Effect of recycled phosphorus and nitrogen blend fertilizers on the growth of viola (*Viola cornuta* L.)

A.A. Robles-Aguilar[1], O. Grunert[2], E. Meers[1] and N.D. Jablonowski[3]

[1]Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium; [2]Aphea.bio, Technologiepark 21, 9052 Zwijnaarde, Belgium; [3]Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences, IBG-2: Plant Sciences, 52428 Jülich, Germany.

Abstract

Viola cornuta **L. is an ornamental plant highly demanded in horticulture. It is becoming more and more critical for greenhouse growers to focus on sustainable production to enhance plant quality, reducing negative environmental impacts. Therefore, assessing the effect of recycled P and N sources on the growth and quality of viola could become very useful for producers and consumers. This experiment aimed to determine the optimal fertiliser composition to grow viola using recovered fertilizer products. The effect of applying the recovered products as fertilizers on the growth of viola was analyzed in a greenhouse trial under controlled conditions at the Institute of Bio- and Geosciences, IBG-2: Plant Sciences, Forschungszentrum Jülich GmbH, Germany. Well rooted viola plugs grew in a standard peat-based growing medium with a well-known fertilizer composition used by the growing media industry (PGMIX14-16-18). Using recycled sources of P and N (struvite and potassium struvite, ammonium sulfate and ammonium nitrate), 15 fertilizer combinations were prepared and tested. Two controls were used: i) a substrate without fertilizer and ii) a slow-release commercial fertilizer (Osmocote 15+9+11+2 MgO and trace elements). Triple-superphosphate (TSP) and potassium sulfate (K_2SO_4) were used together with the recycled nutrients to fulfil the major macronutrient requirements of viola. Plants treated with blends containing the recovered ammonium nitrate showed healthy growth and optimal plant N concentrations. In contrast, combinations using the recovered ammonium-sulfate resulted in an unacceptable increase in electrical conductivity affecting the germination and growth of the plants. The combination of struvite recovered ammonium nitrate and recovered potassium-sulfate has the best chemical composition with non-significant differences in the biomass from the positive controls. Our results indicate that fertilizer blends with recovered fertilizer products (P as struvite and N as ammonium-nitrate) can substitute the use of mineral fertilizer blends to grow ornamental plant species as viola.**

Keywords: struvite, ammonium nitrate, ammonium sulfate, recovered nutrients, greenhouse flowers

INTRODUCTION

The fertilizer industry produces a lot of different fertilising compounds for soil and growing media-grown crops, mainly containing guaranteed contents of nitrogen (N), phosphorus (P) and potassium (K). Still, nowadays, abundant use of these synthetic fertilizers is standard practice. Leached irrigation water from soil-based systems and discharges from soilless cultivation contain high concentrations of nutrients (primarily P and N), which is one of the significant sources of nutrient losses to aquatic systems (Carpenter, 2005; Kalkhajeh et al., 2017). Due to public pressure, environmental pollution has recently become a subject of attention for the governments of the European Community. Moreover, a circular bioeconomy system that closes nutrient loops and gives waste streams a new life is an exciting approach in agriculture but also for the horticultural business.

Besides the consequent environmental problems of their losses to the environment, mineral fertilizers have other drawbacks. There is a complete EU import dependency on P from exhaustible mineral deposits, a partial dependency on fossil fuels for the synthesis of N-fertilizer via the Haber Bosch process (Galloway et al., 2008). Following the idea of a closed nutrient loop, recycled nutrients obtained after the biomass conversion to bioenergy and further treatment of the by-product can be applied as fertilizers (Vaneeckhaute et al., 2013). The recovered fertilizers can also be stable and free of contaminants, thus reducing the need for synthetically produced fertilizers (Haraldsen et al., 2011).

In this experiment, two alternative sources of N recovered from manure (ammonium-nitrate and ammonium-sulfate) were used to grow viola. Manure derived nitrogen fertilisers could be used as chemical nitrogen fertilisers as defined by the Nitrates Directive (91/676/EEC). Also, two promising examples of an alternative source of P were investigated in this study, namely struvite ($MgNH_4PO_4 \cdot 6H_2O$) and potassium-struvite ($MgKPO_4.6H_2O$), both recovered from wastewater. Struvite has previously been proposed as a slow-release fertilizer (Rahman et al., 2014). The slow release of the nutrients can ensure a steady nutrient supply for plants, promoting root development in the early stages and improving nutrient-use efficiency (Robles-Aguilar et al., 2019). Slow-release fertilizers in granulated form, like Osmocote-fertilizer used in this experiment as control, are widely used in modern horticulture.

