A RETROSPECTIVE REVIEW AND CONTEMPORARY ASPECTS OF THE USE OF THE GREEN ALGA *CHLORELLA VULGARIS* IN AGRICULTURE

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Abstract

In recent years great attention has been given to the application of both ecological friendly methods against soil–born pathogens and organic amendments for improving soil quality. Green algae widespread in nature actively take part in improving the soil fertility and increasing the crop yields.

The aim of the review is to present summarized information about different application aspects of the unicellular green alga *Chlorella vulgaris* in agriculture, the world experience as well as the results of our investigation carried out in Bulgaria in the last 10 years. The greatest attention is given to the use of *Chlorella* in horticulture and viticulture. *Chlorella* has a growth factor that strengthens the immunity of the plants and makes them resistant to environmental influences. In the review, a particular attention is paid to studies in which biological products have been used for control of plant-parasitic nematodes which cause more than 50% of crop diseases and in regards to new trends in sustainable agriculture.

Key words: *Chlorella vulgaris*, sustainable agriculture, plant-parasitic nematodes.

INTRODUCTION

Chlorella vulgaris, the object of this study, is characterized with specific growing factor - "Growing factor *Chlorella*" which is proved as very potent immune-stimulator in living organisms. Because of high content in *Chlorella* of protein, amino acids, antioxidants, micro and macro elements and etc. its application has beneficial effect in some serious illnesses of people. The green algae *Chlorella vulgaris* refer to a group of powerful phyto-protectors. There is data in the literature related to the effect of Chlorella as organic fertilizer on plants growth and yield of grapevines, banana trees and lettuce (Abd El Moniem-Eman &, Abd-Allah, 2008; Abd El-Moniem-Eman et al., 2008; Faheed & El-Fattah, 2008). Ectoparasitic nematodes of family Longidoridae, some of them proven as virus - vectors, are widespread on a variety of crops in Europe including in Bulgaria (Alphey and Taylor, 1986; Choleva, 1994). The most economically important nematode-transmitted virus of *Vitis vinifera* is *Grapevine fanleaf nepovirus* (GFLV). It reduces crop yield up to 80% and affects fruit quality (Kovachevski et al., 1995; Andret-Link, 2004). Dagger nematode *Xiphinema index,* the natural vector of GFLV is commonly found in Bulgarian vineyards (Choleva, 1994; Bileva et al., 2009; Bileva and Arnaudova, 2011; Bileva, 2012). Besides being a virus-vector, *Xiphinema index* causes direct damage by feeding on grapevine root tips. Common symptoms of *X. index* feeding are plant stunting, chlorosis, galls and root necrosis (Van Zyl et al., 2012). Combination of nematode feeding and its association with GFLV may lead to grapevine death. During the last years, in organic and sustainable agriculture priority is given to the application of non-synthetic chemical and bio-products to control pathogens and to increase soil fertility. In Bulgaria, there are examples of novel methods including organic fertilizers that were investigated for their effect on improving growth and productivity of different crops and on managing soil health (Bileva and Babrikov, 2007; Choleva et al., 2007, Bileva et al., 2007; 2009; Bileva, 2013).

The aim of the study was to summarize the data on the use of green alga *Chlorella vulgaris* in agriculture and conclude on the effect of its application on the growth of *test grapevine plants and their influence on the root ectoparasite Xiphinema* index.

So far, there are sparse data in the literature regarding the influence of alga *Chlorella vulgaris* on plants. An example thereof for use of *Chlorella* in agriculture is mentioned by Dilov (1985). He describes the experience of Russian researchers who apply algalization of irrigated land in growing cotton. It increased soil fertility and yield of cotton. It was also found that soaking cotton seeds before sowing in the suspension of *Chlorella* increases the biomass of plants. Faheed et al. (2008), tested the effect of *Ch. vulgaris* on germination, growth parameters and physiological response of seeds of lettuce (*Lactuca sativa*). The results showed that the treated variants with significantly increased algae growth and significantly reduced the soluble carbohydrates, proteins and free amino acids compared to the control seeds. Best results were shown by variants treated with dry extract in a concentration of 2,0 and 3,0 g/kg^{-1} soil. Egyptian scientists from the National Research Center in Giza, studied the effect of *Ch. vulgaris* extract (25 to 100%) applied as a foliar fertilizer on the vegetative habits of vine-shrubs (Abd El Moniem-Eman & Abd-Allah, 2008). They found an increase in the number of inflorescences and yield of grapes sprayed with up to 50% extract of *Chlorella* compared to unsprayed variants. So far in Bulgaria, studies related to determining the effects of *Chlorella* on plants are done by Choleva et al., 2005; 2006; 2007; Yancheva and Bileva 2006; Bileva et al., 2007. They study the influence of *Ch. vulgaris* on different varieties and its effect on soil pathogens - nematodes of the genus *Xiphinema* using in-vitro and pot experiments.

