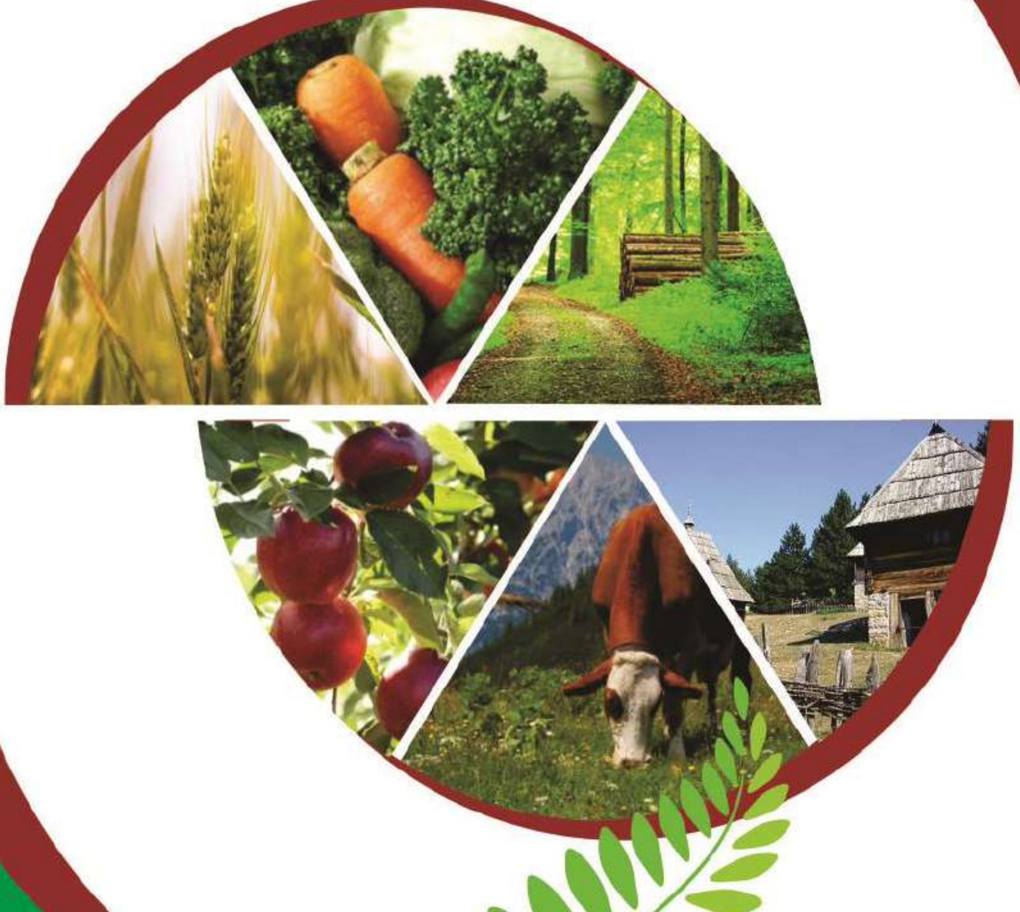
BOOK OF PROCEEDINGS

AgroSym

VIII International Scientific Agriculture Symposium
Jahorina, October 05-08, 2017



agro sym 2017

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VIII International Scientific Agriculture Symposium
"AGROSYM 2017"

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Jahorina, October 05 - 08, 2017

Impressum

VIII International Scientific Agriculture Symposium „AGROSYM 2017“

Book of Proceedings Published by

University of East Sarajevo, Faculty of Agriculture, Republic of Srpska, Bosnia
University of Belgrade, Faculty of Agriculture, Serbia
Mediterranean Agronomic Institute of Bari (CIHEAM - IAMB) Italy
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http://www.agrosym.rs.ba

CIP - Каталогизација у публикацији
Народна и универзитетска библиотека
Републике Српске, Бања Лука

631(082)

INTERNATIONAL Scientific Agricultural Symposium "Agrosym 2017" (8 ; Jahorina)
Book of Proceedings [Elektronski izvor] / VIII International Scientific Agriculture Symposium "Agrosym 2017", Jahorina, October 05 - 08, 2017 ; [editor in chief Dušan Kovačević]. - East Sarajevo =Istočno Sarajevo : Faculty of Agriculture =Poljoprivredni fakultet, 2017

Način pristupa (URL): http://www.agrosym.rs.ba/index.php/en/agrosym/agrosym_2017/BOOK_OF_PROCEEDINGS_2017_FINAL.pdf. - Bibliografija uz radove. - Registar.

