IMPROVING VINE PERFORMANCE UNDER UNFAVORABLE CONDITIONS OF GROWTH BY USE OF TRACE ELEMENTS AND MICROORGANISMS

UTILIZAREA COMPOZIȚIILOR DE MICROELEMENTE ŞI MICROORGANISME PENTRU AMELIORAREA FERTILITĂȚII SOLULUI ÎN PLANTAȚIILE VITICOLE

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Abstract. *Long-term plant cultivation imposed by a monoculture such as the vineyards and orchards, leads to exhaustion of the soil, reduction of microbiological activity in rhizosphere, and accumulation of products from pesticides, such as copper and herbicides. The possibility to elaborate a new advanced technology to maintain vineyard health under sustainable conditions and to increase plantation longevity was undertaken in last 6-7 years. The obtained data demonstrate the possibility of developing an effective process for the control of growing conditions and plant nutrition on the soils contaminated with Cu and pesticides, for productivity and resistance to pollution and low temperatures increase, improve product quality, increase the longevity of the vine, reducing chemical pressing on the environment.*
Key words*: viticulture, trace elements, microorganisms, copper excess, herbicide, plant growth, productivity.*

Rezumat. *Cultivarea pe termen lung a plantelor impusă de către o monocultură precum viile sau livezile, duce la epuizarea solului, reducerea activității microbiologice în rizosferă, și la acumularea produselor din pesticide precum cupru și erbicide. Posibilitatea elaborării unei noi tehnologii avansate de menținere a sănătații podgoriilor în condiții durabile și de creșterea longevității plantațiilor a fost întreprinsă în ultimii 6-7 ani. Datele obținute demonstrează posibilitatea de a dezvolta un proces efectiv pentru controlul condițiilor de creștere și nutriție a plantelor pe soluri contaminate cu pesticide și cupru, pentru creșterea productivității și rezistenței împotriva poluării și temperaturilor scăzute, îmbunătățirea calității produselor, creșterea longevității viei, și reducerea pressingului chimic asupra mediului.*
Cuvinte cheie: *viticultură, microelemente, microorganisme, exces de cupru, erbicid, creșterea plantelor, productivitate.*

INTRODUCTION

The vineyards and orchards constitute a major factor in agricultural productivity of many countries. However, long-term plant cultivation imposed by a monoculture

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such as in vineyards leads to depletion of essential soil nutrients, reduction of microbiological activity in the rhizosphere and soil pollution due to the accumulation of residues from chemical treatments (Veliksar and Toma, 2012). The biomass of microorganisms in the soils under the vineyard is reduced 3- to 4- fold, because of chemical applications and nutrients available to the plants become exhausted. These adverse cultivation conditions negatively affect plant growth by reducing the capability to face abiotic and biotic stresses such as extreme temperatures and pest attacks, and thus plant production by decreasing fruits' quality and quantity.

A new approach to vineyard cultivation aiming at recovering plant productivity by ameliorating the growing conditions is currently requested to maintain adequate levels of the output of this important fruit crop. The goal is to reduce the chemical load on the ecosystem and to correct plant growth in the soils already suffering from heavy metals and pesticide residues. Because the bioavailability of many microelements to perennial plants is limited, a special complex *Microcom-V* containing 6 essentially for grape trace elements was created to improve metabolism in grapes and studied in different conditions (Veliksar *et al.,* 2011; Veliksar *et al.,* 2008). The complex is applied foliar as a 0,15% water solution during vegetation period. It was proposed that beneficial effects of the nutrients in Microcom -V in 0,5 dose will be supplemented with suspensions of living beneficial microorganisms and their metabolites to create a new complex for plant productivity. Such approach have to possess a wide range of action, be ecological friendly, compatible with the standard technology of grape cultivation and offer cost-saving to be competitive on the world market. Creation of the microbial biotechnology is one of the main areas of modern agriculture (Lütfi and Murat, 2009; Salantur *et al.,* 2006). Using the potential of soil bacteria capable to mobilization of nutrients from the soil and the atmosphere is an important achievement of biotechnology and factor in increasing the productivity of agricultural crops. On the basis of the microbial metabolites the various biological composition are created. They are becoming more widely used in crop production.

MATERIAL AND METHOD

The researches have been performed in green house and in field conditions (central region of Moldova) on the technical varieties of grape (Codrinschi, Aligote, Traminer, Chardonnay, Presentable) in the years 2010 - 2014. Plants fertilization involves: a) incorporation in soil of suspension of beneficial microorganisms *Azotobacter chroococcum (suspension #1), Pseudomonas fluorescens (suspension #2),* and *Baccilus subtilis* when planting seedlings and b) foliar treatment of plants with metabolites of microorganisms and complex of trace elements Microcom-V. As a subjects of study were used: vine seeds, vine cuttings of two cultivars (Codrinschii and Prezentable, seedlings grown in plastic pots in green house and nursery, fruitful grape plants. The foliar treatment by the micro fertilizers and bacteria metabolites was conducted three terms – before flowering and at the stage of intensive growth with an interval of 12 to 14 days. Water treated plants were used as control. The following analytical methods were used: the content of free amino acids - using an AAA-300 analyzer, the carbohydrate content - according to Bertran;

photosynthetic pigments determination- using ethanol extraction, trace elements content - using an atomic absorption spectrophotometer Perkin Elmer after dry ashing at 480°C.

