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RESEARCH ARTICLE

Effects of microalgae and compost on the yield of cauliflower grown in low nutrient soil

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ABSTRACT

The application of organic amendments is an increasingly widespread practice, which allows fertilizing and restoring soils in a sustainable and environmentally friendly way. Improving the quality of amendments through the application of microorganisms has been a challenge in recent years. The objective of the study was to determine the effects of microalgae+compost (1:1) mixture on the yield of cauliflower (*Brassica oleracea* L. var. *botrytis* L.) plants grown in a sandy loam soil with low nutrient availability. Yields were compared with the application of compost, cattle manure and two chemical fertilizers. The treatments applied were: T1 prepared with urea (NPK 46-0-0), triple superphosphate (NPK 0-46-0) and potassium saltpeter (NPK 15-0-14); T2 commercial fertilizer (NPK 12-11-18); T3 microalgae biomass (T3); T4 compost; T5 1:1 microalgae+compost mixture; T6 cattle manure; and T0 control (no fertilization). The experiments were conducted in 30 L pots using 40 g soil per plant and 4 g N as a reference to standardize fertilizer application. The results showed that the fresh weight (FW) and dry weight (%DW) of T5 and T6 were not significantly different from those of T1 ($p > 0.05$), on the contrary, FW of T5 and T6 was 43.8% and 40% higher than in T2, as well as the %DW was 0.59% and 0.6% w w^{-1}, also higher than in T2. These results suggest that the microalgae+compost mixture is an alternative fertilizer, equivalent to chemical fertilizers and manure, presenting the advantages of being more stable, sustainable, and environmentally friendly. However, further studies are needed to determine the necessary dose per cultivated plant species.

Key words: Amendments, *Brassica oleracea* var. *botrytis*, cattle manure, chemical fertilizers, compost, microalgae biomass.

INTRODUCTION

Decades of poor agricultural practices have caused a decrease in soils' physical, chemical, and biological quality, which has become one of the significant environmental and agronomic problems worldwide. On the one hand, tillage or intensive soil preparation systems in annual crops have had a negative impact on soil structure, permeability, and compaction, as well as, on nutrient cycling, microorganism diversity and metabolism, and the hydrological cycle of the agroecosystem (Yi et al., 2022). On the other hand, the intensive use of inorganic fertilizers has led to the contamination of agricultural soils and groundwater (Wang et al., 2021). These adverse effects translate into low crop productivity, affecting the planet's food security.

In this context, it has been reported that it is possible to mitigate the damage caused and renew the sustainability of soils by applying adequate amounts of organic amendments (Hale et al., 2021). Positive effects include nutrient supply, improvement of soil physical structure, which in turn allows minimizing nutrient loss by leaching, improvement of biological activity, water retention and soil aeration (Hale et al., 2021), among others. The aptitudes of compost and microalgae as amendments are described below.

Compost is a dark, humus-rich, complex, and pleasant-smelling substance with a high nutrient content, which is slowly released and can improve the physical properties of the soil. This amendment is one of the products resulting from the controlled process of organic waste decomposition (aerobic or anaerobic). Composted manure and plant residues have also been shown to be beneficial amendments and could influence soil chemical properties, such as minimizing the mobility and leaching of important plant minerals and preserving soil fertility (Mehta et al., 2014).

Other less conventional amendments are microalgae, which have numerous and diverse attributes that improve plant growth and productivity (Hamed et al., 2018). On the one hand, microalgae have been characterized by their participation in the biological fixation of N and the P cycle in the soil; they have beneficial effects on microorganisms that stimulate plant growth through the synthesis of phytohormones, symbiotic associations with roots, biocontrol, and soil stabilization (Ronga et al., 2019; Alvarez et al., 2021). Like compost, microalgae release their nutrients slowly into the soil. The microalgae are microorganisms used to produce food, chemicals, biofuels and biofertilizers (Ammar et al., 2022). Into the last area, one of the most documented microalgae genera is *Chlorella* and has been demonstrated that its application in a proportion of 50% w w^{-1} incremented yields in basil (*Ocimum basilicum* L.), enhancing the quality of seedlings and its further development by the fortification of roots (García-Orellana et al., 2016). In the same context, has been found that fertilization of wheat with *Chlorella* produced increments in growth, not only by the contribution with nutrients of this microalgae, but also for its production of phytohormones that enhance roots development, incrementing yield and germination, known as biostimulation effect. This effect is due to the synthesis of plant growth hormones, polysaccharides, antibacterial chemicals, and other metabolites by microalgae of genera like *Chlorella*, *Spirulina*, *Cyanobacteria* (Ronga et al., 2019). Also, the microalgae have a great potential to enhance the soil organic C content by the assimilation of atmospheric CO_2 and N, giving a better soil environment to the plants and the normal microbiota associated (Ammar et al., 2022). While the use of chemical, compost, and manure from different animals as fertilizers has been well documented, the interest on the microalgae as biofertilizer is more recent, from the middle of 2000's and focused mainly on rice, wheat, soybean, and tomato (Gonçalves, 2021).

