenu mikrobiološkog djubriva *Azotobacter chroococcum* – A približava se kategoriji slabe bujnosti, što nije odlika Rizlinga rajnskog u normalnim uslovima. Nedostatak vlage tokom celog perioda vegetacije uslovio je slabiji porast lastara.

Visok sadržaj šećera i nešto niži sadržaj kiselina ostvaren je uz primenu mikrobiološkog djubriva *Azotobacter chroococcum+Bacillus megaterium* – AB. Izmedju tretmana A i kontrole razlike u pogledu ova dva pokazatelja su bile najmanje.

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