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Production of rocket (*Eruca sativa* Mill.) on plug trays and on a floating system in relation to reduced nitrate content

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ABSTRACT

The effect of the growth system on the yield and nitrate content of rocket (*Eruca sativa* Mill.) was evaluated in two experiments. In the first experiment, different sizes of cells in plug trays and different fertilization treatments were tested. In the second, a floating system was used with different substrates and the nitrate content in rocket leaves was analysed. The size of the cells did not affect the yield of rocket significantly but the yield was notably higher (2.02 - 2.21 kg m⁻²) when plugs were fertigated once per week with 6:12:36 + ME + natural biostimulant in comparison with plugs fertigated only with a water soluble fertilizer (1.53 - 1.74 kg m⁻²). The best yield was obtained in vermiculite and perlite in 20 ml cells (2.13 and 1.89 kg m⁻²). The different substrates used in the floating system had no effect on the dry matter content, which was on average 13.7% and was significantly lower than the dry matter content of leaves grown in peat (19.1%). The nitrate content in leaves measured a day before and 10 days after the replacement of the nutrient solution with tap water, fell greatly from 4,288-6,764 mg NO₃ kg⁻¹ FW to 52-634 mg NO₃ kg⁻¹ FW.

Key words: fertigation; biostimulant; rock-wool flocks; vermiculite

IZVLEČEK

PRIDELAVA NAVADNE RUKVICE (*Eruca sativa* Mill.) V GOJITVENIH PLOŠČAH IN NA PLAVAJOČEM SISTEMU IN MOŽNOSTI REDUKCIJE VSEBNOSTI NITRATA

Učinek tehnologije gojenja na pridelek in vsebnost nitratov pri navadni rukvici (*Eruca sativa* Mill.) je bil izvrednoten v dveh poskusih. V prvem poskusu smo primerjali različne velikosti vdolbin v gojitvenih ploščah in različna gnojila. V drugem poskusu pa smo gojili rukvico na plavajočem sistemu v različnih substratih in analizirali vsebnost nitratov v listih. Velikost vdolbin ni vplivala statistično značilno na pridelek. Pridelek je bil največji (2,02-2,21 kg m⁻²), ko smo gojitvene plošče fertigirali enkrat tedensko z vodotopnim gnojilom 6:12:36 + ME in dodatkom naravnega biostimulanta v primerjavi z ploščami, ki smo jih fertigirali samo z vodotopnim gnojilom (1,53-1,74 kg m⁻²). Najboljši pridelek smo dobili v vermikulitu in perlitu v 20 ml vdolbinah (2,13 and 1,89 kg m⁻²). Različni substrati niso imeli vpliva na % sušine v listih, ki je bila v plavajočem sistemu povprečno 13,7% in statistično značilno manjša od sušine v listih navadne rukvice, gojene v šoti (19,1%). Vsebnost nitrata v listih smo merili 10 dni pred pobiranjem, ko so rastline še rastle v hranilni raztopini in na dan pobiranja, ko smo hranilno raztopino nadomestili z navadno vodo. Vsebnost nitratov se je zelo zmanjšala, iz 4,288-6,764 mg NO₃ kg⁻¹ sveže snovi na 52-634 mg NO₃ kg⁻¹ sveže snovi.

Ključne besede: fertigacija; biostimulant; kosmiči kamene volne; vermikulit

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1 INTRODUCTION

Rocket (*Eruca sativa* Mill.) is a fast growing, cool-season crop. The leaves can be cut after 20 or more days and sequentially harvested from re-growth. The main obstacle to sequential production of this crop appears to be early bolting during increasing day-length period (Morales and Janick, 2002). It is a leafy vegetable suitable for growing in plug trays as a fresh-cut vegetable (Nicola et al., 2004). Interest in rocket has been increasing in recent years because of the spicy taste of its leaves. It is mainly used to garnish and flavour salads and a large variety of meals. According to D'Antuono et al. (2009), rocket is a new potential health-promoting vegetable owing to the glucosinolates content.