The proper management of mineral nutrition is a crucial factor in determining the ornamental value of the plants. Growers must know how nutrient uptake relates to viola growth. An excessive fertiliser application could result in excessive seedling size, nutrient toxicity and environmental contamination (Zhang et al., 2012). The application rate applied can influence the shoot dry weight, branch number and length, total leaf area and flower number (Gadagi et al., 2004). Therefore, investigating the effect of applying recycled nutrients to grow viola in its performance is crucial before their recommendation as fertilizers in the new EU fertilizer regulation frame.

This experiment aimed to assess the optimal fertiliser and growing medium composition using recycled nutrient streams to grow viola under greenhouse conditions. To do that, we blended the different recovered nutrients with a nitrogen-phosphorus-potassium (N-P-K) ratio suitable for horticultural crops.

MATERIAL AND METHODS

Recovered nutrients and growing medium characterization

In Table 1, the codification and origin of the recovered nutrients and positive controls that were blended into fertilisers, according to the plants' requirement, are listed. A complete characterization of the different recovered products (ammonium-struvite, potassium-struvite, ammonium-nitrate and ammonium-sulfate) and growing media samples have been carried out in terms of macro/micronutrients (N, P, K, Ca, Mg, SO_4, Fe, Mn, Na, Cl). All these specific analyses were performed in triplicate to ensure reliable and reproducible results. The NH_4^+-N content was determined according to Directive 77/535/EG method 2.1, the P_2O_5 in mineral acid was determined according to directive 77/535/EG method 3.1.1 employing Inductive Coupled Plasma (ICP). The total MgO content was extracted according to 89/519/EG method 8.1, and further determination was conducted with an ICP. The samples were analyzed for total K contents by ICP-OES. The main chemical characteristics of the different recovered nutrients are shown in Table 2.

The final growing medium used was an organic substrate (GB, Grow Bag, Greenyard, Belgium), consisting of a mixture of white peat [80% v/v] and coconut fiber [20% v/v]. According to the Belgian legislation (KB 13th of March 2013), the pH for growing media should range between 4.5 and 7.0±0.3 and exhibit an electrical conductivity below 750 $\mu S\ cm^{-1}$.

Fifteen fertilizing blends were prepared using the different recovered nutrients. Additional controls were used in the test: no fertilizer, a fast release blend based on ammonium-nitrate, triple-superphosphate (TSP) and potassium-sulfate, and a control with a commercial slow-release fertilizer (Osmocote 15+9+11+2 MgO and trace elements). Triple-superphosphate (TSP) and potassium-sulfate (K_2SO_4) were used in the fertilizer blends to compensate for chemical deficiencies. Moreover, TSP and K_2SO_4 have high solubility and are readily taken up by the plants. The blends had an N-P-K ratio of 1:0.26:0.61, and the concentration was 900 g N m^{-3}, 235 g P m^{-3} and 548 g K m^{-3}. The overview of the different combinations of recovered nutrients and standard fertilizers (CRF) for the growth of viola are shown in Table 3. Units are in kg m^{-3}.

Table 1. Codification of the different recovered nutrients and fertilizers used.

Code	Description
A	Potassium-struvite plant in the Netherlands (www.smg.nl)
B	Potassium-struvite Recovered (provided by Lequia)
C	Ammonium-struvite (Product recovered from digested manure (provided by Lequia)
D	Ammonium-struvite WWTP (Waste water treatment plant) (provided by Lequia)
E	Ammonium-struvite (Struvite recovered from digested manure (provided by Lequia)
F	Ammonium-nitrate (provided by BOKU)
G	Ammonium-sulfate (provided by BOKU)
H	Triple-superphosphate (provided by Greenyard)
I	Potassium-sulfate (provided by Greenyard)

Table 2. Overview of chemical composition of the different recovered nutrients.

Recovered nutrients & fertilizers	NH_4^+-N (%)	NO_3^--N (%)	P (%)	K (%)	Mg (%)	S (%)
A[a]	0.00	0.00	5.81	7.34	4.56	0.0
B	0.00	0.03	14.11	0.30	10.63	0.0
C	6.64	0.00	13.18	0.00	10.25	0.0
D	4.97	0.00	5.63	2.45	7.76	0.0
E	8.24	0.00	8.96	0.50	12.60	0.0
F	7.89	8.63	0.00	0.00	0.00	0.0
G	19.47	0.00	0.00	0.00	0.00	0.1
H	0.00	0.00	20.08	0.00	0.00	0.0
I	0.00	0.00	0.00	43.15	0.00	18.0

[a]Letters indicate recovered nutrient or fertilizer used (see Table 1).