RESULTS AND DISCUSSIONS

Effect of microorganisms and trace elements on grape seeds germination and seedlings growth. A promising direction in the improvement of existing technology in viticulture and seedlings production is the application of trace elements and biotechnological products, particularly for vine reproduction from seeds (in the case of breeding new hybrid varieties). Reproduction of vine by seeds is of great importance in the selection too, for new hybrid varieties creation. Vine seeds have low germination energy such as some species of trees and shrubs. Researchers conducted under controlled conditions have shown the possibility to increase seed germination rate of the grape and to improve the quality of planting material by applying trace elements complex Microcom-V and suspension of microorganisms *Ps. fluorescence* - for hybrid seeds obtained by crossing (Tab.1) and *Az. cchroococcum* - for seeds of standard varieties. Recording of seedlings key parameters (height and diameter of shoots) shows that all variants, where seedlings have been treated with suspensions applied separately and in binary combination suspension (1:1), have a better development. These research results show the positive role of bacterial suspension on the growth and development of seedlings.

Table 1

Effect of bacterial suspension on seed germination of hybrid vine obtained by crossing (Madleine Angevine x Moldova)

Variants	% of seeds germina-tion	% to control	the length of shoots, cm	% to control	Diameter of main shoot, cm	% to control
Control	26	100	33,8	100	0,10	100
Seed treatment with Microcom-V	68,5	263,5	35,8	105,9	0,12	120,0
Seed treatment with *Pseudomonas fluorescens*	74,2	285,4	72,9	215,7	0,18	180,0
Seed treatment with *Azotobacter chroococcum*	71,4	274,6	42,1	124,6	0,15	150,0

Effect of microorganisms and trace elements on growth and development of seedlings produced by vegetative propagation. It has been determined the effect of trace elements and microorganisms on the growth of vine cuttings, obtained for vegetative propagation under controlled conditions (in green house). A mixture of two suspensions of microorganisms, incorporated into the soil, and their metabolites used for foliar treatment of plants in pots increased the growth of cuttings, in particular root system. Most intense biomass accumulation of seedlings was observed in variants, were extra root triple treatment of plants by metabolites of two strains of microorganisms and half dose of Microcom-V were used (Tab. 2). Intensive growth and increased biomass accumulation can be explained by the fact that PGPR (plant

growth promoting rhizobacteria) produce auxins, citokinine, gibbereline and adjusts the level of endogenous ethylene in plants (Avis *et al.,* 2008; Martinez-Viveros *et al.,* 2010).

Determination of roots and shoots of seedlings length demonstrates that the total biomass increase was largely due to increased of rhizogenesis process - length and quantity of roots, especially small roots - absorbents hairs. Increased absorption surface of roots is very important to improve plant mineral status and quality of planting material. Significant effect of rhizobacteria and trace elements on growth cuttings is associated not only with major content of active substances in the metabolites of microorganisms, but also with increased growth potential of the vine.

Table 2

Biomass accumulation by vine plants under the influence of microorganisms and trace elements. Green house, variety Prezentabil, g/plant.

Variants	Roots	Above-ground part of seedlings
Control	7.58±2.78	16.09±2.32
Suspension of *Pseudomonas fluorescens* + *Azotobacter chroococcum*, în soil	9.50±1.70	30.96±5.91
Suspension of *Ps. fluorescens* + *Az. chroococcum*, în soil + Microcom (0,5) foliar	9.4±1,89	19.41±4.10
Metabolites *of Ps. fluorescens* + *Az. chroococcum*,- foliar	9.30±1.50	22.42±1,95
Metabolites of *Ps. fluorescens* + *Az. Chroococcum* + Microcom (0,5) foliar	10.68±0.89	28.75±2.64

Effect of microorganisms and trace elements on growth and development of plants under the stress imposed by copper and herbicide accumulations in soil. One of the objectives of the project is highlighting the role of microorganisms and complex of trace elements in reducing the negative effect of herbicides. Currently in viticulture is used most often herbicide Raundap which is quite effective against some weeds, but there is information about the negative effect on grape plants. We included 5 variants in scheme of the experiment: 2 controls - mechanical treatment of soil and without treatment, 1 dose of herbicide Raundap, half dose of herbicide, a consortium of three strains of microorganisms *Pseudomonas putida, Agrobacterium radiobacter, Bacillus subtilis.* Record of the number and type of weeds in mentioned experiment has shown that the number of weeds in variants with microorganisms and herbicide solution sharply drops in comparison to variants with 1 and 0.5 doses of herbicide. This result confirms the information in the literature about the possibility of applying some strains of microorganisms in ecological agriculture.