Production technologies are being investigated in order to simplify harvest, improve the quality of the product and to reduce production costs and environmental impact. A floating system is one of the simplest soilless culture systems, with a number of advantages compared to cultivation in soil; the produced material is clean at harvest, consequently reducing the number of washing treatments; soil disinfection is not necessary; there is full control of the inputs, since substrates are mainly inert (Pasotti et al., 2003; Lazzarin and Giordano, 2007).

Rocket has a short production cycle and can accumulate large amounts of nitrate in leaves (up to 10 g kg⁻¹ FW), forming compounds believed to be potentially toxic to human health (Santamaria et al., 2001; D'Anna et al., 2003; Ferrante et al., 2003). Nitrate *per se* is relatively non-toxic but its metabolites, nitrite, nitric oxide and nitroso compounds, make nitrate of regulatory importance because of their potentially adverse health implications, such as methaemoglobinaemia and carcinogenesis (Nitrate..., 2008; Ferrante et al., 2003). On the other hand, some research has shown that its conversion to nitrite plays an important antimicrobial role in the stomach (McKnight et al., 1999), and other nitrate metabolites also have important physiological/pharmacological roles (Lundberg et al., 2004 and 2006; Bryan, 2006).

A large number of leafy vegetables can accumulate high levels of nitrate. The concentrations depend

on a range of factors, including season, light, temperature, growing conditions, fertilizer use, and storage of the crop (Premuzic et al., 2001; Magnani et al., 2007; Frezza et al., 2005; Kim and Ishii, 2007)

Nitrate predominately enters the human body exogenously from vegetables, water and other foods but is also formed to a limited extent endogenously (Lundberg et al., 2004 and 2006). In plants, nitrate is mainly found in cell vacuoles and is transported in the xylem from the roots to the leaves, from where it is then translocated to the growing points and to the storage organs, such as seeds or tubers. This means that leaf crops such as cabbage, lettuce, spinach and rocket may have fairly large nitrate concentrations, whereas storage organs such as potato tubers, carrots, leeks, onions, seeds and the pods of pea and bean plants have relatively small concentrations (Bottex et al., 2008). Another consequence of the transport system is that young leaves have lower nitrate concentrations than older leaves. Such a relationship has been shown for cabbage, with the highest nitrate concentrations in the outer leaves and much smaller nitrate concentrations in the innermost leaves (Greenwood and Hunt, 1986).

The Regulation on nitrate (Nitrate..., 2008) applies only to 2 vegetable crops: fresh spinach and fresh lettuce. Because of the widely varying climatic conditions, production methods and eating habits in different parts of the European Union, maximum levels for fresh spinach and fresh lettuce are fixed depending on the season. All maximum levels are expressed as mg nitrate/kg fresh weight. The maximum levels for nitrate in those foodstuffs are generally higher for plants that are harvested between 1 October and 31 March than they are for plants harvested between 1 April and 30 September. Moreover, with respect to fresh lettuce, the Regulation differentiates between lettuce grown under cover and lettuce grown in the open air, with lower levels for the latter. Since rocket is becoming more and more popular, its production has been extended over the whole year.

The aim of our study was first to test the plug tray system for growing rocket with a biostimulant and

second, to produce rocket with a low nitrate content on a floating system.

This paper summarizes the results of the two experiments.

In the first experiment, rocket was grown in peat substrate in cells of different sizes, with three fertigation (no fertigation; fertigation with a water soluble fertilizer (WSF); fertigation with WSF and a biostimulant). In the second experiment, we

compared plug trays with various substrates (perlite; rock-wool flocks; vermiculite; expanded clay pellets and peat substrate), using 2 techniques (floating system and growing in a peat substrate in plug trays with fertigation). The nitrate content in leaves was analyzed twice: 10 days before harvest, i.e., just before the nutrient solution was replaced by tap water and at harvest, after the plants had been growing on tap water for 10 days.