Table 3. Overview of the different combinations of recovered nutrients and standard fertilizers (CRF) for the growth of viola.

Combination	A[a]	B	C	D	E	F	G	H	I
1	-	-	-	-	-	5.5	-	1.2	1.3
2	-	-	-	-	-	-	4.6	1.2	1.3
3	-	1.7	-	-	-	5.5	-	-	1.3
4	-	1.7	-	-	-	-	4.6	-	1.3
5	4.1	-	-	-	-	5.5	-	-	1.0
6	4.1	-	-	-	-	-	4.6	-	0.6
7	-	-	-	1.8	-	4.7	-	-	1.3
8	-	-	-	1.8	-	-	4.6	-	1.3
9	-	-	4.2	-	-	4.2	-	-	1.3
10	-	-	4.2	-	-	-	3.6	-	1.3
11	-	-	-	-	2.6	4.1	-	-	1.3
12	-	-	-	-	2.6	-	3.6	-	1.3
13	0.7	-	-	1.5	-		4.1	-	0.08
14					Osmocote (6 kg m^{-3})				
15					Without fertilizer				

[a]See description of the letter code in Table 1.

Experimental setup

Plants were harvested at the onset of flowering (five weeks after planting). Five replicates of a total of 15 treatments were tested. The volume of the pots was 1 L with a final weight of 250 g. For the peat-based growing medium preparation, 33.75 kg of the organic growing medium was mixed with 0.375 kg of lime to raise the pH to the desired value of pH 6. Viola seedlings were transplanted in the middle of the pot. The fertilizer blending process in the organic growing medium is shown in Figure 1 and was as follows: i) Preparation of the different recovered nutrients, ii) blend preparation by weighting the corresponding amount of the recovered fertilizers, iii) blend grinding, iv) labeling of the combinations, v) mixing the blends into the organic growing medium, and vi) liquid ammonium-sulfate addition in the necessary amounts.

Figure 1. A recovered fertilizer products, F1B preparation of the different combinations, F1C all the 15 combinations before application to the substrate.

Plant monitoring

The phenological stage of the viola plants, nutrient deficiency symptoms (color and appearance of leaves), and flowering time were recorded manually weekly. Soil water content, temperature, and electrical conductivity (EC) were measured twice per week after watering using a portable system to ensure that the pots were kept at approximately 50% water holding capacity.

Harvesting was done using secateurs. Plants were cut below the soil surface. Plant fresh weight was measured by balance (Melter Toledo XS205) directly after harvesting. For further analysis, the biomass samples were dried at 60°C until constant weight. Nutrient contents of dried plant samples were determined by elemental analysis via inductively coupled plasma optical emission spectrometry (ICP-OES) (VarioELcube, Elementar). Soil pH was determined using standard electrodes (Hanna Instruments pH 209 pH meter), using 1:5 distilled water extract at 20°C.

Statistical analyses

Statistical analyses were performed using the statistical program R.2.16.3 (R: A Language and Environment for Statistical Computing (2012), http://www.R-project.org/). Measurements were compared with one-way analysis of variance (ANOVA). Data were calculated as arithmetic means ± standard error of the mean of the indicated replicates.

RESULTS AND DISCUSSION

Chemical analyses of the different growing media blends

The recovered nutrients and mineral fertilizer used in this experiment (Table 2) were blended into different combinations according to the viola plants' nutritional requirement and common practice in the growing media industry. The blends were chemically analyzed for plant-available nutrients, not for the total nutrient content. Table 4 shows the concentrations of different nutrients in the growing medium after the addition of the respective blend and their effects on the electrical conductivity and pH.

Table 4. Overview of the chemical composition of the different combinations.