On the other hand, cauliflower (*Brassica oleracea* L. var. *botrytis* L.) is a vegetable of the Brassicaceae family also known as Cruciferae, has become more demanded as a food of high nutritional value in recent years, being one of the vegetables that has increased the most (23%) in terms of cultivated area in Chile, going from 1252 to 1540 ha from 2016 to 2017 (Eguillor, 2020). In fact, the production of cruciferous vegetables is very relevant in Chile because they are mostly grown by medium and small farmers. Cauliflower contains large amounts of vitamins, minerals and anticarcinogenic phytochemicals, and they have high productivity and adaptability in different conditions (Farzana et al., 2016; Abdel Nabi et al., 2020). The use of chemical fertilizers and some organic amendments such as compost, vermicompost and manure are well documented in cauliflower (Yassen et al., 2019; Abdel Nabi et al., 2020), but the application of microalgae or mixtures of microalgae with other biofertilizers such as compost are poorly investigated. Thus, the objective of this research was to determine the fertilizer capacity of microalgae and of a mixture of microalgae+compost, which were compared with other chemical and organic traditional fertilizers. The cauliflower species was selected because of its high production and demand in the area and because of the time of year in which the trial was conducted.

MATERIALS AND METHODS

Site description

The research was carried out in the agricultural property "El Junco", Melipilla (33°42'23.5" S, 71°09'43.5" W), Metropolitan Region, Chile. This agricultural property has large extensions of low fertility soils, caused by the intensive agriculture developed for 40 yr. These soils prevent the adequate and sustainable development of horticultural crops. For this research, 20 subsamples were collected at different points of the field following a zigzag pattern. The subsamples were taken from the first 30 cm of soil and mixed to generate a representative sample of the sector. The soil was sampled according to USDA-NRCS Agriculture (1999), its nutrient availability and physicochemical properties were measured according to Zagal and Sadzawka (2007) and the results were analyzed according to Vistoso and Martinez-Lagos (2019; 2021) and Martinez-Rodriguez et al. (2021).

Plant species

The plant species cultivated was cauliflower (*Brassica oleracea* L. var. *botrytis* L.) of the late-maturing commercial cv. Defender. Seeds were supplied by the company Anasac (Santiago, Chile), and were sown in polyethylene trays with 30 mL cells. The substrate used for sowing was a mixture of blond peat with perlite in a

4:1 ratio. Seeds were incubated in darkness in a TS606/3-1 WTW growth chamber (WTW, Weilheim, Germany) at 20 °C for 24 h to stimulate germination. Then, the trays with germinated seeds were transferred to the greenhouse and kept growing for 35 d, at this time seedlings reached a phenological stage of four true leaves and an average height of 6.6 cm. Finally, the plants were transplanted directly into 30 L pots with 40 kg sieved (0.5 inch sieve) agricultural soil, and two 100 mL irrigations were programmed every 7 d. The crop was maintained until reaching the phenological state of maximum growth of the inflorescence-pre-flowering. The light and environmental conditions during the experiment are described in the Table 1.

Table 1. Monthly air temperature, rainfall, and solar radiation experimental site condition during the study.

Month	Minimal monthly temperature	Maximal monthly temperature	Minimal average temperature	Maximal average temperature	Precipitation	Peak solar radiation
	°C				mm	kWh m^{-2}
April	2.7	32.0	8.7	19.0	0.0	4.09
May	-1.1	25.8	7.0	16.6	3.0	2.62
June	-2.3	21.5	4.7	13.2	42.8	2.17
July	-2.1	22.0	3.3	12.9	8.5	2.38
August	-2.5	31.4	3.8	15.0	0.0	3.08
September	2.2	25.8	7.9	16.7	0.0	4.53

Traditional fertilizers
The traditional fertilizers consisting of a complete mineral fertilization prepared per plant with 2.1 g urea NPK 46-0-0, 6.52 g triple superphosphate NPK 0-46-0 and 18.57 g potassium saltpeter NPK 15-0-14 and a commercial monograin fertilizer NPK 12-11-18 Qrop Complex (SQM, Santiago, Chile) applied as recommended by the manufacturer.

Organic amendments
The amendments were prepared from plant residues, microalgae species provided by the companies ProCycla-Aroma SpA and cattle manure provided by Lechería Pahuilmo (as described before), both companies located in Melipilla, Chile. The preparation of each amendment is described below.

Cattle manure. The manure was obtained from cattle of the Lechería Pahuilmo (Melipilla, Chile) with the animal nutrition based on a concentrate (Concentrado Lechero, CALS, Santiago, Chile) and the addition of fiber to the diet. The manure was collected fresh and after that was stored at room temperature for 14 d until its application. It was not pretreated and was applied directly to the pots with the plants. No hay was added.

Organic compost. Compost was made with a mixture of discarded fruit (citrus, pome fruit and stone fruit) and cattle manure (described before) in a 1.5 m high and 3 m-wide pile system, with turning and irrigation according to the temperature and humidity controls of the mound. The turning was done with a skid steer loader. During the composting process, the temperature was controlled and reached a maximum of 65 °C. During composting, the four phases of the process were reached (mesophilic 1, thermophilic, mesophilic 2 and maturation), and the total process lasted 8 mo.

Microalgae biomass. A mixed culture of *Chlorella* sp. and *Scenedesmus* sp. was used as inoculum (20% v v^{-1}) in a 1500 L pond (raceway type) adding 4 mL L^{-1} Basfoliar 10-04-07 SL culture medium (Compo Expert, Münster, Germany). The culture was incubated for 8 d, under environmental condition. During the assay the environmental condition in Melipilla presented minimal and maximal average temperature of 8.7 and 19.0 °C respectively, and peak solar radiation was 4.09 kWh m^{-2}. After the growth, microalgae were harvested by precipitation using a Z 326 K centrifuge (HERMLE Labortechnik, Wehingen, Germany). The solid fraction was recovered and dried at 70 °C in a model DHG-9077 forced-air drying oven (Nanjing Gengchen Scientific

Instrument, Jiangsu, China) to constant weight and dried biomass was pulverized using a household food grinder (Oster, Boca Raton, Florida, USA).