2 MATERIALS AND METHODS

2.1 Plant growth conditions and media

Both experiments were conducted in a non-heated greenhouse covered with glass in Ljubljana – central part of Slovenia (46 °N, 300 m asl, mean annual T 10 °C). In the first experiment, seeds of rocket were sown on 16th March in polystyrene plug trays with 40 cells (cell volume 60 ml) or with 84 cells (cell volume 35 ml) filled with a peat substrate. In the 60 ml cells 10 seeds were sown per cell and in the 35 ml cells 5 seeds were sown per cell, so the density was 2,400-2,520 plants per m². Three rates of fertilization were used: no fertigation; fertigation with WSF 10:5:26 + ME

and fertigation with WSF 6:12:36 + ME + natural biostimulant, based on lyophilized cattle manure, marine algae and sugar beet pulp, containing 8 % of N, 1 % of P₂O₅ and 1 % of K₂O. Fertigation was performed once a week – 8 times during the trial. Both treatments resulted in a similar amount of added elements (Table 1). There were four repetitions and 1-3 harvests. Plants were cut (harvested) for the first time 37 days after sowing, when 3-4 leaves had formed (22th April). The height and weight of plants grown in the middle of the tray (9 cells per tray) were measured.

Table 1: The amount of nutrients added during fertigation in different treatments of rocket growth

Treatment	Fertilization	Concentrations	Amount of added elements/ m ²
Non-fertigate	-	-	-
NPK	2 g/l of 10-5-26	200 ppm N 100 ppm P ₂ O ₅ 520 ppm K ₂ O	10 g N 5 g P ₂ O ₅ 26 g K ₂ O
NPK + bio stimulant	1.1 g/l of 6-12-36 + 1.7 ml of bio stimulant	200 ppm N 128 ppm P ₂ O ₅ 380 ppm K ₂ O	3.3 g + 6.8 g = 10.1 g N 6.6 g + 0.85 g = 7.5 g P ₂ O ₅ 19.8 g + 0.85 g = 20.7 g K ₂ O

The second (59 days after sowing - 14th May) and third (77 days after sowing - 1st June) harvests were performed only on fertilized treatments, because non-fertilized plants began to flower. The same measurements were done and part of the yield was dried at 60 °C for 2 days, to determine the % of dry matter.

The second experiment was performed in the same glasshouse. Seeds from the same seed company (Semenarna Ljubljana) were sown on 20th January in plug trays with 84 (35 ml; 3 seeds per cell) and 160 cells (20 ml; 2 seeds per cell), which gave us 2

densities – 1,500 and 2,000 seeds m⁻². Cells were filled with 5 different substrates: rock-wool flocks, perlite (3-5 mm), expanded clay with 6-8 mm pellets, vermiculite (3-4 mm) and peat substrate. There were 3 repetitions of each plug tray, so there were 2 densities × 5 substrates × 3 repetitions = 30 trays. Trays with peat were placed on raised benches, with a local heating system under the bench and other trays were placed in an improvised pool – 10 m long, 1.5 m large and 0.05 m deep, filled with water to a depth of 0.03 m. The whole "pool" contained 0.45 m³ of aerated water in which 250 g of WSF 18:18:18 + ME (B (0.05 %), Cu

(0.02 %), Fe (0.14 %), Mn (0.08%), Mo (0.008 %) and Zn (0.05 %)) was dissolved a few days after sowing, when the seeds began to germinate. During the experiment, the water was aerated and topped up several times because of evapotranspiration. When the EC (electro-conductivity) dropped under 1 mS cm^{-1} , the appropriate amount of fertilizer was added to the pool. Altogether, 750 g of 18:18:18 + ME was used on a surface of 15 m^2 , which is equivalent to 90 kg N, 90 kg P_2O_5 and 90 kg K_2O per ha. The control trays with peat were irrigated as necessary and fertigated each week with 100 ppm N, P_2O_5 and K_2O – the same concentration as the nutrient solution of the floating system. Plants were harvested twice - for the first time when 3-4 leaves had formed, 39 days after sowing (23th February) and 57 days after sowing (23th March). The yield of rocket leaves was measured each time and plants from 10 cells per tray were randomly selected and measured in detail – height of plants, number of leaves and their weight (data not shown). At the end of the second experiment, leaves were dried at $60 \text{ }^\circ\text{C}$ for 2 days and dry matter content was determined. Twice per week, the pH, T and EC of the nutrient solution were also measured. Ten days before harvest, the nutrient solution was replaced with water, to reduce the nitrate content in the rocket leaves.

