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Comparison between Chemical Fertilization and Integrated Nutrient Management: Yield, Quality, N, and P Contents in *Dendranthema grandiflorum* (Ramat.) Kitam. Cultivars

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Abstract: To assess the effects of a new integrated nutrient management protocol on yield and cut stem quality, root morphology, N accumulation, nitrogen utilization efficiency (NUE), and P content in tissue, a biennial (2011 and 2012) chrysanthemum cut flower cultivation was carried out. In both years, two nutrition management (CNM: conventional NM and INM: integrated NM) treatments and two *Dendranthema grandiflorum* (Ramat.) Kitamura cultivar ("White CV_1" and "Yellow CV_2") treatments were compared. The treatments were arranged in a split-plot design with three replicates. CNM was fertilized using a recommended dose fertilization of mineral NPK; INM treatment was fertilized using a half dose (50%) of CNM plus a combined usage of N organic fertilizer, seaweed extract (*Ascophyllum nodosum*), and microrganism consortium (*Glomus* sp. and *Bacillus* sp.). Yield at harvest (+19%), number of leaves (+33%), leaf area (+46%), number of flower heads (+27%), and total aboveground dry weight (+40%) were significantly increased by the INM application compared to the control. In terms of the root system, the increase was evident in terms of length (+174%), volume (+167%), projected area (+166%), and surface area (+165%), tips (+175%), forks (+285%), and crossings (+464%). The greatest N accumulation, in both years, was registered by INM treatment at harvest: +94% in 2011 and +55% in 2012. Differences in the NM were evident in the NUE, which was highest in CNM (on average 162) compared to INM (on average 142). In both years the P content in above-ground chrysanthemum tissues was in the order of head > leaves > stems, which was maintained in both INM and CNM treatments. A higher yield (138 stems m^{-2}) was obtained in "CV_2 Yellow" compared to "CV_1 White" (120 stems m^{-2}). Based on our findings, applying INM to chrysanthemum improves yield, cut flower quality, and plant nutrient uptake, in an agro–environmentally sustainable way. A basic economic analysis on fertilizers, cost gross production, and takings difference obtained, was carried out.

Keywords: N organic fertilizer; seaweed extract; mycorrhizal inoculants; phosphate-solubilizing microorganisms; biofertilizers; microorganism consortium

1. Introduction

Fertilization is essential for optimizing crop productivity [1]. Mineral fertilizers, particularly nitrogen (N) and phosphorus (P), are important for plant nutrition [2,3]. However, when used in overly large doses they are also a potential source of environmental pollution [4–6]. Nutrient overapplication has introduced major challenges in terms of soil infertility [7], N and P runoff [8,9], environmental degradation [10], and climate change [11,12].

Today there is an increasing need for a balanced fertilization strategy, minimizing the use of mineral fertilizers to enhance both crop production and quality and nutrient uptake under low input conditions [13]. Mineral fertilizers can be replaced by organic fertilizers [14], plant biostimulants [15], and beneficial microbial inoculants [16].

Possible interventions in conservation agriculture include the combined use of inorganic and organic fertilizers, as well as biostimulants and biofertilizers in order to increase a balanced nutrient supply [17]. Integrated nutrition management (INM) focusing on the optimization of the biological potential improves fertilizer input efficiency, reduces environmental risks, and increases crop productivity, through root/rhizosphere management [18].

In terms of biostimulants, seaweed extracts are used in sustainable agriculture in order to increase growth, quality, and shelf life [19–21]. Many studies have demonstrated the positive effects of seaweed extracts on a wide range of crops, including cereals [22], ornamental and flowering plants [23], vegetables [24], and field crops [25].

Biofertilizers are also an important alternative source of plant nutrients and are key components of integrated nutrient management in crop production. The use of microbial inoculants with P solubilizing activities in soils is an environmental-friendly alternative to further applications of chemical-based P fertilizers [26,27]. Various studies have examined the potential of different bacterial species to solubilize inorganic phosphate compounds. *Bacillus* spp., and in particular *B. subtilis* and *B. megaterium*, may provide the available forms of P to plants, thus considerably improving plant growth performance [28–31].

Other microbial inoculants, such as arbuscular mycorrhiza fungi (AMF), increase the P availability through the expansion of the root surface area by extraradical hyphae formation [32,33].

The various benefits of AMF include increased growth and nutrient uptake (especially N, P, and K) and crop yields [34–38]. The AMF also produce a heat-stable protein called glomalin, which is a glycoprotein that enhances soil aggregation and helps in soil carbon sequestration. Together, glomalin and mycorrhizal hyphae lead to a stable soil structure.

The combined use of N organic fertilizers, biostimulants, and biofertilizers is therefore a new approach that has not been widely investigated in ornamentals, which entails developing many efficient formulations with low mineral inputs, with positive impacts on crops and environment.

Chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura) is a commercial cut flower, belonging to the *Asteraceae* family, with nearly 200 cultivars. It is one of the top ten elite cut flowers globally, due to its different shapes, dazzling colors, varying sizes, and excellent vase life. In Italy, where our research was carried out, there is a considerable demand in both domestic and export markets.

Extracts of the plants (stems and flowers) have many potential medicinal properties, including anti-HIV, antibacterial, and antimycotic [39]. N, P, and K play a vital role in the production of good quality flowers. N is essential for the creation of biomass as well as for the biosynthesis of enzymes in chrysanthemum leaves [40].

The N requirements of chrysanthemums are known to be higher during the first seven weeks of growth, and during this time, deficiencies are more difficult to correct than in later stages of development [41]. Chrysanthemums take up N at an even rate from the time of planting until the flower bud differentiation stage where after N uptake decreases [42]. In chrysanthemums, the need for P is significantly lower than that of nitrogen [43]. K requirements are high, and its presence in the plant favorably affects growth and flower color [44].

To the best of our knowledge, there are no available data on how the INM system based on mineral and organic N fertilizers, seaweed extracts, plus a consortium between AMF (arbuscular mycorrhiza fungi) and PSB (Phosphate solubilizing bacteria), affects yield and quality in chrysanthemums.

The goal of this research was to evaluate the effects of an innovative INM compared to conventional nutrient management, in chrysanthemum cut flower cultivation, on: (1) yield and cut stem quality, (2) N concentration, accumulation, and utilization efficiency and P uptake, (3) root architecture, and (4) soil fertility.

2. Materials and Methods

2.1. Experimental Conditions

Two field experiments were carried out in 2011 and 2012, from August to December, at a floricultural farm located in Sannicandro di Bari (southern Italy: 40°59′24″ N, 16°47′01″E, 181 m a.s.l.). The local climatic conditions are characterized by hot dry summers and mild rainy autumns and winters, typical of the Mediterranean climate. During the plant growth period under natural photoperiod, the mean air temperature was 17.2 °C and 18.2 °C in 2011 and 2012, respectively; minimum air temperature was 3.7 °C in December 2011 and 5.4 °C in December 2012; maximum air temperature was 32.4 °C in August 2011, and 32.6 °C in August 2012.

Seasonal chrysanthemum cuttings (Minstreel Serie, Straathof Plants BV, The Netherlands), ideal for blooming from November to late December, were obtained from a local commercial propagator, with the following characteristics: stem length, 11.6 cm; number of leaves, 8; leaf area, 81.1 cm^2; plant fresh weight, 3.1 g; and plant dry weight, 0.3 g. In both years, plants were transplanted on 6 August into an uncovered tunnel. In the first week of October in both years, the tunnel was covered by ethylene vinyl acetate (EVA) film.

The main soil characteristics (taken from 0 to 25 cm depth) are described in Table 1. Soil pH was determined with a pH meter (P9991, Hanna Instruments, Italy) in a settling suspension on a 60 g sample mixed with 150 mL of deionized water, after shaking for 60 min at room temperature (22 °C). The soil used for our experiment was slightly sub-alkaline (pH = 7.34, near to neutrality) and it was representative of Apulian soils in which chrysanthemum was cultivated with remarkable production results. Chrysanthemum plants generally grow with a pH ranging between 6 and 7.2 [45].

The electric conductivity (EC) was measured on water extract (1:5 *v/v*) with a conductivity meter (HI 4321, Hanna Instruments, Italy). Soil organic carbon (SOC) was determined by wet oxidation. Based on USDA classification, experimental soil was classified as clay loam soil. Experimental soil was moderately provided with organic matter and CEC was also classified as moderate [46].

The total Kjeldahl N (TKN) was measured using 1 g samples of both growing media and plant tissues using the Kjeldahl method after 96% H2SO4 hot digestion. Total phosphorus was determined (P) by the colorimetric molybdovanadate phosphoric acid method. Exchangeable K, Ca, and Mg were determined using 0.2 g of dry sample (105 °C for 24 h) after acid digestion in a microwave oven (CEM Mars Xpress, Cologno al Serio, IT). Substrate digests were filtered, diluted, and analyzed by atomic absorption spectrometry (Perkin-Elmer Aanalyst 200, Waltham, MA, USA). The analyses were carried out in triplicate.

The soil was sandy clay with a slightly alkaline pH of 7.3 (IUSS), EC of 1.77 dS m^{-1}, and moderately high CEC (cation exchange capacity) of 23.8 Meq 100 g^{-1}.

Table 1. Initial soil physico–chemical characteristics (mean ± standard error). Data are the means of three samples.