MATERIALS AND METHODS

The study was conducted during 2004, 2007 and 2010 in the Laboratory of Zoology, Department of Ecology & Environmental protection at Agricultural University of Plovdiv, Bulgaria.

Pot experiment with seedlings of *Vitis vinifera* L. *cv.* "*Cabernet Sauvignon*" grafted on rootstock SO4 cultivated in posts with 1 000 cm^3 sterilized soil (a plant per pot in three repetitions) was carried out during 4 months (2004). The grapevine seedlings were pre-soaked under scheme by the producer in a solution of Humustim (on the base of potassium humates - 3% N, 1.14% P2O5, 7.83% K2O, 3.92% Ca, 1.1% Mg, Cu, Zn, Mo, Mn Co, B, S; Agrospeis Ltd., Bulgaria). After planting, test-plants were artificially infested with *Xiphinema index* and treated with *Chlorella vulgaris* (dry extract of monocellular green alga *Chlorella vulgaris*, "The Golden apple"- Plamen Barakov, Bulgaria). The nematodes were taken from the rhizosphere of naturally *Xiphinema index* infested grapevines in the region of Pomorie town – South-East Bulgaria (42.55 ^{o} N 27.65 ^{o} E). They are placed in a pot with *Ficus carica* for artificial multiplication. The nematodes were extracted from 200 cm^3 of soil samples by Cobb's sieving and gravity method (Cobb, 1918) in combination with Baermann funnel method. Soil sieving (2 mm, 150 µm и 63 µm) was used and a 48 h decanting period through a Baermann funnel. Counting of nematodes was carried out with stereoscopic microscope. Numbers of *X. index* in the infested variants are calculated on the base of six specimens /100 cm^3 soil which is the minimum number of nematodes for Bulgarian population of *X. index* that cause damage to the roots of vine (as found by Choleva, 2000). The trials with *Ch. vulgaris* included application of 0.5 g, 1.0 g and 2.0 g algae per plant/pot dissolved in 100 ml water. The plants were watered once with solution. The variants were: V_1 (infested + 0.5 g *Ch. vulgaris*), V_2 (infested + 1 g *Ch. vulgaris*), V_3 (infested + 2 g *Ch. vulgaris*), V_4 (infested and non-treated plants), V_5 (uninfested and non-treated plants), V_6 (uninfested + 0.5 g *Ch. vulgaris*), V_7 (uninfested + 1 g *Ch. vulgaris*), V_8 (uninfested + 2 g *Ch. vulgaris*), V_9 (uninfested + Humustim), V_{10} (infested + Humustim).

An eight-variaties experiment with ungrafted grapevine seedlings *cv*. "*Palieri*"(a plant per pot with 1000 cm^3 sterilized soil in three replications) and *X. index* was carried out for 6 months in 2007. In this assay, the population of *X. index* and green algae were identical to that described in the previous test. Numbers of nematodes in the infested variants were calculated as in previous experiment. The trials with *Ch. vulgaris* included applications of 0.5 g, 1.0 g and 2.0 g algae per plant/pot dissolved in 100 ml water. The plants were watered once with that solution. The variants were V_1 to V_8, as described above