The temperature in the greenhouse was at least $10 \text{ }^\circ\text{C}$ during nights and air humidity was mainly more than 90 %. The temperature of the nutrient solution was between 17 and $19 \text{ }^\circ\text{C}$, pH 5.6-8 and EC $0.8\text{-}1.6 \text{ mS cm}^{-1}$. When the solution was replaced with water, the EC was 0.48 mS cm^{-1} .

2.2 Determination of nitrate content in leaves

The concentration of nitrate in leaves was analyzed twice - before and after the change of nutrient solution with tap water. For each sample, about 2.5 g of well homogenized fresh leaves were put in a 50 ml test tube with 20 ml of distilled water and disintegrated with Ultrathurax T25. The solution was then heated in a water bath ($60\text{-}70 \text{ }^\circ\text{C}$) for 20 min, cooled and filtered through Whatman No 41 filter paper into a 50 ml polypropylene centrifuge tube with a screw cap (ISOLAB, Germany) filled to 50 ml with DI water and stored at $-20 \text{ }^\circ\text{C}$ until analysis. Three replicate extractions per treatment were performed.

The content of nitrate in defrosted samples was determined according to ISO13395:1996 using a continuous-flow analyzer (Flowsys, Alliance Instruments, Salzburg, Austria). No nitrite was detected in any of the samples.

The collected data were subjected to analysis of variance and Duncan's Multiple Range Test at 95 % confidence level.

3 RESULTS AND DISCUSSION

3.1 First experiment

Rocket plants were harvested three times, except for the control plants, which were not fertilized and began to flower after the first cut. Plants were cut when they had 3-4 leaves and were around 10 cm high. Where a biostimulant was added, the plants grew faster and were significantly higher than plants that had received almost the same amount of

nutrients but no biostimulant. The weight of leaves was understandably much higher in 60 ml cells in which 10 seeds were sown than in 35 ml cells with five seeds. Again, the treatment with biostimulant was significantly better than the treatment without the addition of biostimulant. Measurements are presented in Table 2.

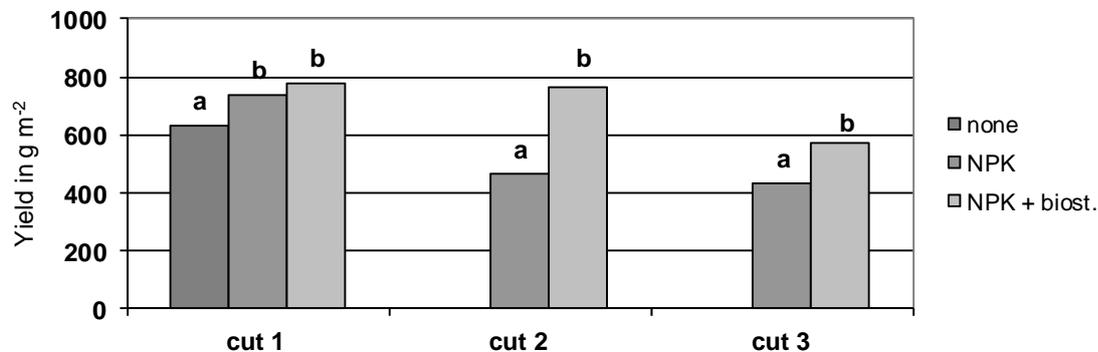
Table 2: Average height and weight of rocket plants grown in 35 and 60 ml cells and cut once or several times. The data represent means (\pm SD of 36 plants in 4 replicates).