Parameter	Value
pH (soil:H_2O ratio 1:2.5)	7.34 ± 0.2
Electric Conductivity (EC) (soil:H_2O ratio 1:5) (dS m^{-1})	1.77 ± 0.08
Cation exchange capacity (CEC) (Meq100g^{-1})	23.8
Sand (%)	52 ± 3
Silt (%)	16 ± 2
Clay (%)	32 ± 4
Total C (g kg^{-1})	12.54 ± 1.2
Organic matter (g kg^{-1})	21.61 ± 1.9
T Kjeldahl-N (g kg^{-1})	1.15 ± 0.13
P (mg kg^{-1})	71.25 ± 0.9
Available K (mg kg^{-1})	579 ± 10.1
Available Ca (mg kg^{-1})	2160 ± 22
Available Mg (mg kg^{-1})	495 ± 31

2.2. Treatments and Experimental Design

In both years, four treatments in total consisting of two nutrition management (NM) and two *Dendranthema grandiflorum* (Ramat.) Kitamura cultivar (CV) were compared as follows:

1. Conventional NM (CNM or control) and integrated NM (INM);
2. "White CV_1" and "Yellow CV_2".

Treatments were carried out using a split-plot design with three replicates, with NM as the main plot and CV as the subplot. The surface of each experimental plot measured 2.2 m^2.

CNM treatment was applied through a fertigation system using a recommended dose of mineral NPK: 17 g m^{-2} N, 16 g m^{-2} P_2O_5, and 17 g m^{-2} K_2O plus microelements, starting one week after transplanting, every week, for 12 weeks, the last one during the second week of November (flower bud differentiation).

INM treatment was applied by fertigation at a half dose (50%) of CNM plus a mixture of an N organic fertilizer, seaweed extract and microorganism consortium as shown in Table 2, starting from transplantation. Commercial products were applied at the manufacturer's recommended rates.

NPK doses added with INM fertilization were the following: 11.8 g m^{-2} N, 8 g m^{-2} P_2O_5, and 12 g m^{-2} K_2O. N organic fertilizer added to the mineral NPK dose mentioned above, was derived by hydrolyzed animal epithelium, beet molasses extract, and brown seaweed extract.

In the second year, the same treatments were repeated.

In both years, the growing density was 34 plants m^{-2}.

Table 2. Combined use of N organic fertilizer, seaweed extract, and microorganism consortium applied in two experiments (2011 and 2012).

Type and Commercial Product (*)	Content	Total Rate (g/100m^2)	Weeks of Applications (*n*) (**)
N organic fertilizer (Euroflorid)	N = 5% *w/w* (N = 0.5 g/m^2)	75	I, II, III, IV, V
N organic fertilizer (Amminostim-bio)	N = 6% *w/w* (N = 0.9 g/m^2)	25	VI, VII, VIII
Seaweed extract (Euroalg)	*Ascophyllum nodosum* (L.) Le Jol. 32% *w/w*, N = 1.5% *w/w* (N = 0.9 g/m^2), K20 = 5.0% *w/w*	58	I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII
Microrganism consortium (Micotric L)	*Glomus mosseae* and *G. intraradices* (2 spores g^{-1}) and *Bacillus megaterium var. Phosphaticum* 6 × 107 CFUs g^{-1}	90	I, II, III, IV
Microrganism consortium (Europlus)	*Glomus mosseae* (2 spores g^{-1}) + *Trichoderma viride* and *Bacillus megaterium var. Phosphaticum* 6 × 107 CFUs g^{-1}	162	I, II, III, IV

(*) by Eurovix SpA, Entratico (BG), Italy; (**) fertigation from transplant (week 1) to flower bud differentiation (week 12).

During the experiments, all field management procedures (e.g., irrigation and pest control) were the same among treatments. The irrigation system was a micro drip; each drip line was placed between two plants rows with an emitter (pressure compensating) discharge rate of 2.0 L h^{-1}. Except for nutrition, production was carried out using the grower's standard practices. Cut flowers were harvested when 50% of flower heads had opened.

Morpho–biometric measurements were carried out at the Department of Agro–environmental and Territorial Sciences (DISAAT), University of Bari, Italy. Plants were sampled for aboveground and ground biomass and N and P content (%) at 55, 93, and 131 DAT (days after transplant) in both growing periods.

The growth and yield observations were recorded on twelve randomly selected plants from each treatment.

In both years, at harvest (second ten days of December), the soil was washed from roots, and aboveground plants were divided into stems, leaves, and flowers, which were oven dried at 70 °C until they reached a constant mass to measure the respective dry weights.

At flower harvest, the measurements involved: yield (secondary branches = stems m^{-2}), stem length (cm), inflorescence (*n* and diameter, cm), leaves (*n*), and leaf area (cm^2), Chlorophyll SPAD

(Single-photon avalanche diode) index (Minolta Chlorophyll Meter SPAD-502), dry and fresh weight of leaves, stems, inflorescences, roots, and whole plants. In order to perform root morphology analysis, only in the first year, roots were spread out, washed, and then scanned at 300 dpi on an HP DeskScan II scanner (HEWLETT PACKARD C6261A, Palo Alto, CA, USA). Root analysis was performed using the WinRHIZO® image analysis system (V 4.1c Régent Instruments, Quebec, Canada); measurements involved total root length, average root diameter, projected and surface area, tips, forks, and crossings.