A third pot experiment with seedlings *Vitis vinifera* L. *cv.* "*Rubin*" grafted on rootstock SO4 was carried out during May-October in 2010. The following variants were established: V_1 (infested + 1 g *Ch. vulgaris*), V_2 (infested + 2 g *Ch. vulgaris*), V_3 (1 g *Ch. vulgaris*), V_4 (2 g *Ch. vulgaris),* V_5 (uninfested and non-treated plants), V_6 (infested and non-treated), V_7 (infested + Humusil), V_8 (Humusil). Test-plants were cultivated in pots with 1 000 cm ^{3} naturally *X. index* infested soil and 150 ml coconut (with one plant per pot in five repetitions). The soil was taken from rhizosphere of naturally *X. index* infested vines from the Experimental station of Department of Viticulture, Agricultural University of Plovdiv. Sterilized soil was used as *X. index* free control. The nematodes were extracted from 200 cm^3 of soil samples by Cobb's method (Cobb, 1918) in combination with Baermann funnel method. Counting of nematodes was carried out with stereoscopic microscope. In the infested variants, the initial population (IP) of ectoparasite *X. index* was IP=84/sp./100 cm^3 soil.

After completion of the experiment, the soil of infested variants was mixed well to be homogenized. Nematodes were extracted from soil by sieving and gravity method (Cobb, 1918) in combination with Baermann funnel method from 100 cm^3 soil of each repetition. Nematode suspensions were fixed in T.A.F (Formalin (= 37% formaldehyde) 7.6 ml; Triethylamine 2 ml; Distilled water 90.4 ml) and then were transferred to glycerine with the glycerine-ethanol method (Seinhorst, 1959) for analyses of nematode communities.

Morphological data, on the base of measurements of plant height, root length, fresh and dry weight, were analyzed. At the end of experiments, the Final nematode Population (FP) was recorded. Data were analyzed using the single factor analysis for field experiments by BIOSTAT program (ANOVA) (Dimova & Marinkov, 1999).

RESULTS AND DISCUSSION

In pot experiments with grapevine seedlings *cv. Cabernet Sauvignon* very good results have been obtained in trials with *Ch. vulgaris*. Strong stimulating effects of 1 g *Ch. vulgaris* on plant growth and development were observed in grape seedlings infested by *X. index* (Table 1). A strong suppressing effect on obligate ectoparasite *X. index* (FP = 14 sp./pot) in comparison with initial density was observed in infested and treated with 1 g *Ch. vulgaris* plants. Nearly four times higher than the initial populatin of *X. index* has been reported in variants with infested and non-treated plants (FP = 227 sp./pot). In infested control, the grape seedlings have greatly reduced roots, with coral-like malformation and typical initial necrosis caused by ectoparasite *X. index*.

The biometrics of Humustim plants are close to those of control variant (untreated and uninfested grape seedlings). Proven suppression of root ectoparasite *X. index* was observed in trials with Humustim in comparison with the infested control soil.

The effect of *Ch. vulgaris* on grape seedlings cv. *Palieri* showed promising results, confirmed by plant growth. The results showed that the infested and non-treated test plants were with a poorly developed vegetative part, in comparison with uninfested and non-treated plants (Table 2). Application of 1g *Ch. vulgaris* significantly improved plant development and decreased final nematode population in variants of infested and treated plants FP = 21 sp./pot, in comparison with IP = 65 sp./pot. Compared to the initial population, a triple number of dagger nematodes was recorded in infested control (V_4) FP = 214 sp./pot and plants showed clear nematode damages. There is a weak suppression of *X. index* FP = 43 sp./pot in application of 0.5 g *Chlorella* (V_1). A phytotoxic effect in the variant with 2 g Chlorella (V_8) was observed (Table 2). The plants are not fully grown, with least developed vegetative part and roots, and with morphometric parameters close to the control variant.