Physicochemical characterization of organic amendments

The nutrient availability and physicochemical properties of the organic amendments were obtained using different techniques. The pH and electrical conductivity (EC) were determined by potentiometry. Organic matter, organic C, C:N ratio, N, P (in the form of phosphorus oxide; P_2O_5), K (in the form of potassium oxide K_2O), Ca (in the form of calcium oxide; CaO) and other metals were analyzed by atomic absorption, colorimetric methods, and titration techniques, according to methodology described by Zagal and Sadzawka (2007).

Determination of amendment per treatment

The amount of total N recommended for cauliflower was obtained from Rincón Sánchez et al. (2001), who determined an amount of 175 kg ha^{-1} N considering 44000 cauliflower plants ha^{-1}, equivalent to 4 g N cauliflower $plant^{-1}$, value in agreement with the recommendation by the Instituto de Investigaciones Agropecuarias (INIA), ranged 125-280 kg ha^{-1} N considering a cauliflower yield of 25-40 t ha^{-1} (Hirzel and Salazar, 2021). The N supplement for each treatment was calculated using the information from physicochemical analysis, considering the N recommended shortage of the N in 40 kg agricultural soil used in each treatment.

Experimental design

The experimental design was a completely randomized design, which consisted of seven fertilization treatments with five replicates each. The experimental unit was a 30 L pot containing 40 kg agricultural air-dry soil and one seedling with four true leaves. The treatments applied (T1-T6) are described in Table 2 and includes a negative control (T0) without fertilization. The application of each treatment was the following: (1) Traditional chemical fertilizers were applied according to the manufacturer's instructions; (2) soil microalgae biomass, compost and manure were incorporated directly into the soil.

Table 2. Treatments applied to an intensively cultivated land.

Treatment	Fertilization type	Composition
T0	Without fertilizers	Intensive cropland
T1	Mixed traditional chemical	Urea NPK 46-0-0, triple superphosphate NPK 0-46-0 and potassium saltpeter NPK 15-0-14, in a ratio 1:3:9
T2	Commercial chemical	Fertilizer Qrop Complex, monograin fertilizer (NPK 12-11-18)
T3	Organic amendments	Microalgae biomass
T4	Organic amendments	Compost
T5	Organic amendments	50% w w^{-1} microalgae biomass+compost mix
T6	Organic amendments	Dairy cattle manure

Biometric analysis of the cauliflower crop

Plant height (cm) was defined from the point of cotyledon abscission to the base of the apical bud; number of leaves and area of the plant or rosette (cm^2) were defined as the result of multiplying the largest diameter of the rosette and its perpendicular diameter. Measurements were taken every 15 d until 120 d.

At the end of the trial, yield was obtained based on the fresh weight (FW) of inflorescence, stem and leaves using a semi-analytical balance (0.0001 g) (BAS 31 plus, Boeco, Hamburg, Germany). The plant biomass was dried in a drying oven (DHG-9077, Spark Electronics, Wuxi City, China) at 60 °C for 48 h to constant weight or dry weight (DW) and the percent dry weight (%DW) was calculated with respect to the FW. The total DW of the aerial part of the plant was calculated as the sum of the percent dry biomass of the inflorescence, stem, and leaves. The phenological stage of crops at harvest (end of the experiment) was the complete maturity of inflorescences.

Statistical analysis

All results were obtained by calculating the mean and standard deviation (SD) of at least five replicates evaluated. The significance of the differences was evaluated by ANOVA, after verification of normal distribution of data, followed by a Bonferroni post-hoc test. The significance level of the null hypothesis, i.e., that significant differences exist was $p \leq 0.05$. Pearson's correlation coefficient r ($-1 < r < 1$) was obtained to determine the degree of association between the variables. A value greater than 0 indicates a positive association, considered significative only when the parameter p for correlation was < 0.05.

RESULTS

Physicochemical properties of agricultural soil, fertilizers, and amendments

Initially, the agricultural soil of the Fundo El Junco farm was analyzed. Physical observations of the soil indicated that it is a sandy loam soil, with very low water saturation and medium-high pH (Table 3). According to the chemical analysis, the soil had a medium availability of organic matter, low to very low availability of elemental macronutrients for plant development such as N, P and K, except for S, which had a high level. However, it had very high and medium levels for salt-forming macronutrients such as Ca, Na and Mg (Table 3). In addition, chemical analysis showed that micronutrients such as Al, B, Cu, Zn, Fe and Mn varied between medium and high levels (Table 3).

Table 3. Physicochemical analysis of soil. EC: Electrical conductivity; Sum of bases: Ca + Mg + K + Na; ECEC: effective cation exchange capacity; Al saturation (exchangeable Al divided sum of bases plus exchangeable Al) multiplied per 100.

