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RESEARCH & INNOVATION CENTRE

IZC_NEREA Validation

Greenhouse basil, lettuce, gerbera production and chrysanthemum rooting

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Overview

Zeolite is a crystalline hydrated aluminosilicate with a honeycomb structure that has been widely used in multiple industries like water/gas purification, slow release fertilizer production, heavy metal removal and soil amendment. Zeolites are ubiquitous and environmentally friendly, which has attracted attention in recent years owing to their multidisciplinary benefits accrued in agricultural activities. They are negatively charged and have a high cation exchange capacity. Because of their unique porous structure, zeolite can hold water and nutrients in the root zone until plants are ready to utilize them, therefore reducing nutrient leaching[1].

From July to December 2021, Vineland Research and Innovation Center (Vineland) evaluated the use of natural zeolite and NEREA, which is a surface modified zeolite that has been embedded with all the nutrients that plants require. The substrates and mixtures were tested on four different potted plant species in the greenhouse. This report includes the laboratory results for physical and chemical properties testing of NEREA mixtures, as well as 4 individual growing trials, including chrysanthemum rooting, gerbera, lettuce and basil plants.

Objectives

1. Understand the physical and chemical properties of NEREA as a growing substrate

2. Assess the viability of NEREA for rooting vegetative cuttings (chrysanthemum) and compare to industry standard substrate mixes/forms.

3. Validate the NEREA product for potted crop (i.e., lettuce, basil, gerbera) production compared to other commonly used substrates or substrate mixtures, and investigate their optimal irrigation regimes.

Approach

The HYPROP 2 (METER Group, Inc. USA) was used to evaluate the water holding and release capacity of NEREA mixtures (25% NEREA and 50% NEREA mixed with peat moss). The HYPROP 2 took over 100 readings in the 0 to -1 MPa moisture range for volumetric water content and soil water potential via the evaporative method, as the soil gradually dries out over an extended period of time in the lab.

All potted plants trials were conducted in one greenhouse compartment with supplemental lighting (set point at 200 $\mu mol/m^2/s$) using Pro-mix GSX, NEREA and natural zeolite mixtures created by mixing with pre-charged and amended peat moss (Lambert AFM-3) at different

[1] Szerement J., A. Szatanik-Kloc, R. Jarosz, T. Bajda and M. Mierzwa-Hersztek. Contemporary applications of natural and synthetic zeolites from fly ash in agriculture and environmental protection. 2021. Journal of Cleaner Production. 311(2021)-127461.

concentrations. Plants grown under different treatments were either irrigated with fresh water (indicated by +W) or with soluble fertilizer (indicated by +F).

Chrysanthemum ('Breeze Orange') unrooted cuttings were ordered from Ball Seed Company (IL, USA) and were transplanted to seedling trays (1.5" square by 2.25" deep) filled with different percentages of NEREA, zeolite and pro-mix mixtures on Sep. 27th (**Figure 3**). 30 cuttings were grown under each treatment. All trays were soaked with fresh water or fertilizer at 75 ppm before they were put on the bench with a misting system. The system was on 10 seconds every 10 minutes for the first 4 days, and switched to 5 seconds every 10 minutes from 7am-7pm and 5 seconds every 30 minutes during the night. The longest root length, shoot length, root and shoot fresh weight, and root and shoot dry weight were measured at the end of the trial on October 7th.

Gerbera ('Flori Line Maxi') seedlings were ordered from Orchard Park Growers (St. Catharines, ON) and transplanted to 6-inch pots on September 22nd when seedlings had four true leaves. The fertilizer treatments were irrigated on flood tables once a day, while the fresh water treatments were sub irrigated on flood tables twice per day. All gerberas were harvested on November 10th and were measured for total flower count, largest flower diameter, total leaf count, and leaf chlorophyll content using a SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies Inc.). Leaf tissue samples were sent to SGS Laboratories for macronutrient and micronutrient analysis. Substrates were sent out to A&L at the end of the trial for chemical analysis.

Basil (*Ocimum basilicum*, 'Aroma 2') and lettuce (*Lactuca sativa*, 'Rex') were started from seeds on Oct. 8th. At that time, 20-30 basil seeds were sowed directly into 4 inch pots, and 30 pots were grown under each treatment. For lettuce, 15 plants were started directly in 4 inch pots while the other 15 plants were sowed in seedling trays and transplanted to 4 inch pots 24 days after sowing (DAS). A second lettuce trial was started October 22nd to confirm the discrepancy in germination rates between treatments that was found in the first trial. The plants were first misted for 10 seconds every 15 minutes and irrigated using flood tables when seedlings were established. Basil and lettuce plants were harvested on November 9th and November 22nd , respectively, and were measured for plant height, total fresh weight, total dry weight, and chlorophyll content. Macronutrient and micronutrient analysis was conducted on leaf tissue samples either by SGS or A&L Canada Laboratories for basil and lettuce, respectively.