The total Kjeldahl N (TKN) content was measured, both in the first and second years, using 1 g samples of foliar and radical tissues, using the Kjeldahl method after 96% H_2SO_4 hot digestion. On the other hand, the P-Olsen measurement was only used during the first year.

Nitrogen utilization efficiency (NUE) was estimated by the ratio of dry biomass to plant N accumulation at harvest.

2.3. Economic Analysis

A basic economic analysis about fertilizer costs (for CNM and INM), gross sealable production, and profit raised was developed.

2.4. Statistical Analysis

The data were analyzed by three-way ANOVA using CoStat-Statistics Software. Treatment means were separated with Student–Newman–Keuls (SNK) ($p \leq 0.05$).

3. Results

The overall aims of this research were to evaluate the effects of an innovative INM compared to CNM, in a biennial chrysanthemum cut flower cultivation, on (i) yield and cut stem quality; (ii) root morphology; and (iii) N accumulation, NUE, and P content in plant tissue.

The main effect of NM was found to be highly significant for most of the parameters investigated.

Yield at harvest, as determined by the harvestable number of cut stems per plant (Table 3), increased significantly in INM (140 stems m^{-2}, +19%) compared to those under CNM (118 stems m^2).

Genotype influenced marketable yield: CV_2 registered the highest value (138 stems m^{-2}), surpassing that of CV1 by 15% (on average 120 stems m^{-2}).

Concerning the Y factor, the yields were not different (133 stems m^{-2} on average).

Table 3. Main effects of nutrient management, cultivar on yield, stem height, leaf number, leaf area, chlorophyll index, and number of flower heads in chrysanthemum plants over the two years of application.

Treatments	Yield (no. stems m^{-2})	Stem Height (cm)	Leaves (no./plant)	Leaf Area (cm^{-2})	Chl. Index (SPAD)	Flower Heads (no. $stems^{-1}$)
Nutrition Management (NM)						
Conventional NM (CNM)	118a	103	60b	2064b	44.8	6.6b
Integrated NM (INM)	140b	106	80a	3017a	46.9	8.4a
Significance	*	ns	*	**	ns	**
Cultivar (CV)						
CV 1	120b	105	64	2416	45.1	8.2
CV 2	138a	104	76	2665	46.6	6.9
Significance	*	ns	ns	ns	ns	ns
Year						
2011	133	116a	82	2795	46.6	8.1
2012	133	92b	59	2486	45.1	7.3
Significance	ns	*	*	ns	ns	ns
Interaction						
NM × CV	*	ns	ns	ns	ns	*
NM × Year	ns	ns	ns	ns	ns	ns
CV × Year	ns	ns	ns	ns	ns	ns
NM × CV × year	ns	ns	ns	ns	ns	ns

Different letters within each column indicate significant differences according to SNK test ($p \leq 0.05$). NS not significant * $p < 0.05$ and ** 0.01, indicate level of significance.

Table 3 also shows the influence of the treatments on the commercial quality parameters of the cut stems at harvest. The stem height is an important parameter that is used for the classification of the stems for marketing and sales, and in fact, customers often prefer flowers with a longer stem. Stem height was not found to be significant between both NM and CV treatments; however, it showed significant differences among Y: in 2012 it was 20% lower (92 cm) than 2011 (116 cm).

Regarding the number of leaves per plant, the INM treatment led to an increase of 33% (80 leaves/stem) compared to CNM (60 leaves/stem); in 2012 the number of leaves (59) showed an average decrease of 28% (82 leaves) compared to 2011.

The INM treatment also showed a significant increase of 46% (3017 cm^2) in the leaf area value compared to CNM (2064 cm^2).

The chlorophyll index SPAD was not significant in any of the treatments.

The number of flower heads per stem was highest (8.4) with an increase of +27% when plants were treated with INM, compared to CNM (6.6). No differences were found between the cultivars and years.

Concerning the leaves, stems, flower heads, and aboveground dry weight, Table 4 shows the statistically significant differences in favor of INM compared to CNM. Leaf values showed a 38% increase, stem value a 37% increase, and flower heads a 55% increase, which were reflected in the increase of aboveground dry weight (+40%). No difference was found between the cultivars.

Table 4 also shows that 2011 had the highest aboveground dry weight value, which decreased to 25% during 2012.

Table 4. Main effects of nutrient management, cultivar on dry weight of various organs, and above-plant on chrysanthemum over the two years of application at harvest time.