Parameters	Result	Level	Reference
Organic matter, mg kg^{-1}	2.20	Medium	Martínez-Rodríguez et al., 2021
pH	8.19	Medium high	Martínez-Rodríguez et al., 2021
EC, dS m^{-1}	2.00	Lightly saline	
Available N, mg kg^{-1}	14.00	Low	
Available P, mg kg^{-1}	3.30	Very low	Vistoso and Martínez-Lagos, 2021
Available K, mg kg^{-1}	84.00	Low	Vistoso and Martínez-Lagos, 2021
Available S, mg kg^{-1}	16.00	High	Vistoso and Martínez-Lagos, 2021
Interchangeable Ca, $cmol_{(+)}$ kg^{-1}	30.41	Very high	Vistoso and Martínez-Lagos, 2021
Interchangeable Mg, $cmol_{(+)}$ kg^{-1}	0.97	Medium	Vistoso and Martínez-Lagos, 2021
Interchangeable K, $cmol_{(+)}$ kg^{-1}	0.22	Low	Vistoso and Martínez-Lagos, 2021
Interchangeable Na, $cmol_{(+)}$ kg^{-1}	0.56	Very high	Vistoso and Martínez-Lagos, 2021
Sum of bases, $cmol_{(+)}$ kg^{-1}	32.16	Very high	Vistoso and Martínez-Lagos, 2021
Interchangeable aluminum, $cmol_{(+)}$ kg^{-1}	0.06	Very low	Vistoso and Martínez-Lagos, 2021
ECEC, $cmol_{(+)}$ kg^{-1}	32.18		
Al saturation, % w v^{-1}	0.08	Very low	
B, mg kg^{-1}	0.74	Medium	Vistoso and Martínez-Lagos, 2019
Cu, mg kg^{-1}	6.78	High	Vistoso and Martínez-Lagos, 2019
Zn, mg kg^{-1}	0.80	Medium	Vistoso and Martínez-Lagos, 2019
Fe, mg kg^{-1}	24.00	High	Vistoso and Martínez-Lagos, 2019
Mn, mg kg^{-1}	9.91	Medium	Vistoso and Martínez-Lagos, 2019

Subsequently, the prepared organic amendments were analyzed physiochemically. The results of the analysis of each amendment are presented in Table 4, as well as the information available for the traditional fertilizers used. In general, the pH of the amendments was higher than that of traditional fertilizers, with the microalgae biomass having the highest pH (10). Regarding chemical analysis, it was observed that compost and cattle manure were the amendments with the highest level of organic matter (86% w w^{-1}); C:N ratio was variable among amendments, compost showing the highest ratio (C:N 25); microalgae biomass was the amendment with the highest levels of N and

P, Ca and Mg; K was found at higher levels in compost (Table 4). All the levels in % w w^{-1} of nutrients described for the traditional fertilizers are higher than those of amendments.

The N results obtained from the chemical analysis were used to determine the amount of amendment applied in each treatment, considering that the amount of N required by the cauliflower plant grown directly in agricultural soil is 4 g $plant^{-1}$. The N in the agricultural soil was 14 mg kg^{-1}, which is equivalent to 0.56 g in 40 kg soil, used in each pot, so it was necessary to apply 3.44 g N per amendment. The total amount of amendment needed to supply the missing N is presented in Table 5. Before the application of the amendments The EC of the soil used in this study was 2 dS m^{-1} and after the fertilization EC ranged 2 ± 0.02 dS m^{-1}.

Table 4. Physicochemical analysis of chemical fertilizer and amendments. EC: Electrical conductivity; ND: not described; Mix fertilizer: mixture of urea, triple superphosphate, and potassium saltpeter.

Parameters	Qrop Complex	Mixture fertilizer	Microalgae biomass	Compost	Cattle manure
pH	6.00	6.00	10.00	6.90	5.50
EC, dS m^{-1}	1.02	0.86	4.40	3.20	6.70
Organic matter, % w w^{-1}	ND	ND	56.00	86.00	85.50
Organic C, % w w^{-1}	ND	ND	31.10	48.00	47.50
C:N ratio	ND	ND	8.30	25.00	15.80
N, % w w^{-1}	12.00	13.80	3.75	2.30	3.00
P (P_2O_5), % w w^{-1}	11.00	11.00	1.40	0.05	2.00
K (K_2O), % w w^{-1}	18.00	9.56	0.49	4.02	0.78
C (CaO), % w w^{-1}	ND	ND	14.10	0.15	3.10
Mg (MgO), % w w^{-1}	0.02	ND	1.20	0.40	1.20

Table 5. Quantity of fertilizer used in each treatment.

Fertilizer	N%	Total amendment applied per plant	Total N applied
	w w^{-1}	g	g
Qrop Complex	12.00	29.00	3.44
Mixture of urea, triple superphosphate, and potassium saltpeter	46 in urea and 15 in potassium saltpeter	4.59 of urea and 8.86 of potassium saltpeter	3.44
Microalgae biomass	3.75	92.00	3.44
Compost	2.30	150.00	3.44
Microalgae biomass+Compost	3.75 in microalgae and 2.3 in compost	46.0 of microalgae and 75.0 of compost	3.44
Cattle manure	3.00	115.00	3.44

Growth of cauliflower plants

All cauliflower plants grown in pots with agricultural soil and the different treatments showed a periodical height increase (Figure 1). Measurements were made every 15 d and nonsignificant differences ($p > 0.05$) were present between treatments. However, a different situation occurred with the area results. Even though the plant area increased gradually with respect to the cultivation period, during the first 30 d analyzed there were nonsignificant differences ($p > 0.05$) in the size of plants, however, during the measurements recorded after 45 d, differences were observed ($p < 0.05$). The final area in T2 and T3 were as T0 ($p > 0.05$), which are lower compared with T1, T5, and T6, without significant difference ($p > 0.05$) between them (Figure 2).

On the other hand, Figure 3 shows the development of the number of leaves periodically, where nonsignificant differences ($p > 0.05$) were observed between treatments. At the beginning of the trial, seedlings had an average of 3.7 ± 0.7 leaves each and at the end of the trial, after 120 d cultivation, plants had significantly increased their number of leaves with an average of 20.3 ±1.7 leaves.