Fertilization	Cell volume	Plant height (cm)			Weight of leaves per cell (g)		
		1 st cut	2 nd cut	3 rd cut	1 st cut	2 nd cut	3 rd cut
None	60 ml	8.1 \pm 1.17	-	-	2.2 \pm 0.80	-	-
	35 ml	8.1 \pm 0.87	-	-	1.5 \pm 0.42	-	-
	Average	8.1	-	-	1.8	-	-
NPK	60 ml	10.5 \pm 1.52	11.6 \pm 1.15	10.1 \pm 0.99	3.5 \pm 0.81	1.9 \pm 0.62	1.8 \pm 0.60
	35 ml	9.3 \pm 0.84	10.7 \pm 1.02	8.9 \pm 1.46	1.3 \pm 0.42	1.0 \pm 0.33	0.9 \pm 0.30
	Average	9.9	11.2	9.5	2.4	1.4	1.3
NPK+bio-st.	60 ml	12.2 \pm 1.54	14.4 \pm 1.42	11.7 \pm 1.35	3.1 \pm 0.96	3.2 \pm 1.01	2.1 \pm 0.64
	35 ml	11.0 \pm 0.78	12.9 \pm 1.45	10.9 \pm 1.57	1.6 \pm 0.52	1.5 \pm 0.61	1.3 \pm 0.48
	Average	11.6	13.7	11.3	2.4	2.3	1.7

Biostimulants appear to work best when the plants are under some kind of stress, either environmental, such as poor growing conditions, or due to disease or lack of nutrients (Blake, 2002). This was definitely the case in our experiment, since plants were growing in plug trays with limited space and a limited amount of substrate (20-60 ml).

Figure 1 shows that the biostimulant added to the fertilizer had a positive influence on the growth of

rocket in all 3 cuts. The cumulative yield of rocket was 2.1 kg m⁻² and was significantly higher than in the treatment fertilized only with WSF, in which the cumulative yield was only 1.6 kg m⁻² (Fig. 2). The size of the cells did not significantly influence the yield (Fig. 3). The addition of a natural biostimulant in our study resulted in 29 % higher yield, even though the amount of added nutrients was approximately the same as in fertilization alone. Plants without fertilization bolted very quickly (after the first cut).

**Fig. 1:** Average yield of rocket from the 1st experiment in g m⁻². Mean values of each cut followed by the same letter are not significantly different according to Duncan's Multiple Range test at $P < 0.05$

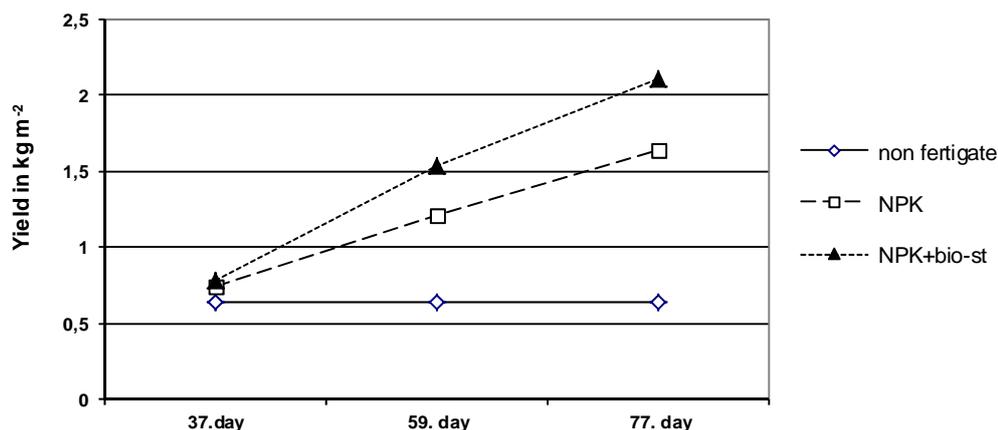


Fig. 2: Average cumulative yield of rocket (kg/m²) from all 3 cuts (exp. 1)