ISBN 978-99976-718-1-3

COBISS.RS-ID 6954776

WINTER GREENHOUSE VEGETABLE PRODUCTION ENHANCED BY THE BROWN SEAWEED EXTRACTS

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Abstract

Owing to the content of hormones, vitamins and enzymes, brown algae extract, *Ascophyllum nodosum* L. (ANE) has plant growth promoting effect despite the small content of the major plant nutrients (N, P, K etc.). In addition, due to its natural content of physiologically active substances, ANE can alleviate the effects of stress in treated plants. This may have a particularly great importance for the winter production of vegetables in greenhouses in which the soil is usually highly saturated with nutrients. In order to determine the plant growth promoting effects of three different ANE, the experiment was conducted in the greenhouse of the Institute of vegetable crops in Smederevska Palanka, Serbia, on the vertisol soil type. The low-temperature tolerant species radish (*Raphanus sativus* var. *radicula* L.), spinach (*Spinacia olerace* L.) and garlic (*Allium sativum* L.) were sown in late autumn and treated with three different ANE. Plant height, plant mass, the number of leaves per plant and yield were measured. Comparing to untreated plants, examined traits on radish treated with ANE were not significantly different. However, ANE enhanced the plant mass and yields of spinach and onion. The application of ANE for vegetable cultivation in greenhouses during the winter can be recommended for conventional as well as for organic production systems.

Keywords: *Greenhouse cultivation, Sustainable production, Fertilizers*

Introduction

During the last few decades, the awareness for environment and ecosystems preservation was increased. Due to application of heavy doses of mineral fertilizers, contamination with nitrogen, phosphorus and some accompanying heavy metals became serious problem in some agricultural soils and ground waters (Stojanović et al. 2006; Huang et al. 2007; Sebilo et al. 2013). Eutrophication of rivers, lakes, water-ways and water accumulations is another great problem caused by excessive application of mineral fertilizers (Carpenter 2005). Many trials highlighted the necessity of precise and reasonable fertilizer utilization management, as well as the use of some biostimulants and plant growth promotion matters in agricultural practice (Khan et al. 2009; Diacono et al., 2013).

Brown algae or rockweed, *Ascophyllum nodosum* L., is an important member of marine ecosystems which provides habitat, nitrogen and carbon storage services (Schmidt et al. 2011). It is used as a raw material in a foodstuff and pharmaceutical industry (Khan et al. 2009). Due to content of certain organic acids, polysaccharides, phenols, enzymes and hormones, brown algae have application in agricultural production (Audibert et al. 2010; Craigie 2011; Yuan and Macquarrie 2015).

Recent studies, showed the effects of *Ascophyllum nodosum* L. extract (ANE) as a plant growth promoter (Khan et al. 2009; Zodape et al., 2010; Alam et al., 2013). The application of ANE on some plant species is also connected with increment of phenolic antioxidant content (Fan et al., 2011). The findings of Vera et al (2011), as well as Abkhoo and Sabbagh (2016) suggested the possibility of application ANE as a stimulant and protection against plant pathogens. The most interesting issue of ANE is the improvement of stress tolerance of cultivated plants (Guinan et al 2012; Nair et al 2012). Particularly important is the increase in tolerance to low temperatures (Nair et al 2012).

In this experiment our main goal was to evaluate biological nutritional value (growth promoting effect) of three different ANE and to determine their usefulness for the winter vegetable production in the greenhouses without additional heating.

Materials and Methods

The experiment was conducted in the experimental glasshouse of the Institute of vegetable crops, Smederevska Palanka (44^{o} 22' N, 20^{o} 57' E, altitude 101 m above sea level), in Serbia. The experiment was set up as a completely randomized block design with three replications. The soil type was vertisol with pH 6.6, 3.83% of organic matter, 0.19% of nitrogen, 1.29% of calcium carbonate, 838.8 ppm of available phosphorus and 392.6 ppm available potasium.

The previous crop was tomato (grown as monoculture; more than 8 years). Plant residues were removed following the tomato fruits harvest. Soil was watered by sprinklers and tilled by rototiller. During the first decade of December 2016, radish (*Raphanus sativus* var. *radicula* L. cv. *Saksa*) and spinach (*Spinacia olerace* L. cv. *Matador*) seeds were sown at the high rate density in rows separated 20 cm from one another and the garlic bulbs (*Allium sativum* L. cv. *Domaći jesenjak*) were planted in similar distance rows with 7.5 cm distance between bulbs inside the row. Each plot measured 4m^{2}. After sowing and planting, the drip irrigation system was installed and the plots were cowered by agro textile tiles in order to accumulate heat in rhizosphere and speed up emergence of crops.