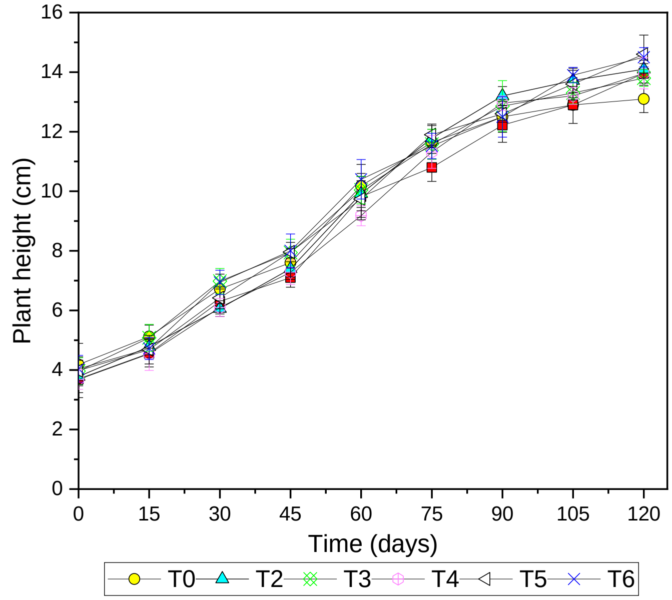

Figure 1. Cauliflower plant height in all periods evaluated. All results were obtained by calculating the mean and standard deviation (SD) of at least five replicates evaluated. T1: Prepared with urea (NPK 46-0-0), triple superphosphate (NPK 0-46-0) and potassium saltpeter (NPK 15-0-14); T2: commercial fertilizer (NPK 12-11-18); T3: microalgae biomass (T3); T4: compost; T5: 1:1 microalgae+compost mixture; T6: cattle manure; and T0: control (no fertilization).

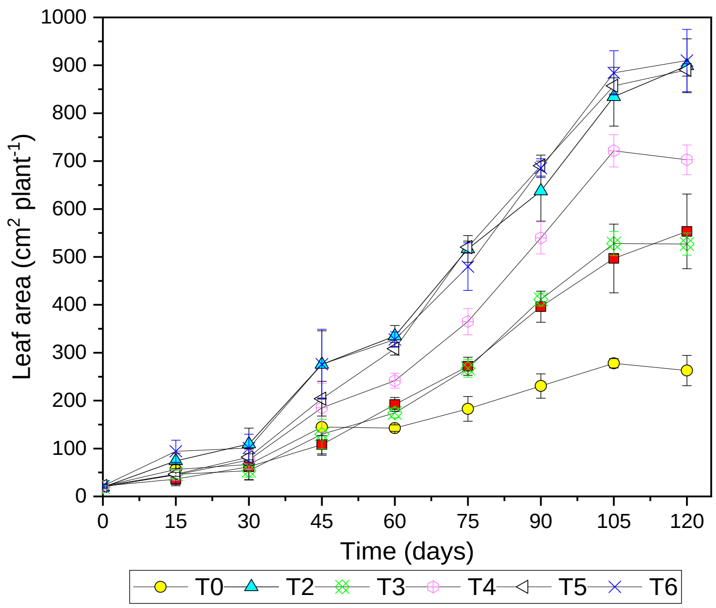

Figure 2. Cauliflower plant area in all periods evaluated. All results were obtained by calculating the mean and standard deviation (SD) of at least five replicates evaluated. T1: Prepared with urea (NPK 46-0-0), triple superphosphate (NPK 0-46-0) and potassium saltpeter (NPK 15-0-14); T2: commercial fertilizer (NPK 12-11-18); T3: microalgae biomass (T3); T4: compost; T5: 1:1 microalgae+compost mixture; T6: cattle manure; and T0: control (no fertilization).

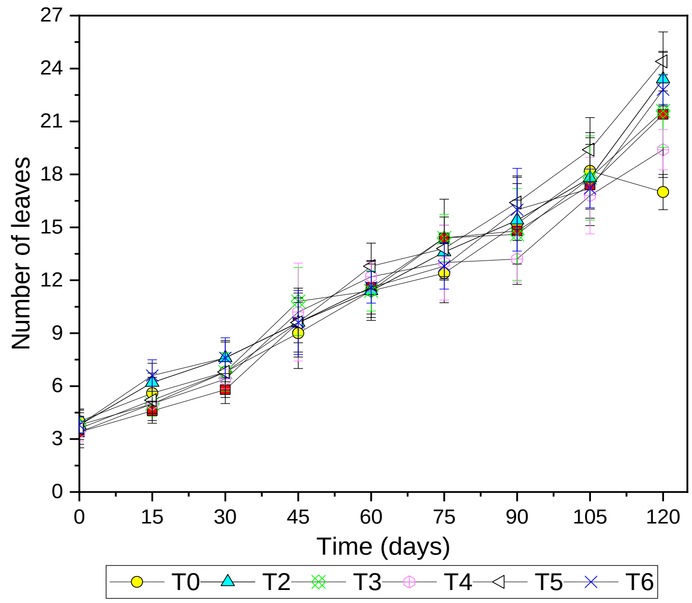

Figure 3. Number of leaves of cauliflower plant in all periods evaluated. All results were obtained by calculating the mean and standard deviation (SD) of at least five replicates evaluated. T1: Prepared with urea (NPK 46-0-0), triple superphosphate (NPK 0-46-0) and potassium saltpeter (NPK 15-0-14); T2: commercial fertilizer (NPK 12-11-18); T3: microalgae biomass (T3); T4: compost; T5: 1:1 microalgae+compost mixture; T6: cattle manure; and T0: control (no fertilization).