All data was analyzed using R statistical software (Version 1.4.1106) with treatment effects determined by a one-way analysis of variance (ANOVA). The separation of treatment means was performed using the Tukey's honestly significant difference test at $P \leq 0.05$. For all tables and graphs in this report, means sharing the same letter or bars sharing the same letter are not significantly different by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

Results

Physical and chemical properties of NEREA mixture

Bulk density of NEREA substrates increased correspondingly to increased percentages of zeolite/NEREA. A linear regression was generate to determine the substrate bulk density with percentage of zeolite incorporated with peat moss, and can be used to estimate substrate weight (**Figure 1**). The bulk density of the peat-based substrate mixed with 25% NEREA/zeolite was around 3 times higher than when mixed with the same amount of perlite (**Table 1**). For container production, previous research inferred that the degree of water availability has cutoffs of easily available water (between –1 and –5 kPa) and water buffering capacity (between –5 and -10 kPa)[2], with water becoming completely unavailable at -1500 kPa (permanent wilting point). The easily available water and water buffering capacity decreased when zeolite percentages increased in the growing substrates. Most growing media have an air space of 10%-30%, and all zeolite mixtures fell into this recommended range. In general, 25% zeolite provided a similar level of easily available water and water buffering capacity compared to 25% perlite, while further increasing zeolite percentages decreased those water contents and are not recommended for growing substrates.

The combined volume of the aqueous and the gaseous phases of the medium are defined as its total porosity, which is related to is related to the particle shape, size and distribution within the substrates. In general, growing substrates have a total porosity of 70–95%. Increased zeolite percentages generally decreased total porosity, which is potentially correlated to the particle size of zeolite compared to peat moss.

Volumetric water content (VWC) was logged during a 3-day period during the trial. VWC in Pro-mix and 25% NEREA substrate maintained around 35% to 40% and increased by 5% after each irrigation. VWC decreased as percentages of NEREA increased in the substrate, and remained at only 10% with 100% NEREA (**Figure 2**).

[2] Fields. J.S., J.S. Owen and H.L.Scoggins. The influence of substrate hydraulic conductivity on plant water status of an ornamental container crop grown in suboptimal substrate sater potentials. 2017. HortScience. 52(10).

Table 1. Physical properties of peat-based substrates mixed with different percentages of zeolite or perlite.

Substrates [z]	Bulk density (g/cm^3)	Total porosity (%)	Air space (%)	Container capacity (%)	Easily available water (%)	Water buffering capacity (%)	Field capacity (%)	Permanent wilting point (%)
25% Zeolite	0.32	68.47	19.54	48.93	21.73	7.86	19.34	1.36
50% Zeolite	0.52	65.54	10.72	54.82	17.99	6.04	30.79	10.29
70% Zeolite	0.70	55.36	26.10	29.26	13.15	3.46	12.65	3.28
25% Perlite[3]	0.10	78.00	-	68.00	26.4	9.4	-	12.3%

[z] Easily available water refers the amount of water released from water potential of −1 and −5 kPa; water buffering capacity refers the amount of water released from water potential of −5 and −10 kPa; field capacity measures volumetric water content at -10kPa; and permanent wilting point measures volumetric water content -1.5MPa.

[3] Extracted from Londra P., A. Paraskevopoulou and M. Psychogiou. Hydrological behaviour of peat- and coir based substrates and their effect on begonia growth. 2018. Water. 10 (772).