Table 1. Morphological and productive parameters of grape seedlings *cv. Cabernet Saugvinon* in pot experiment in relation to treatments

Treatments	**Plant height/cm**	**Fresh weight/g**	**Dry weight/%**
Control (untreated and uninfested grape seedlings) $\mathbf{V_5}$	103	40	27
$\mathbf{V_1}$ (*X. index* + 0.5 g Chlorella)	105	43	25
$\mathbf{V_2}$ (*X. index* + 1 g Chlorella)	110	56	34
$\mathbf{V_3}$ (*X. index* + 2 g Chlorella)	98	37	20
$\mathbf{V_4}$ (*X. index*)	56	55	14
$\mathbf{V_6}$ (0.5 g Chlorella)	107	59	38
$\mathbf{V_7}$ (1 g Chlorella)	168	62	25
$\mathbf{V_8}$ (2 g Chlorella)	99	51	21
$\mathbf{V_9}$ (Humustim)	97	42	15
$\mathbf{V_{10}}$ (*X. index* + Humustim)	90	39	24
GD 5 % 1 % 0.1 %	4.10 5.52 7.34	2.91 3.93 5.21	

Table 2. Plants biometric data from a pot experiment with grape seedlings cv. *Palieri*

Treatments	Plant height/cm	Root length/cm	Fresh weight/g		Dry weight/g	
			Leaves	Roots	Leaves	Roots
Control (uninfested and non treated plants) **V_5**	47	17	10.929	3.066	2.562	1.084
V_1 (*X. index* + 0.5 g Chlorella)	31	16	5.947	1.847	1.297	0.811
V_2 (*X. index* + 1 g Chlorella)	45	20	11.757	6.190	3.179	2.699
V_3(*X. index* + 2 g Chlorella)	38	18	8.153	2.334	2.275	0.758
V_4 (*X. index* and non treated plants)	29	10	4.435	1.277	0.896	0.677
V_6 (uninfested + 0.5 g Chlorella)	42	13	5.261	2.864	2.001	1.656
V_7 (uninfested + 1 g Chlorella)	53	23	12.298	4.249	4.348	2.253
V_8 (uninfested + 2 g Chlorella)	40	19	7.722	2.308	1.561	0.689
GD 5 %	3.600	2.632	2.361	1.753	0.789	0.897
1 %	4.884	3.571	3.204	2.378	1.071	1.218
0.1 %	6.555	4.793	4.300	3.190	1.438	1.634

After 180 days, a positive impact of *Ch. vulgaris* and liquid fertilizer Humusil on *X. index* infested grapevines cv. Rubin was found (Bileva & Vuchkov, 2013). Application of 1 g *Ch. vulgaris* (V_1 and V_3) significantly improved plant development. Data showed increased plant growth and double reduction in treatment with infested test plants with *X. index* +1 g *Ch. vulgaris*. A stimulation effect on predators and increase in omnivorous nematodes in infested and treated 1 g Chlorella variant was observed.

In this assay with grape seedlings *cv. Rubin*, poorly developed test-plants with yellow leaf colouring in treatment with infested and treated with 2 g *Ch. Vulgaris plants* and variant with uninfested and treated 2g *Ch. Vulgaris* test plants were also observed. The conclusion is that applying 2 g dry extract of *Ch. vulgaris* per pot/plant has a phytotoxic effect on grapevines.

The best results obtained in this study were in trials with 1 g *Ch. vulgaris*. They are in accordance with the results established by Choleva et al., 2005; 2007; Yancheva and Bileva, 2006. *Chlorella vulgaris* in this concentration, i.e.1 g dry extract/plant, has a suppressive effect on root ectoparasite *X. index*. Another benefit of application of additional organic fertilizers is the better and stronger root system, better developed vegetative part of grapevine and growth boost.

The results show that *Ch. vulgaris* have significant and positive effects on both plant growth as well as suppressive effect on ectoparasitic nematode *X. index*.

CONCLUSIONS

1. The preventive actions for nematode control before planting of grapevines, including the use of certified virus-free material and resistant rootstocks, is always preferable.

2. In agricultural conditions, when no alternative preventive technologies are available, data has shown that the application of some organic products such as green algae increases plant growth and lowers nematode densities. This bio-product may help farmers to reduce the problem of nematode damages whilst avoiding environmental contamination due to pesticides.

3. The beneficial effect of microalgae *Chlorella vulgaris* can be attributed to increased plant immunity and improved soil health. The grapevines treated with *Ch. vulgaris* have a strong immune system which makes them resistant to ectoparasite *X. index.*

4. Based on the study results, we may recommend to farmers to include dry extract of *Chlorella vulgaris* when replanting vineyards, especially in areas threatened by the presence of dangerous pathogen *Xiphinema index* vector of GFLV.

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