Plant yield

During the analyses, 100% cauliflower plants showed development of inflorescences. The yield of FW and DW of the inflorescence, leaves, and stems, at 120 d was different between some treatments ($p < 0.05$), (Tables 6 and 7). The FW was higher in leaves compared to the biomass of inflorescences and stem in all treatments (Tables 6 and 7). The stem was the organ that contributed less weight to the entire plant weight. However, the comparison between treatments indicates that FW of the three organs was lower in T0 and the highest weights in T1, T5 and T6, without significant differences ($p > 0.05$).

Table 6. Yields expressed in fresh weight (FW) of inflorescence, leaves, stems, and total yield of the cauliflower plant. Data are presented as mean of at least five replicates. Bonferroni post hoc test was performed. Means with equal letters are not significantly different ($p > 0.05$). T1: Prepared with urea (NPK 46-0-0), triple superphosphate (NPK 0-46-0) and potassium saltpeter (NPK 15-0-14); T2: commercial fertilizer (NPK 12-11-18); T3: microalgae biomass (T3); T4: compost; T5: 1:1 microalgae+compost mixture; T6: cattle manure; and T0: control (no fertilization).

	FW Yield			
Treatments	Inflorescences	Leaves	Stem	Total mean
	g			
T0	185.0D	284.7D	45.04D	440.7D
T1	610.0A	944.0A	150.0A	1704.0A
T2	417.0B	645.9B	107.1B	1170.0B
T3	310.0C	481.6C	78.6C	870.2C
T4	483.0B	747.6B	118.5B	1349.0B
T5	603.0A	931.0A	148.8A	1682.0A
T6	586.0A	907.0A	145.6A	1638.0A

On the other hand, total FW (Table 6) was different among some treatments, but it was also higher in T1, T5 and T6, with nonsignificant differences among them ($p > 0.05$). While the %DW yields of the three organs analyzed showed nonsignificant differences among treatments, except T0 (no fertilizer application) which was significantly lower (Table 7). Values were higher than 11% w w^{-1} in treatments T1 to T6 and 9% ww^{-1} in T0. In addition, the comparison of DW% means for each organ evaluated showed nonsignificant differences (Table 7).

Table 7. Dry yields expressed in percentage of the fresh weight of inflorescences, leaves, stems, and total yield of the cauliflower plant. Data are presented as mean of at least five replicates. Bonferroni post hoc test was performed. Means with equal letters are not significantly different ($p > 0.05$). T1: prepared with urea (NPK 46-0-0), triple superphosphate (NPK 0-46-0) and potassium saltpeter (NPK 15-0-14); T2: commercial fertilizer (NPK 12-11-18); T3: microalgae biomass (T3); T4: compost; T5: 1:1 microalgae+compost mixture; T6: cattle manure; and T0: control (no fertilization).

	Dry yield			
Treatments	Inflorescences	Leaves	Stem	Total mean
	% w w^{-1} FW			
T0	10.31B	9.32C	8.98C	9.53C
T1	11.47AC	11.14B	10.72B	11.83A
T2	13.19BC	11.25B	11.22B	11.35B
T3	10.91B	10.74B	11.31B	10.89B
T4	11.89ABC	11.38B	11.01B	11.53B
T5	13.36A	11.21B	10.71B	11.94A
T6	13.54A	11.10B	10.88B	11.95A

DISCUSSION

The physicochemical analysis allowed classifying the agricultural soil, used in this study, as a sandy loam soil with low water retention, medium availability of organic matter and medium to low in nutrients, indicating that it is a soil with low fertility (Vistoso and Martínez-Lagos, 2019; 2021; Martínez-Rodríguez et al., 2021). To improve soil nutrition, different fertilization treatments were applied (Table 2). The trial was conducted in pots and the final development and yield of cauliflower plants were evaluated. Cultivation of cauliflower in pots has been described in several previous studies, in which containers of different sizes ranging from 2.4 to 48.0 L were used (Rahman et al., 2013; Singh et al., 2013). In this study, a 30 L pot with 40 g soil was used. The technique of using a soil mixture with fertilizer for potted plant cultivation has some advantages compared to using fertilizers in inert substrates, such as perlite and vermiculite, or directly in the soil. For example, it significantly improves the adsorption of fertilizer nutrients and the retention of fertilizers against leaching (Bunt, 1988; Farzana et al., 2016). With this, it is possible to calculate and apply the right doses of nutrients ensuring their availability throughout plant development.

Fertilization applied with each treatment shows a clear positive effect in this study, as the results indicate that cauliflower plants increased in size and yield in all treatments compared to the control (T0, no amendment). On the one hand, it was observed that the number of leaves evaluated in each experimental unit had more than 14 leaves up to a maximum of 26, results that exceed what was previously reported (Farzana et al., 2016). The height and number of leaves of cauliflower plants did not show significant differences between treatments, unlike previous studies in which height and number of leaves did vary as a function of the amendment used (Farzana et al., 2016). However, the differences may be related to the fact that treatments were applied under nutrient control conditions in pots, unlike the reported applications that were direct applications to the soil, where there is a greater possibility of nutrient leaching and greater root extension depending on the soil condition and the type of amendment added (Farzana et al., 2016). On the other hand, when analyzing the effect of treatments at 120 d, it was observed that the best results in cauliflower development were obtained in the treatment with microalgae+compost (T5) and bovine manure (T5) amendments. The highest yields were observed in the FW and DW of leaves and inflorescences (Tables 6 and 7). However, the greatest importance lies in the fresh inflorescence known as cauliflower flower (Table 6), which is the plant organ that generates commercial interest as food (Farzana et al., 2016).