Treatments	Dry Weight (g)			
	Leaves	**Stem**	**Heads**	**Above-plant**
Nutrition Management (NM)				
CNM	8.30 b	14.61 b	5.65 b	28.56 b
INM	11.10 a	20.05 a	8.75 a	39.90 a
Significance	**	**	**	**
Cultivar (CV)				
CV 1	9.40	17.10	8.05	34.55
CV 2	10.00	16.60	7.95	34.55
Significance	ns	ns	*	ns
Year				
2011	10.80	19.80	8.70 a	39.30 a
2012	8.60	14.40	6.50 b	29.5 b
Significance	*	**	*	**
Interaction				
NM × CV	ns	ns	ns	ns
NM × Year	*	ns	*	*
CV × Year	ns	ns	ns	ns
NM × CV × year	ns	ns	ns	ns

Different letters within each column indicate significant differences according to SNK test ($p \leq 0.05$). NS not significant * $p < 0.05$ and ** 0.01, indicate level of significance.

In 2011 the root morphology (Table 5) was evaluated. Parameter values for the plants under INM treatment were higher than CNM as follows: root length (+174%), area projection (+166%), surface area (165%), root volume (+167%), tips (+175%), forks (+285%), and crossings (+464%).

Regarding the CV, the best performing root system was White (CV_1) compared to Yellow (CV_2): root length (+63%), area projection (+37%), surface area (+38%), root volume (+19%), tips (+100%), forks (+109%), and crossings (+197%).

Table 5. Main effects of nutrient protocol management and cultivar on total root length (TRL), area projection (AP), surface area (SA), root volume (RV), root tips (RT), root forks (RF), and root crossings (RC) at 2011 harvest period in chrysanthemum plants.

Treatments	TRL (cm)	AP (cm^{-2})	SA (cm^{-2})	RV (cm^3)	RT (no.)	RF (no.)	RC (no.)
Nutrition Management (NM)							
CNM	382.1 b	21.2 b	66.7 b	0.9 b	1264.8 b	948.4 b	34.7 b
INM	1049.2 a	56.3 a	177.0 a	2.4 a	3486.6 a	3655.4 a	195.6 a
Significance	**	**	**	**	**	**	*
Cultivar (CV)							
CV 1	975.0 a	48.7 a	153.0 a	1.9 a	3535.2 a	3501.8 a	208.2 a
CV 2	597.4 b	35.5 b	110.8 b	1.6 b	1768.3 b	1676.0 b	70.0 b
Significance	*	*	*	*	**	**	**
Interaction							
NM × CV	*	*	*	*	**	**	**

Different letters within each column indicate significant differences according to SNK test ($p \leq 0.05$). NS not significant * $p < 0.05$ and **0.01, indicate level of significance.

Concerning the plant N accumulation (gm^{-2}) at every DAT in both years (Table 6), the maximum value was obtained under INM, which was the result of a simultaneous increase in dry weight (Table 4). The highest N accumulation, in both years, was at harvest (131 DAT), in 2011 with an increase of 94%, and in 2012 with an increase of 55%. No significant difference was found between the CVs, except for the flower head value at 131 DAT in both years.

Table 6. Main effects of nutrient management and cultivar on N accumulation ($g\ m^{-2}$) at three different days after transplant (DAT) in chrysanthemum plants over the two years of application.

Treatments	DAT		
	55	93	131
		First Year	
Nutrition Management (NM)			
CNM	4.33 b	5.47 b	6.20 b
INM	6.28 a	9.13 a	10.39 a
Significance	*	*	*
Cultivar (CV)			
CV 1	5.55	7.20	8.99
CV 2	5.61	7.68	8.48
Significance	ns	ns	ns
Interaction			
NM × CV	ns	ns	ns
		Second Year	
Nutrition Management (NM)			
CNM	2.67	5.05	6.37 b
INM	3.31	6.12	9.90 a
Significance	*	*	**
Cultivar (CV)			
CV 1	2.93	5.42	8.47 a
CV 2	3.03	5.72	7.57 b
Significance	NS	NS	*
Interaction			
NM × CV	ns	ns	ns

Different letters within each column indicate significant differences according to the SNK test ($p \leq 0.05$). NS not significant * $p < 0.05$ and **0.01, indicate level of significance.

Table 7 shows that in both years CNM treatment statistically influenced N accumulation (gm^{-2}) in all plant epigeal organs and on all sample dates. In the first year, the highest N accumulation was observed compared to CNM in the leaves (+48%) at 55 DAT, stems at 93 DAT (+85%), and flower buds (+79%) at 131 DAT. Regarding INM, in the second year, the highest value was recorded in leaves (+28%) at 55 DAT, stems (+46%), and flower buds (+117%) at 131 DAT. In both years the CVs did not influence N accumulation.

Table 7. Main effects of nutrient management and cultivar on N accumulation (g m^2) in different organs at three different DAT in chrysanthemum plants over the two years of application.