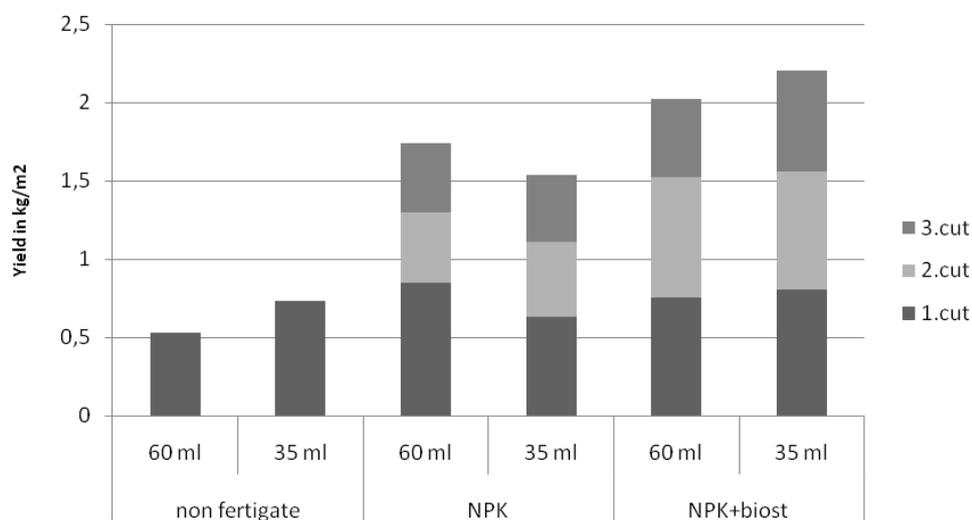


Fig. 3: Yield of rocket (kg m⁻²) with regard to cell volume and fertigation treatment (exp. 1).

The biostimulant may enhance the metabolism, increase chlorophyll efficiency and production, increase antioxidants and enhance nutrient availability. However, the nature and the effects of biostimulants may vary widely. After an extensive series of experiments with different biostimulants in a nursery, Thompson (2004) concluded that biostimulants do not improve growth when all the factors are optimal, but act more as an insurance policy to protect crops against the vagaries of nature.

3.2 Second experiment

The second experiment was conducted on a floating system, and the germination of seeds was also recorded. Statistical analysis showed no significant differences between the substrates except for the expanded clay, which turned out to be an inappropriate substrate for raising seedlings. Germination was very poor, probably due to a low water holding capacity of the substrate. The impact of cell volume on the germination of seeds was insignificant in all tested substrates (Fig. 4).

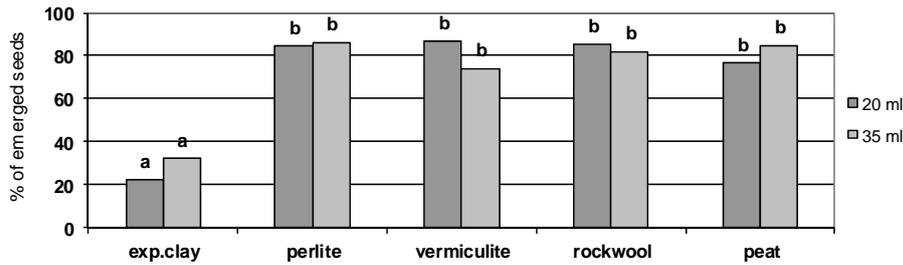


Fig. 4: Percentage of emerged seeds of rocket in different substrates and cell volumes (exp. 2).