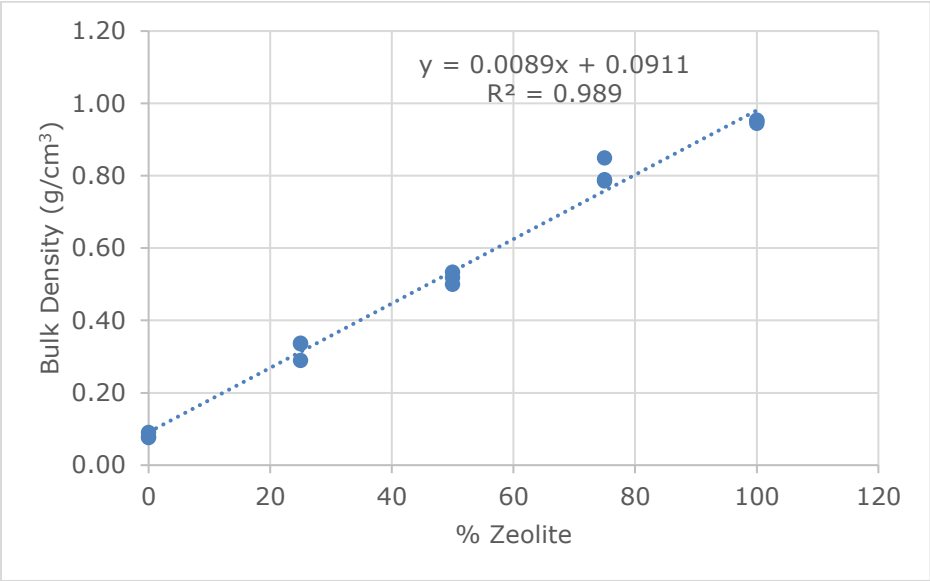


Figure 1. Linear regression between bulk density and percentage of zeolite in zeolite and peat moss mixtures.

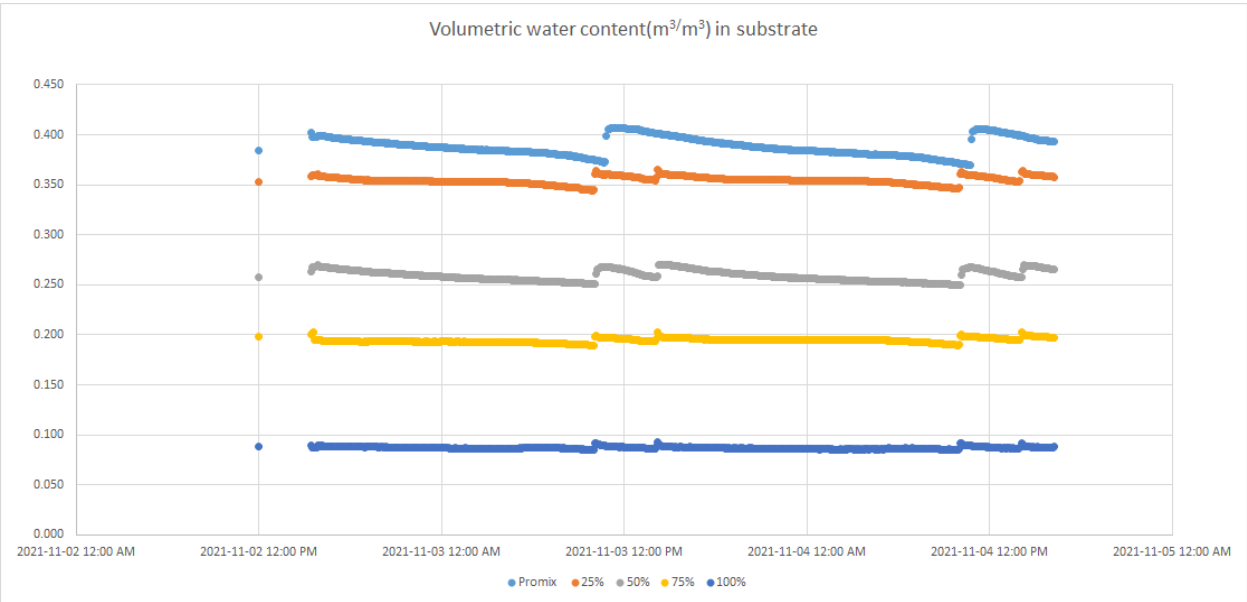


Figure 2. Changes of volumetric water content (VWC) within the substrates before and after irrigation.

The findings demonstrate that 100% NEREA + F still maintained a higher level of cation exchange capacity (CEC), pH, EC and nutrient content compared to 25% NEREA+W and 25% NEREA + F, as well as 25% zeolite + F. The findings demonstrate that 25% NEREA

supplemented with fertilizer throughout the trial resulted in higher EC and nitrate content with substantially lower sodium content (**Table 2**).

Table 2. Chemical tests of different substrate mixtures at the end of the potted plant trial.