Combination	pH (H_2O)	EC (µS cm^{-1})	NO_3-N (mg L^{-1})	NH_4^+-N (mg L^{-1})	P (mg L^{-1})	K (mg L^{-1})	Ca (mg L^{-1})	Mg (mg L^{-1})	SO_4 (mg L^{-1})
1	4.9	713	44	61	226	718	900	205	917
2	5.1	1800	8	690	170	540	1028	233	1945
3	5.3	528	0	12	88	530	858	285	902
4	5.3	1330	0	492	85	428	918	300	1761
5	6.5	708	44	75	357	928	878	905	997
6	6.5	2030	8	838	302	723	1085	845	2191
7	5.8	652	26	111	256	693	873	398	928
8	5.7	2200	0	897	236	690	1013	410	2215
9	6.0	730	17	123	335	745	945	470	965
10	5.8	1760	0	667	317	748	980	495	1922
11	6.2	820	26	208	474	858	903	700	956
12	6.2	2100	7	834	511	933	965	710	2033
13	6.1	270	0	68	260	193	950	563	269
14	5.1	156	0	7	16	105	773	218	235
15	5.2	137	0	5	10	90	705	200	213

Blend 14 (Osmocote 15+9+11+2 MgO and trace elements) and blend 1 (mineral fertilizers) were used as a positive control. Blends with even numbers (2 to 12) contained ammonium-sulfate. Odd-numbered blends (3 to 11) had ammonium nitrate (see Table 2 for the detailed composition of each combination).

Violaceae prefer a pH between 5.5 and 6.5, but only combinations 5-13 were according to this pH. However, very high electrical conductivities observed in the blends 2, 4, 6, 8, 10, 12 that had in common the addition of ammonium sulfate. This resulted in permanent damage of the plants, showing that the electrical conductivity was the most important factor for a good plant performance rather than the pH. When the electrical conductivity is too high, plants suffer osmotic stress and, consequently, decreased water uptake and even chemical burning of the roots. We assume that the high electrical conductivity observed when ammonium-sulfate was included in the blends (which increased up to 2,000 µS cm^{-1}) might be caused by the high ammonium concentrations rather than the sulfate concentrations. This is in line with previous studies where the use of ammonium-sulfate impacted the electrical conductivity (Martikainen, 1985). Blends with ammonium-sulfate had an ammonium concentration near 900 g N m^{-3}; however, blends with ammonium-nitrate had an ammonium concentration around 100 g N m^{-3}.

In all the blends, the ammonium concentration was higher than the nitrate concentration. Ideally, the nitrate/ammonium ratio should be 0.6. However, in our blends, the ratio was much lower than 0.6. Combinations, where we found the requested 900 g N m^{-3}, showed that the electrical conductivity was unacceptably high. That means that combinations 6, 8 and 12 were not suitable for good plant growth. Combination 7 and 13 had the requested amount of 35 g P m^{-3}; however, all other combinations had more significant P contents. It seems that combination 7 (blend of ammonium-struvite, ammonium-nitrate and potassium-sulfate) had the best chemical composition, followed by combination 13 (blend of potassium-struvite, ammonium-struvite, ammonium-sulfate and potassium-sulfate.

Plant performance

Fertilizer companies already use struvite as an additive or substitute of raw material in standard fertilizer production technology (Li and Zhao, 2002). Moreover, the results of a previous study highlight the suitability of most struvites to enter the EU fertilizer market (Muys et al., 2021). Nevertheless, the additional use of ammonium and potassium to formulate a balanced NPK fertilizer together with struvite is necessary. For struvite to be part of a fertilizer blend suitable for green horticulture or sustainable agriculture, it needs to be combined with other nutrients, ideally also from a biobased origin. In this experiment, the extra ammonium was added in the form of ammonium nitrate or ammonium sulfate. Blends 2, 4, 6, 8, 10, 12 had in common the addition of ammonium sulfate. Plants growing in those blends died due to the high electrical conductivity of the growing medium (Figure 2). Plants growing with ammonium nitrate grew healthy (Figure 2). The negative effect of adding ammonium sulfate was observed in the color and size of leaves, as well as in the significantly reduced plant growth. Plants were not able to flower.

Figure 2. From left to right, the appearance of viola plant growing under combination 6, 12 (treated with ammonium sulfate) and combination 11 (with ammonium nitrate).

The biomass of viola plants treated with different fertilizer blends was compared at the end of the experiment (flowering stage of the control plant) (Figure 3). Blends 5, 7, 9 and 11 are those with clean recovered struvite (laboratory grade or pilot-scale). There were no significant differences in biomass among them. Blend 1 was the positive control made from commercial mineral fertilizers. Blend 3 (containing potassium-struvite) resulted in lower biomass and a lower concentration of ammonium and phosphorus inside the plant's tissue; however, the leaf nutrient concentrations were not lower than combination 14 (Osmocote-15+9+11+2 MgO and trace elements), which had a higher biomass production.