The rocket was cut twice, when the plants had reached a height of 12-14 cm and had 3-4 leaves. Because of poor germination, the yield was lowest in expanded clay. The highest yield was recorded in vermiculite and perlite when smaller cells were used, but the yield in rockwool flocks was not

significantly lower. Smaller cells and a higher plant density (2.000 seeds per m²) gave better results and did not affect plant quality. The yield in peat was significantly lower than in vermiculite, regardless of the cell volume (Fig. 5).

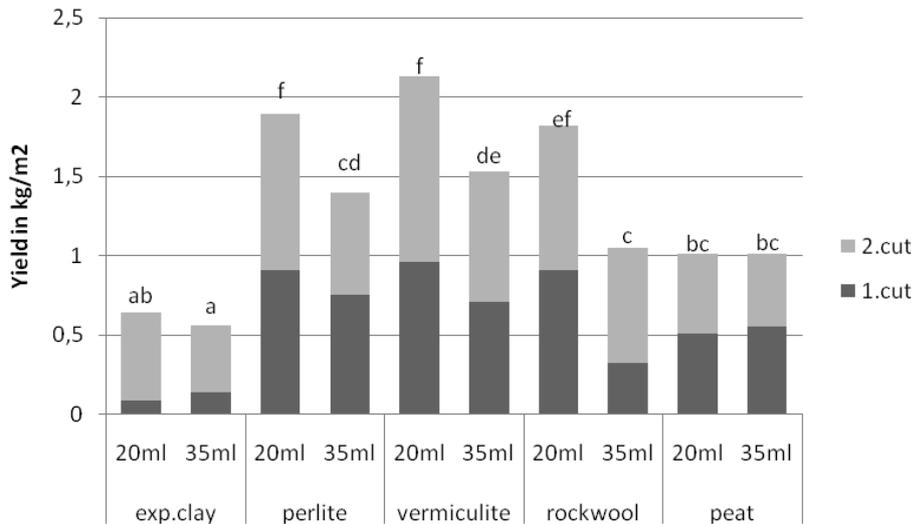


Fig. 5: Cumulative yield of rocket (kg m⁻²) in different substrates and volumes of cells (exp. 2).

Mean values followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's Multiple Range test

Nicola et al. (2004) reported that rocket plants produced more leaf area and fresh weight when growing in a mixture of peat and perlite (3:1) than in rockwool. In their experiment, rockwool media gave in general the worst results. Growing rocket in vermiculite has not previously been reported.

Growing rocket in a plug tray has proven successful. The technology is not new and has already been described by several authors (Fontana and Nicola, 2009). Nicola et al. (2005) compared a

traditional and soilless culture system with overhead irrigation to produce rocket. The soilless culture had about 75 % higher yield than rocket grown in local soil and peat. The highest yield (around 2 kg m⁻²) was obtained with the highest plant density (2.134 plants m²), which is similar to our results.

Rocket leaves grown in peat substrate had significantly higher dry matter content (19.1 %) compared to those grown in a floating system (the

average in different substrates was 13.7 %) in which the influence of the substrate on dry matter content was insignificant (Fig. 6). One of the disadvantages of plants grown in a floating system

is the higher content of water in the products. Nicola et al. 2005 reported that dry matter in rocket leaves grown in a traditional way was 15.9 %, and those grown in a soilless culture was 11.2 %.

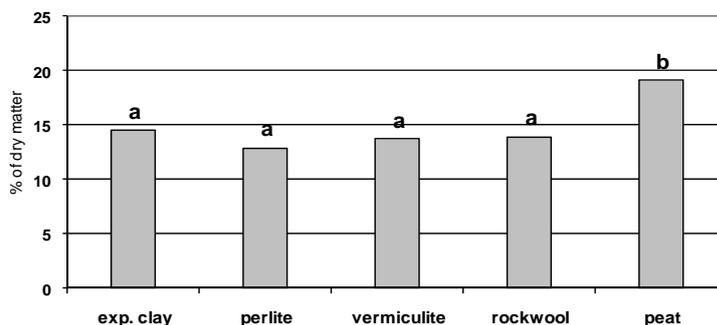


Fig. 6: Dry matter content (%) of rocket grown in different substrates.