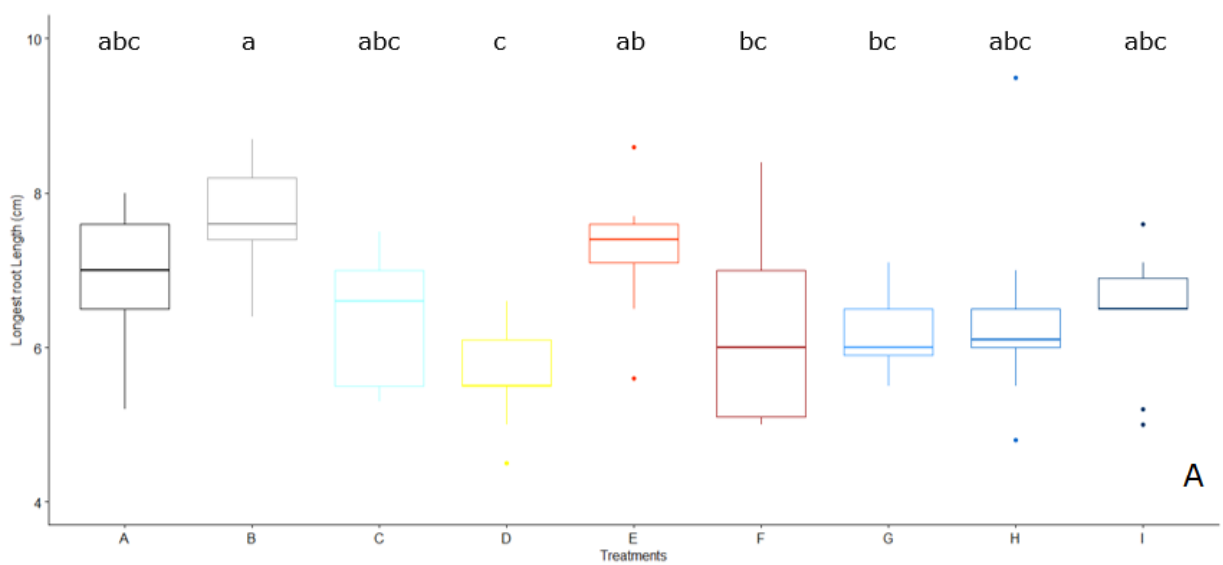
Parameters	**100% NEREA+W**	**25% NEREA+W**	**25% NEREA+F**	**25% Zeolite + F**
CEC meq/100g	50	30.9	28.9	19.6
PH	6.8	5.8	5.4	6.8
SALT	1.42	0.75	1.54	1.18
NO3-N (ppm)	83	10	171	126
NH4-N (ppm)	947	109	284	11
P (ppm)	806	286	330	76
BICARB P (ppm)	174	70	85	25
K (ppm)	3266	873	862	398
Ca (ppm)	4666	2429	2110	1608
Mg (ppm)	403	503	344	353
S (ppm)	718	455	299	169
Na (ppm)	4566	1183	861	641
Zn (ppm)	3.3	2.8	2.6	2.1
Mn (ppm)	53	20	15	14
Fe (ppm)	132	104	84	80
Cu (ppm)	0.5	0.3	0.3	0.4
B (ppm)	0.1	0.1	0.8	1