We studied the nutrient content in the soil under the fruitful grape plants after herbicide application. Over a month after application of Raundap in recommended dose (1 dose) it was observed a decrease in the content of mobile forms of basic nutrients not only from 0-30 cm and 30-60 cm. In the variant with incorporation into the soil of the consortium of microorganisms with the application of herbicide dose of 0.5 it was mentioned a tendency to maintain the contents of the nutrients in the soil at a

stable level. Interesting data was obtained by determining the capacity of a soil nitrification after Raundap application. Herbicide application reduced soil nitrification capacity from 6.8 up to 6.2 mg $N\text{-}NO_3$ / 100g soil, which also indicates about the negative effect of the herbicide on the soil condition and properly - plant productivity. Soil nitrification capacity increased before 8.8 mg $N\text{-}NO_3$ / 100g soil after the common application of herbicide with microorganisms.

It was clearly demonstrated that the use of the recommended dose of herbicide Raundup on the vines violates the nutrient status of plants in the soil (0-60 cm), where are the majority of roots, that adversely affect the productivity and viability of plantations. Adding half of the dose of the suspension of the three strains of microorganisms with halved dose of herbicide is more effective to the weeds than a single dose of a herbicide. More even, they contribute to the maintenance of the mineral status of the soil to the control.

One of the goals of presented study was increase *the vine resistance to excess of one of the heavy metals - Cu***.** Data from the literature and those obtained in our previous experiments show that the accumulation of heavy metals in the surface layers of soil brings unbalance in the soil- plant - atmosphere system, multiple deviations in plant nutrition, worsening of health. The vineyards during the agricultural year are treated repeatedly with different pesticides at the same time and with preparations containing copper and zinc. Cu, which is accumulation in some soils of Moldova exceeds 10-15 times compared to normal soils (background), presents a particular danger. The obtained data demonstrate that addition of suspension of microorganisms decreases obvious negative effect of toxic doses of Cu. It is well pronounced tendency to decrease the toxicity of the metal in variants with suspension of consortium of microorganisms application, especially when adding the complex of micronutrients Microcom-V - foliar treatment (Tab. 3). This confirms our hypothesis about possibility to reduce the toxicity of heavy metals by derivatives of microorganisms applying and foliar fertilization by Microcom-V.

Table 3

The length of shoots and roots of plants in experience with $CuSO_4$, variety "Prezentabil" (10/02/2013), the average of 10 plants

Variants	roots length, cm	shoots length, cm	shoots maturati-on cm	% to control		
				roots length	shoots length	shoots matur.
Control	292.2±28.8	26.2±2.6	18.8±2.1	100	100	100
Cu – 1200 mg/kg	229.7±52.4	24.4±4.4	14.8±4.7	178,6	193.1	178.7
Cu + suspension of microor. #1 în sol	302.2±55.1	25.9±1.9	15.33±0.5	103.4	99.2	81.4
Cu + suspension #2 în sol	301.8±84.4	28.8±4.1	19.00±2.6	103,3	110.1	100,9
Cu –+ suspens.# 1+2 în sol	304.3±55.2	31.0±3.8	21.63±2.4	1104.1	118.5	114.8
Cu + suspen. 1+2 in sol+ Microcom-V- foliar	359.2±62.9	29.6±9.6	21.9±8.7	122.9	113.3	116.7

The study of the effect of trace elements and micro-organisms on the fruit-bearing vines. In the fruit-bearing plant leaves were determined some indices, which are necessary for plant status characterization after fertilization: photosynthetic pigments content, proline, intensity of photosynthesis. The optimization of the metabolic processes in the course of plant vegetation through application of trace elements and metabolites of microorganisms was mentioned, which resulted in shoot growth and maturation, enhancement of plant resistance to frost during the period of dormancy. It contributes to the formation and a fuller manifestation of genetically based potential of frost and winter resistance of vine, increase of plant productivity and longevity.

CONCLUSION

It was highlighted for the first time the possibility common use of complex of trace elements Microcom-V and saprophytic microorganisms to enhance the growth and development of vine cuttings and fruitful plants. It was established beneficial effect of saprophytic microorganisms on vine growth under unfavorable conditions (copper excess, low temperature, application of herbicides). It was revealed the ability to decrease by 50% the dose of herbicide Raundap adding consortium of 3 microorganisms with the same effect on weed control, maintenance of nitrification activity in soil and plant nutrient status to a higher level. The obtained data demonstrate the possibility of developing an effective process for the control of growing conditions and plant nutrition on the soils contaminated with Cu and pesticides, for productivity and resistance to pollution and low temperatures increase, improve product quality, increase the longevity of the vine, reducing chemical pressing on the environment.

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