When each crop emerged, excessive plants were removed and crop densities were decreased to terminal number of plants (80, 50 and 60 plants/m^{2} of radish, spinach and onion respectively).

At the stage of two regular leaves, each crop was watered with three different ANE solutions, according to producers’ instructions (Table 1.). The fourth treatment was water (control). Different ANE solutions were prepared as follows: four barrels (20l) were half filled with water. In three barrels, the ANE extracts were added (20, 40 and 40 ml of Biocomplex, ASCO Forte and SIB AF respectively) and the barrels were mixed by hands. Each ANE solution (10l) was sprinkled by hand-can with sieve as well as water used for control treatment. The experimental plots were previously watered by drip irrigation system. The can and sieve were carefully washed after each treatment. The same treatments were repeated after ten days.

During the experiment it was not noticed any pest or plant diseases appearance on experimental plants so the usual treatments with pesticides were not applied and all other measures of vegetable growing practice were in accordance with organic principles.

Table 1. Basic chemical properties of examined ANE and their concentration of application

Parameter	Biocomplex	Asco Forte	SIB AF 1/17
pH	4.1	6.4	8.4
Dry matter (%)	15.2	15.5	15.1
Organic matter (%)	9.2	11.2	11.6
N total (%)	0.27	0.22	0.24
C total (%)	4.5	5.5	5.7
Concentration of application (%)	0.2	0.4	0.4

When the crops reached commercial maturity, samples of 20 plants of radish, spinach and onion were taken and measured for examined parameters via analytical and technical scale (yields); radish (9.02.2017.): fresh plant mass, number of leaves per plant, hypocotyl mass and biomass yield per plot; spinach (14.03.2017.): fresh plant mass, number of leaves per plant, leaves mass and biomass yield per plot; onion (21.03.2017.): fresh plant mass, number of leaves per plant, plant height and biomass yield. Hypocotyl index (radish) was calculated and used for calculation of radish hypocotyl yield per area unit (Hypocotyl yield = Hypocotyl index * biomas yield per ha; Hypocotyl index = average hypocotyl mass/fresh plant mass). Spinach commercial yield per unit area (ha) was calculated via average fresh plant mass, leaves mass and biomass yield per plot i.e. Spinach commercial yield (ha) = (average leaves mass / average fresh plant mass) * biomass yield per plot * 2.500.
One way ANOVA was performed for each examined parameter of both crops. The differences between average parameter values were compared by Duncan-test ($p < 0.05$).

Results and Discussion

The average temperature in December was 0.2°C and the sum of sunshine hours was 103, which slowed down the sprouting and initial growth of plants. In January, the average temperature was -4.3°C with 12 days with temperatures below 0°C throughout the whole day, and sum of 99 hours of sunshine, which additionally slowed down the growth, but also caused the freezing and decaying of plants that were not at appropriate stage of development. In February, average temperature was 5.1°C with sum of sunshine being 114 hours, which influenced the growth and development of crops very favorably. In March, average temperature was above average, 10.4°C and the sum of sunshine was 184 hours. All this influenced the growth and development of plants beneficially.
In case of radish plants treated with ANE, recorded were the average larger plant weight, higher number of leaves per plant, higher hypocotyl mass, higher hypocotil index and higher yield of hypocotyl per unit area compared to the control group of plants. However, only in the yield of the hypocotil per unit area in ASCOforte and SIB AF treatments, this difference was statistically significant (Table 2).

Table 2. Average plant mass, number of leaves, hypocotyl mass, hypocotyl index and radish hypocotil yield, recorded in control treatment and in treatments of the tested ANE.

Treatment	Plant mass[ns] (g)	Number of leaves[ns]	Hypocotyl mass[ns] (g)	Hypocotyl index[ns]	Hypocotyl yield (t/ha)*
Control	24.47	7.87	10.82	0.44	4.81[a]
Biocomplex	25.51	8.2	12.07	0.47	5.23[ab]
ASCOforte	26.62	8.01	11.79	0.44	5.42[b]
SIB AF 1/17	25.28	7.91	11.57	0.46	5.57[b]

Values within the same column followed by the same letter do not differ significantly ($p<0.05$)

Compared to the control of ANE treated spinach plants, larger plant mass, higher number of leaves, higher root mass, higher mass per plant, and higher market yield was recorded. In the ASCOforte and SIB AF treatment, mass of the plant, root mass and market yield, and in regards to the control, the difference was statistically significant ($p<0.05$). Regarding the control treatment but also the other two ANE, there was no statistically significant difference in yield in the Biokomplex treatment (Table 3).