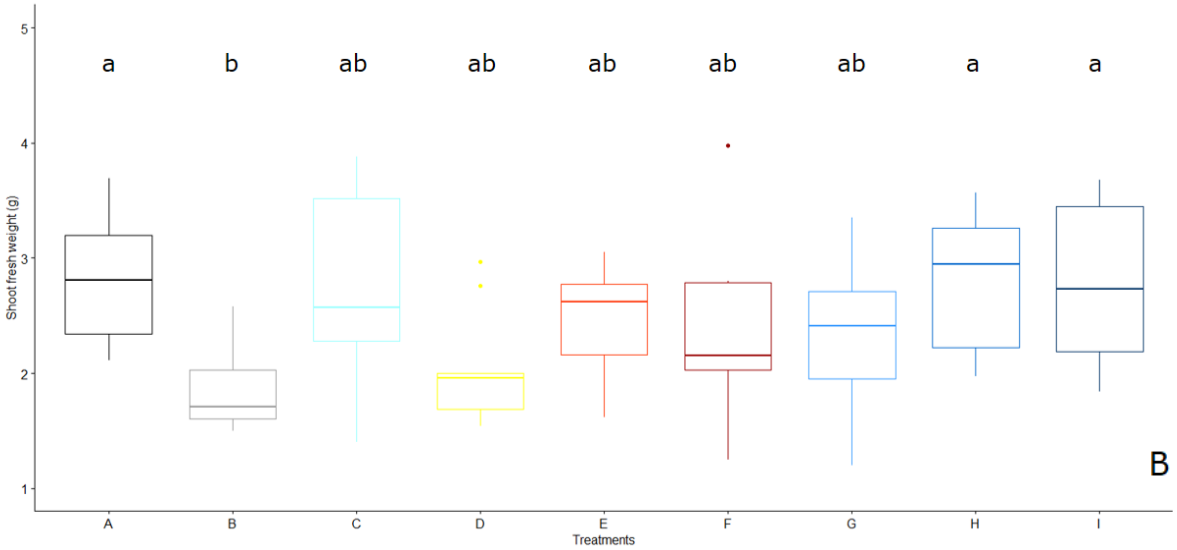
Chrysanthemum rooting trial

The chrysanthemum rooting trial was conducted from September 27th to October 7th at Vineland. By the end of the trial, 50% NEREA + F and 75% NEREA + W promoted shoot length by 18% and 17% compared to QuickPlug, respectively. The longest shoot length was not affected when cuttings were rooted in QuickPlug, Pro-mix, 25% NEREA+F or 50% zeolite + F, 50% or 75% NEREA with fresh water (**Figure 3.B**). However, cuttings grown with 25% zeolite or 100% zeolite with fertilizer, or 25% NEREA with water had a shorter longest root compared to Pro-mix (**Figure 3.A**). QuickPlug, 50% NEREA and 75% NEREA with water promoted shoot fresh weight by 53%-54% compared to Pro-mix. QuickPlug promoted root fresh weight by 45%, 52% and 52% compared to 25% NEREA with fertilizer, 50% NEREA and 75% NEREA with water, respectively. There was no difference on shoot or root dry weight among all the zeolite and NEREA mixtures with Promix, while Quickplug generally promoted shoot and root dry weight compared to the other treatments.

Table 3. Summary of treatments in chrysanthemum rooting trial.

A	B	C	D	E	F	G	H	I
Quick plug	Pro-mix PGX + F	25% NEREA + F	25% Zeolite + F	50% Zeolite + F	100% Zeolite + F	25% NEREA + W	50% NEREA + W	75% NEREA + W