Regarding total FW and DW the results have a similar trend, the results indicate that both yields increased with all treatments compared to the control (T0, no amendment); however, T1, T5 and T6 produced significantly higher values compared to the other treatments (Tables 6 and 7; $p < 0.05$). Reviewing the effect of traditional chemical treatments (Table 6) on cauliflower plant yield, we observed that FW at T2 (FW: 1170 g plant^{-1}) had a similar effect to those previously described in the literature (1050-1130 g plant^{-1}), while T1 (FW: 1704 g plant^{-1}) produced a significantly higher effect (Jahan et al., 2014). Similarly, FW obtained with the organic treatments, T5 (1:1 mixture microalgae+compost) and T6 (manure), were good and equivalent to the yields obtained with T1 (Table 6; $p > 0.05$), thus demonstrating that it is possible to replace chemical fertilization with sustainable and environmentally friendly organic amendments. Along with the above, T5 and T6 generated better yields of pot-grown cauliflower (Table 6) compared to other studies (900-940 g FW plant^{-1}) where compost, vermicompost and manure were used (Jahan et al., 2014; Priyono et al., 2018). Even, FW results in T5 and T6 were better than results obtained in field-grown cauliflower (1034-2607 g plant^{-1}), where manure and compost were used as fertilizers (Yassen et al., 2019; Abdel Nabi et al., 2020). This information is related to the fact that the crop was grown in pots with low nutrient leaching and that plants were grown independently without suffering from the effect of spacing on soil crops (Farzana et al., 2016).

The fertilization properties of the treatments applied in this study were determined by physicochemical analysis. Where microalgae biomass and compost presented nutrient values (Table 4) similar to those described in the literature (Jimenez et al., 2020; Ajaweed et al., 2022), while cattle manure showed higher nutrient proportions (Table 4).

The pH of microalgae and compost (Table 4) were similar to those described by other research groups, ranging from 6 to 10 (Jimenez et al., 2020; Ajaweed et al., 2022). Specifically, the pH of the compost used in this study (Table 4) was found within the range 6.0-8.5, recommended by the California Compost Quality Council (CCQC) and the British Standards PAS-100 (BSI) for compost used in agricultural application. As for bovine manure, it showed a moderately acidic pH (Table 4), a property that can increase the availability of P in the soil (main limiting factor for cauliflower yield) and increase cauliflower growth (Pedersen et al., 2017), which is closely related to T6, one of the treatments with the highest yield in this study (Tables 6 and 7).

Another important analysis obtained from the physicochemical tests of soil and amendments is the EC, which depends on the amount of dissolved salts, in which N has a prominent impact. Among the chemical species of N, nitrate (NO_3^-) causes a large increase in the salinity of the mixture. In addition, although ammonium produced from urea does not ionize appreciably and is therefore not detected in EC-based salinity measurements, this N source contributes to the osmotic potential or total salt stress experienced by the plant (Bunt, 1988).

Regarding the above, first the analysis of compost EC (3.2 dS m^{-1}; Table 4) was presented within the optimal range for mixed organic materials of 2.2 to 17.5 dS m^{-1} (Al-Turki et al., 2013); however, the result is lower than the upper limit of 4 dS m^{-1} recommended by CCQC and BSI for agricultural use of compost (Al-Turki et al., 2013). The microalgae biomass resulted in an EC of 4.4 dS m^{-1}, higher than the conductivity found by other research groups, which ranged 1.2-3.8 dS m^{-1} and similarly occurred with the EC of cattle manure which was higher than the average 3 dS m^{-1} (Coppens et al., 2016; Zitnik et al., 2019). Although the EC of microalgae and cattle manure was slightly higher, their application did not significantly affect the final soil EC from 2.00 to 2.00-2.01 dS m^{-1}. Considering that cauliflower is moderately tolerant to salinity, with an EC threshold ranging from 1.52-3.20 dS m^{-1}, these results suggest that the EC of organic amendments did not significantly affect cauliflower plant development (Da Costa et al., 2020). This statement is reinforced by the fact that the traditional mixed fertilizer (T1) had nonsignificant differences with T5 and T6 in terms of EC (Table 6; $p > 0.05$).

Second, the maximum amount of mineral N that can be safely added in base fertilizer varies by plant species but is only in the order of 0.20-0.25 g L^{-1} mixture. Quantities exceeding this figure may result in specific toxicity or high salinity risk (Bunt, 1988). Thus, the total N in 40 kg soil, in this study, should not exceed 8-10 g, which is a higher range than the N added in all treatments (Table 5), so N toxicity is not suggested in this case. Furthermore, plant area and number of leaves were positively correlated with fresh biomass and total yield ($r > 0.5$), and all parameters analyzed increased with time. This fact strongly suggests that the amendments used in this work and their EC did not have a negative effect on physiology and normal plant development.

This study indicates that amendments with a mixture of microalgae+compost could replace chemical fertilization for cauliflower cultivation. The results are favorable for the farmer considering that chemical fertilizer prices are high and organic amendments such as those based on microalgae can save 24%-40% of the high cost of chemical fertilizers (Ammar et al., 2022). No comparable studies have been published on cauliflower cultivation using microalgae or a mixture of microalgae+compost as fertilizer. However, the use of microalgae species *Spirulina platensis* as a biofertilizer for growing kailan or chinese kale (*Brassica oleracea* var. *alboglabra*) and pak-choy (*Brassica rapa* subsp. *chinensis*) has been documented (Wuang et al., 2016). The results showed that *S. platensis* improved FW by 110% and DW by 155.8% compared to the control (Wuang et al., 2016). Good results were also obtained by using the microalgae *Monoraphidium* sp. on tomato (*Solanum lycopersicum*) crops, where DW was increased by 32% (Jimenez et al., 2020). Similarly, the use of microalgae as biofertilizer has been tested in lettuce, rice, eggplant, cucumber, resulting in improved plant growth, among other benefits (La Bella et al., 2022).