Treatments	DAT						
	55		93		131		
	Leaves	Stems	Leaves	Stems	Leaves	Heads	Stems
				First Year			
Nutrition Management (NM)							
CNM	3.05	1.28	3.31	2.16	2.83	1.87	1.50
INM	4.52	1.76	5.14	3.99	4.50	3.34	2.55
Significance	**	*	**	**	**	**	**
Cultivar (CV)							
CV 1	4.09	1.46	4.08	3.13	3.52	3.48	2.00
CV 2	4.04	1.57	4.53	3.26	3.75	2.73	2.00
Significance	ns	ns	ns	ns	ns	*	ns
Interaction NM × CV							
				Second Year			
Nutrition Management (NM)							
CNM	1.84	0.83	3.07	1.88	2.75	1.28	1.75
INM	2.35	0.96	3.57	2.55	3.30	2.78	2.57
Significance	*	*	*	*	*	**	*
Cultivar (CV)							
CV 1	2.07	0.87	3.23	2.19	3.09	1.71	2.54
CV 2	2.11	0.91	3.52	2.20	2.95	1.97	1.77
Significance	ns	ns	ns	ns	ns	ns	*
Interaction NM × CV							

Different letters within each column indicate significant differences according to SNK test ($p \leq 0.05$). NS not significant * $p < 0.05$ and **0.01, indicate level of significance.

Figure 1 shows that in both years the N utilization efficiency (NUE) value was highest in CNM (on average 162) compared to INM (on average 142); no significant difference was found between the CVs.

In both years, the P content (%), at harvest, in above-ground vegetative tissues (leaves, stems, and heads) of INM plants was higher than those of CNM plants (Table 8). In the first year, the increase in INM compared to CNM was 11% in the leaves, 20% in the stems, and 21% in the flower heads. In the second year, the increase in P content in the leaves under INM was similar to that recorded in the first year (12%), while it was lower for stems (+12%) and flower heads (+14%). In both years the P content in above-ground vegetative tissues were in the order of head > leaves > stems, which was maintained in both INM and CNM treatments.