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Shoot fresh weight (g)
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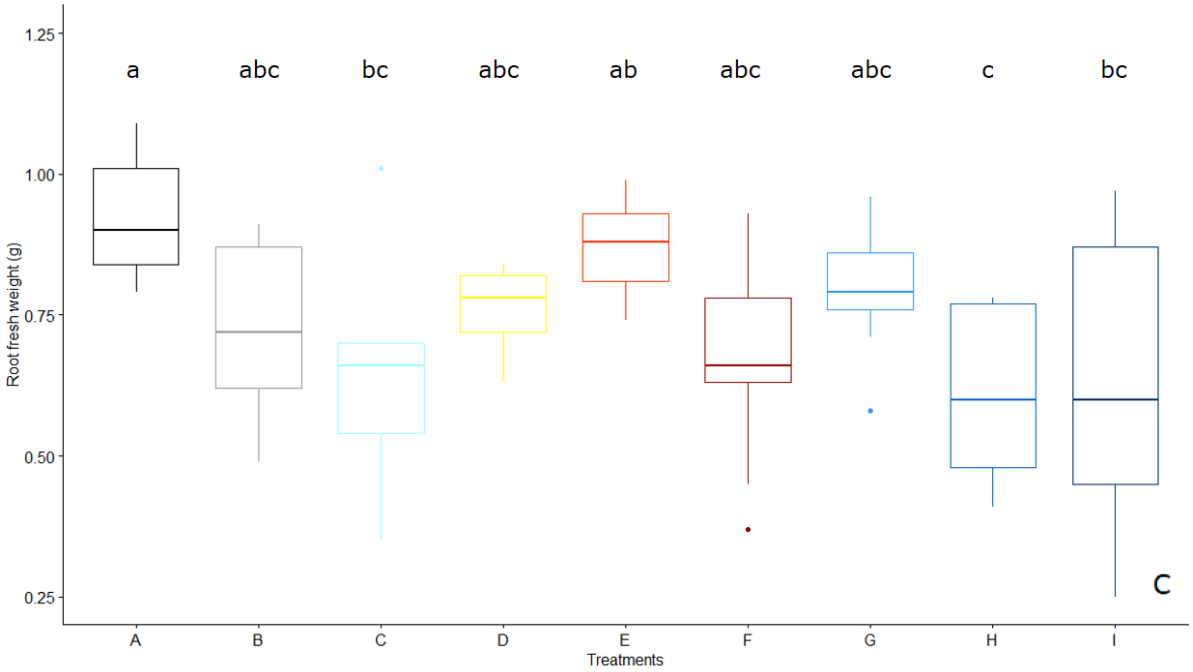
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Root fresh weight (g)
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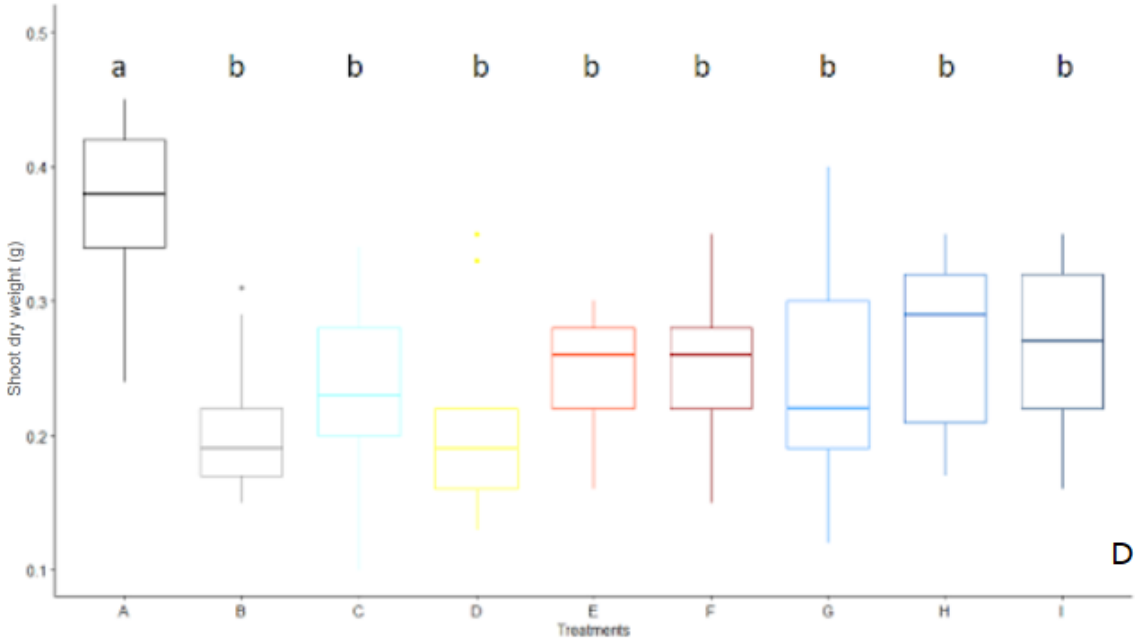
Treatments