Values followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's Multiple Range test

The nitrate concentration in rocket leaves was reduced drastically after the plants were grown for 10 days in tap water. The nitrate content in rocket leaves grown on a floating system in a nutrient solution was between 4288.4 and 6763.9 mg kg⁻¹ FW, which was significantly higher than that of plants grown in peat (2067.7-2356.3 mg kg⁻¹ FW). After 10 days of "water treatment", the nitrate concentrations in leaves dropped significantly and were between 51.9 and 633.8 mg kg⁻¹ FW in

different substrates. No nitrate was detected in leaves grown in peat (Table 3). Ferrante et al. (2003) reported that soil grown rocket usually contains a high level of nitrate - about 7.000-8.000 mg kg⁻¹ and, during the winter period, this value can easily surpass 9000 ppm. Our results thus demonstrate that the floating system offers an excellent means of reducing the nitrate content in rocket leaves.

Table 3: The mean nitrate concentration ± SE (mg kg⁻¹ FW) in the leaves of rocket grown in different substrates in plant nutrient solution until 10 days before harvest and in tap water until the day of harvest.

Substrate	No. of cells	NO ₃ -N in mg/ kg FW.		NO ₃ in mg/kg FW	
		before harvest	on harvest	before harvest	on harvest
perlite	84	1422.6±70.81	93.6±19.89	6299.9±313.61	414.7±88.18
	160	1290.2±85.09	11.7±85.09	5713.9±376.79	51.9±22.51
Exp. clay	84	1423.3±151.36	82.5±41.85	6303.1±670.36	365.2±185.4
	160	1527.3±118.86	108.5±17.90	6763.9±526.37	480.5±79.22
peat	84	532.1±64.95	0.0±0.0	2356.3±287.63	0.0±0.0
	160	466.9±102.77	0.0±0.0	2067.7±455.09	0.0±0.0
vermiculite	84	1068.1±69.39	85.9±14.30	4730.2±307.29	380.3±63.38
	160	1283.8±45.81	55.6±15.96	5685.3±202.88	246.3±129.22
rockwool	84	968.3±67.93	143.1±21.45	4288.4±300.88	633.8±94.95
	160	1478.7±67.55	122.3±21.52	6548.4±299.25	541.7±95.29

Santamaria et al. (2002) suggested that vegetables constitute the major dietary source of nitrate. Though rocket is mainly used to flavour salads, the ingestion of only 100 g of raw vegetables with a nitrate concentration of 2.500 mg kg⁻¹ FW would already lead to an intake of 250 mg NO₃.

Consuming this item alone, the amount of nitrate would exceed the ADI (acceptable daily intake) for a person of 60 kg, by 13 %. Assuming the partial conversion of nitrate to nitrite (5 %) after ingestion, the current SCF (Scientific Committee on Food) ADI for nitrite (0.06 mg kg⁻¹ body weight) would be exceeded by 247 %.

4 CONCLUSIONS

The plug tray system is suitable for growing rocket and the addition of a biostimulant can be beneficial, since plants are growing in a small amount of peat substrate. Smaller cells and a higher plant density (2.000 seeds per m²) gave better results and did not affect plant quality. Statistical analysis showed no significant differences between used substrates (rock-wool flocks, perlite (3-5 mm), vermiculite (3-4 mm)) except for the expanded clay, which turned out to be an inappropriate substrate for raising seedlings.

Since the yield of rocket leaves grown in a floating system was more than 80 % higher than the yield in plug trays filled with peat (1.01 kg m⁻² in peat; 1.83 kg m⁻² in vermiculite), farmers could be expected to prefer to use a floating system for the production of rocket, in spite of the high nitrate content. So the suggestion of replacing the nutrient solution with water for the last 10 days before harvest can be useful for the consumer and for the producer, since it would allow higher and safer consumption of rocket leaves.

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