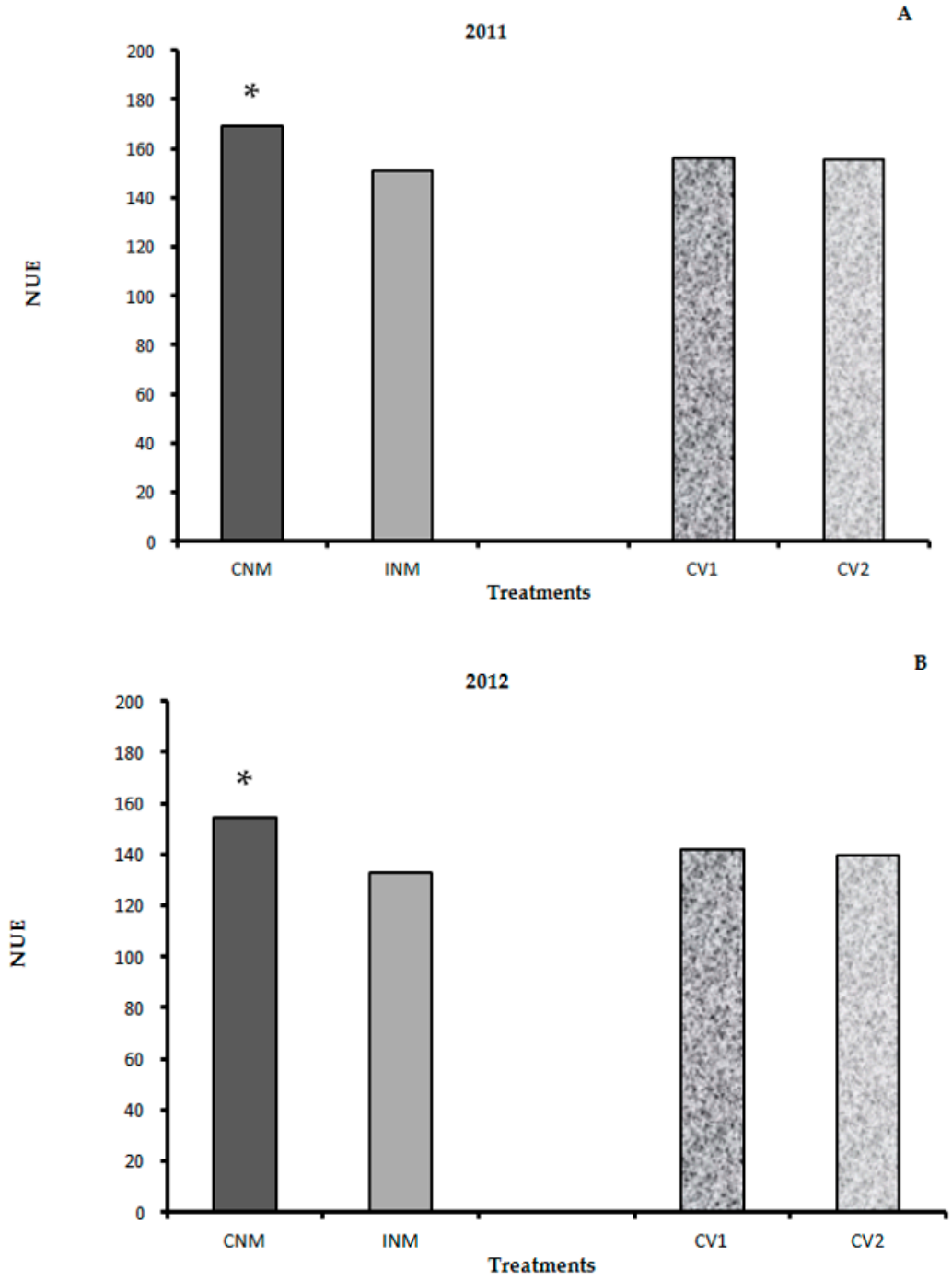

Figure 1. Main effects of nutrient management and cultivar on N utilization efficiency (NUE) in first (A) and second (B) year in chrysanthemum plants at harvest time (* indicates the level of significance at $p < 0.05$).

Table 8. Main effects of nutrient management and cultivar on phosphorus content (%) in different organs at harvest in chrysanthemum plants over the two years of application.

TMTS	Leaves	Stem	Flower Heads
		First Year	
Nutrition Management (NM)			
CNM	0.19 b	0.15 b	0.33 b
INM	0.21 a	0.18 a	0.40 a
Significance	*	*	*
Cultivar (CV)			
CV 1	0.20	0.16	0.32
CV 2	0.21	0.17	0.31
Significance	ns	ns	ns
Interaction			
NM × CV	ns	ns	ns
		Second Year	
Nutrition Management (NM)			
CNM	0.17 b	0.16 b	0.29 b
INM	0.19 a	0.18 a	0.33 a
Significance	*	*	*
Cultivar (CV)			
CV 1	0.18	0.19	0.31
CV 2	0.18	0.18	0.30
Significance	ns	ns	ns
Interaction			
NM × CV	ns	ns	ns

Different letters within each column indicate significant differences according to SNK test ($p \leq 0.05$). NS not significant * $p < 0.05$ and **0.01, indicate level of significance.

Concerning an economic point of view, the increased yield obtained with INM (140 stems m^{-2}) compared with CNM (118 stems m^{-2}) led consequently to an increase of gross production of exactly €50,600.00 (takings difference). This amount was much greater than the cost increases needed for INM compared to CNM (Table 9).

Table 9. Basic economic analysis of fertilizers cost, gross production, and takings difference obtained.

	Fertilizer Cost	Yield	Gross Production	Takings Difference
	(€ ha^{-1})	(stems m^{-2})	(€ ha^{-1}) *	(€)
CNM	1945.00	118	271,400.00	
INM	3144.00	140	322,000.00	+50,600.00

* Chrysanthemum price calculated at 2012–2013: 0.23 €/stem (ISMEA/2012–2013).

4. Discussion

In our study, mineral nutrient management (CNM) and integrated nutrient management (INM) were compared in chrysanthemum cultivation. The INM protocol, which combined the application of half the rate of CNM and seaweed extract, organic and biofertilizer (AMF + PSB), improved yield, cut stem quality traits, root morphology, as well as N accumulation and P content in tissues. Based on other research about INM practices [47–49], this protocol seems to be suitable in order to obtain advantages on profits and sustainability. Our aim was to verify a new mixture in order to reduce mineral fertilizer application, making chrysanthemum cultivation more sustainable, as well as highly profitable.

Compared to CNM, the INM protocol led to a significantly higher yield in terms of the number of secondary branches per m^{-2} (Table 3). This could be attributed to a better nutrient translocation in the plant, which led to the production of a greater number of axillary buds and therefore of secondary axes,

in line with Kale et al. [50] in *Salvia* and Nethra [51] in the Chinese aster. In other studies regarding biostimulant applications, yield also increased in seaweed treated plants influenced by cytokinin content, which enhances nutrient mobilization in plant organs [52].

Regarding cut stem quality traits (Table 3), our results are in agreement with Verma et al. [53], who applied an INM on chrysanthemum CV Roja. The treatment that consisted of *Azospirillum*, PSB, vermicompost, and 50% of recommended mineral NPK recorded the highest plant height, number of branches, and flowers per plant. Similar results were reported in *Crossandra* [54] and Dahlia [55]. The combination of biofertilizers with the recommended NPK dose yielded a higher flower production in *Limonium* [56] and *Calendula* [57].