The results of T5 (microalgae+compost) are not only based on the benefits of microalgae, but also on the synergistic effects provided by microalgae+compost, suggested by the significant difference in yield between T4 (compost) and T5 shown in Tables 6 and 7. On the one hand, it has been reported that green algae and cyanobacteria, positively interfere in plant physiology and in the proliferation of beneficial microbial communities in the soil, stimulating plant growth, thanks to their ability to produce metabolites such as plant hormones and polysaccharides, of high nutritional content, among others (Hamed et al., 2018; Gonçalves, 2021). On the other hand, compost is also characterized for being a promoter of the growth of beneficial soil microorganisms and a disease suppressor (Mehta et al., 2014), in addition, to being a contribution of different macro and micronutrients necessary for plants (Hamed et al., 2018; Gonçalves, 2021). There is a possible interaction between the microorganisms contributed by the compost and the degradation of microalgae, which in turn justifies better fertilization results of the mixture.

Similar result to those of microalgae+compost was obtained with bovine manure, which has been reported as a beneficial fertilizer in light and sandy soils with low C, as is the case of the sandy loam soil used in this research (Risberg et al., 2013). However, manure has the disadvantage of being odorous and incorporating pathogenic microorganisms into the soil if not previously composted (Risberg et al., 2013). Composting manure adds value to this resource by decreasing the negative effects of manure contaminants, such as pathogenic bacteria and antibiotics used in animal production, which represents a microbiological advantage also for the rhizosphere of plants (Staley et al., 2021). Some studies reported that there was nonsignificant effect on pathogenic bacteria counts (2.5-3.2 log count range: CFU g^{-1}), following single application of raw dairy manure or dairy manure-derived fertilizer up to 4 mo prior to vegetable harvest; almost at the limit of the U.S. Food and Drug Administration (FDA) recommendation for biological soil amendments of animal origin (3 log CFU g^{-1} total solids). But, at this point, the real problem is that the presence of pathogenic bacteria such as *Escherichia coli* and antibiotics in an amendment is the perfect breeding ground to promote the selection and success of antibiotic resistant pathogenic bacteria, not only *E. coli* and *Salmonella* spp., but other pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, which are pathogenic in humans and other animals. These bacteria are environmentally versatile and acquire antibiotic resistance genes through horizontal gene transfer mechanisms (Hidalgo et al., 2022), so the initial composting process would be essential to avoid this proliferation.

Another benefit of composting manure is that it accelerates its entry into the soil, more easily balancing soil density. This improves nutrient retention avoiding frequent fertilization; it balances soil pH between 6-8, promoting nutrient availability to plants (Mehta et al., 2014); it promotes healthy plant root development, providing greater resistance to pests and diseases and reducing weed growth (Mehta et al., 2014); in addition, it improves water retention, a great advantage in these days of water scarcity in Chile and around the world.

In summary, the results of these trials stand out from what has been previously reported, since the effect of different amendments on cauliflower yield was evaluated, including a mixture of microalgae with compost not previously evaluated in this crop. It also highlights the ideal requirement of N applied per plant, as usually reported by many fertilization studies, since N is the main macronutrient absorbed by cauliflower during its cultivation, especially in the peak growing season (Priyono et al., 2018). The application of constant N in each treatment allowed to compare the fertilization capacity of the different treatments and to evaluate the possible effects of other properties such as EC.

Finally, the decision to opt for the use of organic amendments instead of chemical fertilization can be based on the one hand, on the various ecosystem analyses in the soil that contemplate multiple benefits such as increased nutrient content and remediation, improved water uptake and increased beneficial microbiology, resulting in higher yields and sustainability of agricultural soils (Farzana et al., 2016; Hale et al., 2021). The most relevant results in this study were obtained with the microalgae plus compost mixture and its application is recommended. In some cases, microalgae-based amendments may have a higher production cost (La Bella et al., 2022). However, scientific advances to reduce costs are growing by leaps and bounds and it has been reported, for example, the production of microalgae using wastewater as a source of nutrients, to improve the production process from nutrient recovery and water recovery from these effluents (La Bella et al., 2022).

CONCLUSIONS

The results of this study demonstrate that the use of bovine manure and microalgae+compost mixture in cauliflower cultivation produce yields similar to those of chemical fertilizers. The latter being widely applied in intensive agriculture. On the other hand, the microalgae+compost mixture produces significantly higher yields than compost alone, suggesting a synergistic effect of microalgae and compost. The advantages of processed amendments such as the microalgae+compost mixture over manure have been exposed, thus demonstrating the feasibility of totally replacing chemical fertilizers with the organic amendments composed of microalgae+compost in the sandy loam soil studied. This type of biofertilizer or biostimulant also provides the multiple benefits reported for amendments, such as the restoration and permanent sustainability of agricultural soils. Finally, this study aims to contribute to the development of environmentally friendly agriculture and to safeguard food security in the study area.

Author contributions

Conceptualization: F.D., R.D., F.H. Methodology: F.D., R.D. Formal analysis: G.V. Research: F.D., R.D., F.H., E.V., G.V. Resources: F.H. Data curation: F.D, R.D. Writing-original draft: G.V. Writing-revising and editing: G.V., E.V. Visualization: G.V. Supervision: G.V., E.V., F.H. Project administration: F.D., R.D., G.V. Funding acquisition: F.H. All coauthors reviewed the final version and approved the manuscript before submission.

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