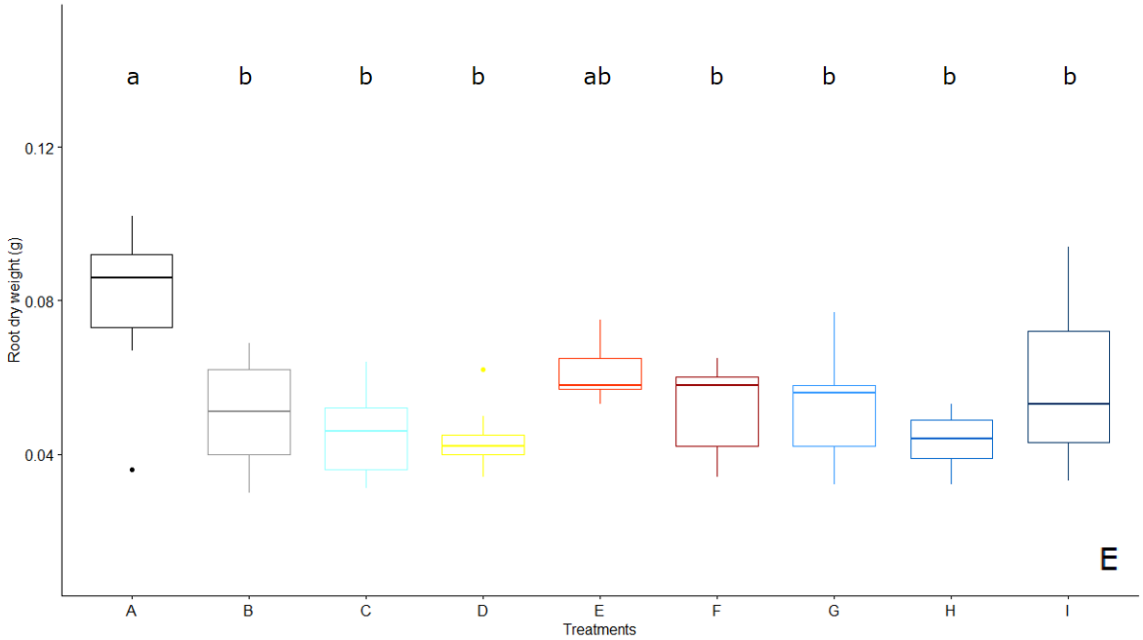


Figure 4. Longest root length (A), shoot fresh weight (B), root fresh weight (C) , shoot dry weight (D) and root dry weight (E) of chrysanthemum cuttings grown in different substrate mixtures. Bars sharing the same letter are not significantly different by Tukey's honestly significant (HSD) difference test at P ≤ 0.05.

Gerbera trial

The Gerbera trial was conducted from September 22nd to November 10th 2021 at Vineland using hydroponic flood tables. During the establishment stage after transplanting, gerberas grown under 75% and 100% NEREA were hand watered at least once in addition to the regular irrigation schedule.

First flower was initiated under 25% NEREA+F and 50% NEREA+W 21 days after transplanting. All 25% NEREA+F plants (25/25) had flowers when harvested, followed by Pro-mix and 25% NEREA+W (**Figure 4**). The total number of leaves per plant was not significantly different under each treatment. The diameter of the flowers was the largest under 25% NEREA+F as well, which could be 29% to 60% larger compared to 25% zeolite+F, 25% NEREA+W and 100% NEREA+W. 25% NEREA +F promoted flowering time, while maintaining comparable numbers of flowers, numbers of leaves and chlorophyll content index compared to Pro-mix under the same fertigation regimes. Using 25% NEREA with 75% peat moss, irrigated with fertilizer promoted gerbera days to flower, flower diameter as well as CCI compared to the same substrate mixture with only fresh water (**Table 4**). The results of gerbera leaf tissue analyses were summarized in **Table 5**. Comparing the treatments that resulted in the most flowered plants at the end of the trial, 25% NEREA+F increased phosphorus and manganese content, and decreased copper content compared to Promix + F.

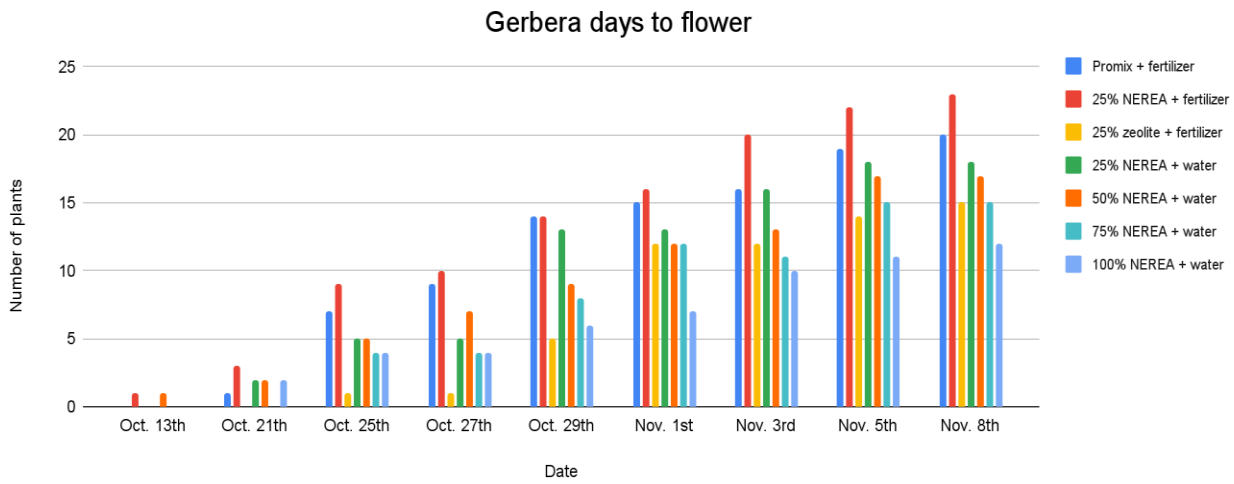


Figure 4. Number of gerbera flowering plants under each treatment from Oct. 13th to Nov. 8th.