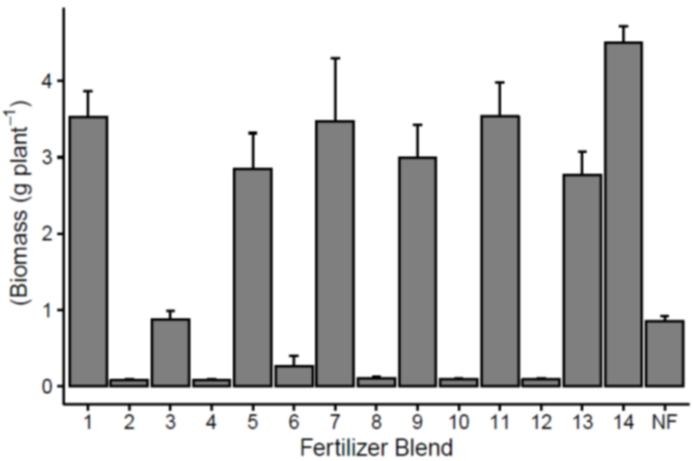

Figure 3. Average biomass (g dry weight plant^{-1}) (n=7) of viola plants for each fertilizer blend. Numbers refer to each specific blend that combines different recovered nutrients applied at the same final dose. Blend 1 is a positive control, blend 14 is Osmocote, a commercial slow-release fertilizer, and NF indicates "no fertilizer".

The concentrations (mg 100 g^{-1}) of P, K and Mg were analyzed in plant tissues of viola for the blends that allowed the plant has a healthy growth at the end of the experiment. There were no significant differences in the plant concentration of P, Mg and K among the different blends in this case. The N analyses were done in dried plant tissues harvested following growth on all blends. It showed that combinations with ammonium-sulfate (even combinations from 2 to 12) had N concentrations considered excessive or toxic (from 5.8 to 6.8% DW). Plants treated with blends containing the recovered ammonium nitrate showed healthy growth and resulted in optimal concentrations of N in mature leaf tissue. The blends with struvite and ammonium-nitrate (5, 7, 9 and 11) and the positive control blend with Osmocote (15+9+11+2 MgO and trace elements) (14) and mineral fertilizer (1) had a sufficient or normal concentration (from 2.5 to 3.4% DW). The blend with no fertilizer addition and blend with K-struvite (blend 13 and 3) showed low concentrations of nitrogen (<2.5%).

Besides the positive controls, it seems that blend 7 (combination of struvite from wastewater, recovered ammonium-nitrate and potassium-sulfate) had the best chemical composition and resulted in non-significantly different biomass from the positive controls. Fertilizer blends with recovered products (P as struvite and N as ammonium-nitrate) can substitute the use of mineral fertilizer blends to grow ornamental plant species such as viola. Still, the preparation of green fertilizer blends requires exhaustive control and knowledge of every product added. The effect of struvite as a P source to add into a fertilizer blend needs to be further evaluated to enable a successful combination with other recovered materials. Specific combinations will significantly affect soil chemical properties and, therefore, plant growth, and those results might be species dependent.

CONCLUSIONS

From the results obtained, it can be concluded that we can use different recovered nutrients as potential fertilizers that can be blended into a growing medium. As observed, mixing the different blends of recovered nutrients into a growing medium impacts the chemical composition of the final product. The fertilizer blend influenced the pH, electrical conductivity, and total nitrogen, phosphorous and potassium content.

In previous studies, it was shown that struvite applied alone as fertilizer might not efficient; however, it has been demonstrated that mixing it with other recovered sources of N can positively affect plant performance. Struvite might also increase the pH of the soil; therefore, another advantage might be the chemical equilibrium in the growing medium when it is applied as a blend with other nutrients such as ammonium nitrate.

The use of ammonium-sulfate impacted the electrical conductivity causing osmotic stress, resulting in a decreased water uptake and chemical burning of the roots. A blend of ammonium-struvite, ammonium-nitrate and potassium-sulfate (combination 7) had the best chemical composition, followed by a blend of potassium-struvite, ammonium-struvite, ammonium-sulfate and potassium-sulfate (combination 13). Interestingly combination 13 had lower nitrogen levels than combination 7; however, there was a potential slow-release effect of this fertilizer blend resulting in better chemical conditions in the substrate in the long run.

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