In our study, a higher leaf area value was found in chrysanthemum plants under INM. According to De Lucia and Vecchietti [58], the application of seaweed extract (*A. nodosum*) in *Lilium* CV Brindisi, greatly affected these parameters (12.3 cm^2 of treated plants, compared with 10.3 cm^2 of untreated plants). This was potentially due to the direct effect of the biostimulant containing betaine. The nutrient concentration present in both the N organic fertilizer and seaweed extract biostimulant cannot on its own explain the positive response as an increase in aerial organ dry weight (Table 4). In fact cytokinins have a considerable influence on nutrient mobilization in vegetative and reproductive organs [59].

Microbial inoculants are also good supplement with half the recommended mineral dose of fertilizer. Wu et al. [60] reported that *G. mossae* plus *B. megaterium* on maize increased plant growth and NPK assimilation. As regards the effect of applying INM, the chrysanthemum root development exhibited a remarkable increase in all parameters compared to CNM (Table 5). The root growth promoting activity has been observed in snapdragon, when a biostimulant was applied [61]. Previous research has shown that the brown seaweed extract, rich in auxin, improved lateral root formation when applied to mung bean [62]. A study carried out by Mancuso et al. [63] on potted *Vitis vinifera* under seaweed extracts, showed an increase in the total volume of the root system.

Concerning the nutrient uptake, Biswas et al. [64] and Adesemoye et al. [65] showed that PGPR (plant growth-promoting rhizobacteria) also influences this parameter through a more pronounced development of the root surface area. The INM seems to encourage a better uptake of mineral nutrients by plants, which results in a higher number of branches as well as leaf area, and more flowers [66].

The N uptake by chrysanthemum plants may be enhanced by the use of biofertilizers, possibly because they stimulate better root architecture or due to the influence of growth hormones contained in seaweed extracts. These substances can increase the ability of nutrient absorption as well as enzymatic activity, in agreement with Kumari et al. [67].

The N accumulation (g m^{-2}) value in the INM treatment could be caused by the better availability and uptake of nutrients facilitated by the application of both mineral and organic fertilizers, biostimulants, and biofertilizers (Tables 6 and 7). Mahadik et al. [68] showed that the increase in N and P uptake by chrysanthemum plants was the highest with the application of *Azotobacter* plus PSB, 50% of RDF (Recommended Dose of Fertilizers) (100:100:100 kg ha^{-1} NPK), and 10 t ha^{-1} of vermicompost. Regarding the P content in plant tissue (Table 8), our findings are validated by similar results found in a number of earlier studies on bacteria.

Shirmardi et al. [69] reported that PGPR solubilizes the inorganic phosphate and produces IAA, thus improving plant growth by increasing P-uptake from the soil and its transport to plant shoots. A significant increase in sunflower growth parameters, including plant P content, was found in inoculated plants after inoculation with *Bacillus* sp., possibly due to the P-solubilizing, IAA-synthesizing, and root-colonizing of these strains [70], which increase nutrient uptake.

Richardson (1994) and Rodríguez and Fraga [71] studied the influence of several soil bacteria on the supply of P to plants as a consequence of their capacity for inorganic or organic P solubilization and, therefore, for improving plant growth performance. In addition, in a 1994 study, Garbaye [72] postulated that some PSB behave like mycorrhizal helper bacteria with a synergistic interaction.

Compared to the non-treated control, the combined application of mycorrhizal fungus and rhizobacteria significantly increased growth parameters, i.e., total fresh weight, aerial dry weight,

shoot length, and leaf area, in bananas. The leaf mineral content, i.e., N, P, and K, also increased significantly following the combined application of both microorganisms [73].

Finally, integrated nutrient management practices could be viable for sustainable floriculture on a commercial and profitable scale. Our data on the economics of chrysanthemum flowers are in agreement with those Verma, who showed that the cost of fertilizer can be saved with inoculation of both Azospirillum and PSB, obtaining higher flower yield compared to CNM. Angadi too, carried out a study that shed light on the combination of Azospirillum, PSB, 50% vermicompost, and 1/2 recommended NPK dose, giving the maximum net returns per euro invested.

5. Conclusions

The quality and quantity of fertilizers are the key factor affecting the growth, yield, and quality of cut flowers. Since chrysanthemum is an energy-intensive ornamental crop with a very high input of fertilizers, several experiments have been aimed at using alternative methods, reducing mineral fertilizers, and in particular the INM.

Our results shows that the INM protocol, 50% mineral RDF with N organic fertilizer plus biostimulant (seaweed extract) plus biofertilizer (microbial consortium of *Glomus* sp. and *Bacillus* sp.), is effective in enhancing yield, quality, root morphology, and nutrient uptake compared to RDF. This indicates the possibility of the sustainable, eco-friendly cultivation of chrysanthemum. In order to discern the influence of each component of INM mixture on yield and quality traits, future research is needed.

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