Table 3. Average plant mass, number of leaves, root mass, laves mass, yield imdex and market yield of spinach, recorded in control treatment and treatment of tested ANE.

Treatment	Plant mass (g)*	Number of leaves[ns]	Leaves mass (g)[ns]	Root mass (g)*	Commercial yield (t/ha)*
Control	12.54[a]	7.93	6.85	1.62[A]	2.58[a]
Biocomplex	15.79[ab]	7.97	7.96	2.06[AB]	3.25[ab]
ASCOforte	17.55[b]	8.38	8.87	2.37[B]	3.89[b]
SIB AF 1/17	15.73[ab]	8.22	8.38	2.2[B]	3.91[b]

Values within the same column followed by the same letter do not differ significantly ($p<0.05$)

In garlic plants treated with ANE, comapred to the control, noted were the higher plant mass, greater number of leaves, larger plant height and higher yield. In all three ANE treatments, difference was statistically significantly higher (Table 4)

Table 4. Average plant mass, number of leaves, height of plants and garlic yield, recorded on the control treatment and the treatment of the tested ANE.

Treatment	Plant mass (g)*	Number of leaves[ns]	Plant height (g)[ns]	Yield (t/ha)*
Control	7.33[a]	5.40	36.9	3.08[A]
Biocomplex	9.77[b]	5.95	40.74	4.4[B]
ASCOforte	10.23[b]	5.49	40.55	4.39[B]
SIB AF 1/17	9.18[b]	5.73	37.15	4.11[B]

Values within the same column followed by the same letter do not differ significantly ($p<0.05$)

Many studies indicate that application of seaweed increases the yield of vegetable crops (Jayaraman et al 2011; Jannin et al 2013; Đurić er al 2014; Abkhoo and Sabbagh, 2016) but there is also a number of studies with opposite results (Lola-Luz et al 2013; Yusuf et al 2015).

Testing the effects of ANE foliar application on cabbage, Lola-Luz et al (2013) determined qualitative improvements i.e. increase in phenol and flavonoid compounds, however, no significant increase in yield relative to control was found. The absence of effects in the above case could be attributed to the method of ANE application, since it was noticed that watering had better effects than foliar application of ANE (Abkhoo and Sabbagh, 2016).
Increase in growth of plants treated with ANE is associated with better health conditions and greater resistance to plant diseases (Jayaraman et al 2011). In our study, no harmful insects or diseases were observed, both on plants treated with ANE and on control treatment.
Đurić et al (2014) and Yusuf et al (2015) have found that ANE can influence the increase in vegetative mass of the plant, in particular the increase in root mass, i.e. Hypocotyl. The effect on treated plants seems to depend on the type of ANE used. By examining different ANE on Brassica napus, no statistically significant increase in root biomass and overheads was observed in all ANE treatments (Jannin et al 2013).
Our results are in accordance with experiments of Janin et al. (2013), Đurić et al (2014), Yusuf et al (2015), especially when it comes to garlic and spinach where statistically significant increase in average root mass and average plant mass was determined.
On the other hand, the absence of a statistically significant effect of the ANE application in the observed properties in radish is most likely due to short vegetation period of the investigated species, and in particular due to the short period of time, since the moment of treatment with the investigated agents, until the moment of harvesting.
An increase in yield of radish could be attributed to a larger number of plants that survived negative temperatures, which is in accordance with results of Nair et al (2012), which confirmed the increase in tolerance to low temperatures on Arabidopsis talian plants.

Conclusion

Based on the results of the experiment, use of the tested ANE can be recommended for winter vegetable production in greenhouses. All three investigated ANE showed positive effects on yield and observed properties. Given the relatively low content of the main nutrient macroelements in ANE and relatively small dose of application, it can be concluded that the cause of increased growth and yield of treated plants, is not the main macroelements, but some physiologically active substances found in ANE. The obtained results on radish, spinach and garlic lead to assumption about the effectiveness of ANE on other plant species during the winter production season in greenhouses. The special benefit of ANE is that their production is renewable and can be applied both in conventional production and in organic farming systems.

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