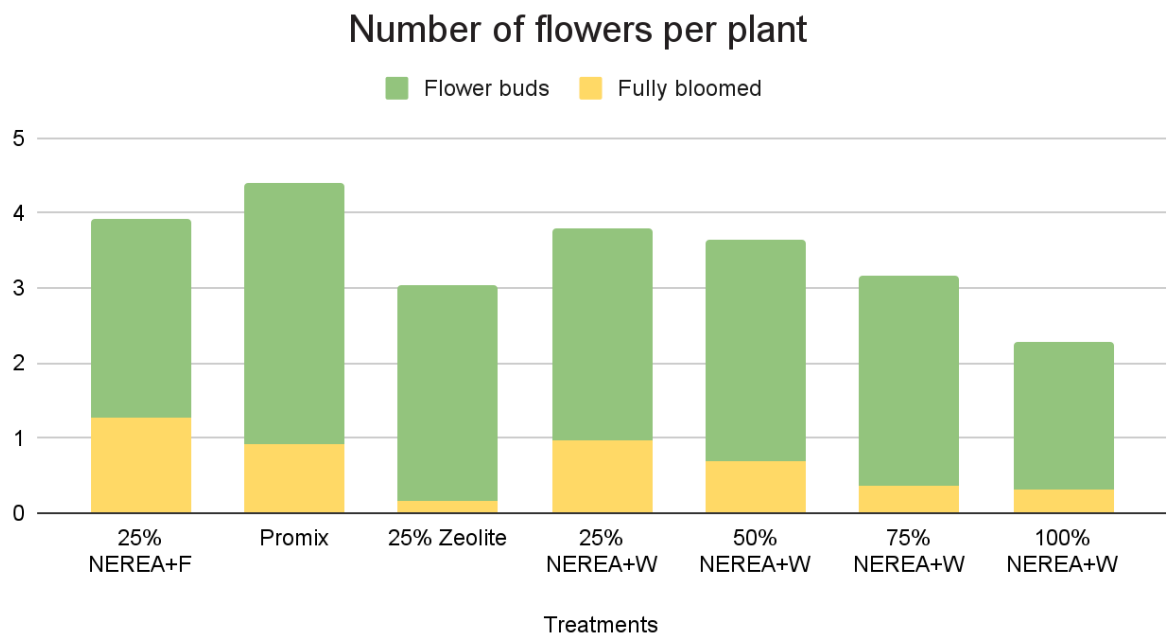


Figure 5. Number of fully bloomed flowers and flower buds under each treatment when harvest.

Table 4. Average leaf count, flower diameter and chlorophyll content under each treatment when harvest.

Treatments	Total number of leaves	Flower diameter (cm)	SPAD
25% NEREA+F	19.50 a	11.50 a	62.69 a
Promix+F	18.25 a	9.83 ab	57.72 ab
25% Zeolite+F	16.17 a	7.20 c	47.85 c
25% NEREA+W	20.58 a	8.92 bc	54.88 bc
50% NEREA+W	20.58 a	9.75 ab	54.01 bc
75% NEREA+W	16.25 a	9.69 abc	52.38 bc
100% NEREA+W	20.08 a	8.25 bc	54.18 bc

Table 5. Macronutrients (%) and micronutrient (ppm) content of gerbera leaves grown under different growing substrates (2 samples from each treatment).

Treatment	N%	P%	K%	Mg%	Ca%	S%	Zn (ppm)	Mn (ppm)	Cu (ppm)	Fe (ppm)	B (ppm)
25% NEREA+W	3.58	1.01	3.42	0.60	1.68	0.22	44.26	340.56	1.47	111.08	23.85
50% NEREA+W	4.03	1.12	3.80	0.49	1.58	0.22	40.99	354.93	1.83	96.46	20.91
75% NEREA+W	3.83	1.29	2.98	0.51	1.91	0.20	32.98	465.73	1.60	59.15	18.36
100% NEREA+W	3.90	0.91	2.96	0.53	1.52	0.19	18.77	207.91	2.38	101.52	9.44
Promix+F	3.89	0.48	4.59	0.34	1.32	0.20	48.22	209.46	5.79	210.31	23.80
25% NEREA+F	3.96	0.96	3.97	0.59	1.59	0.20	42.08	308.67	2.04	295.46	27.79
25% ZEOLITE+F	4.46	0.56	4.27	0.60	1.16	0.22	27.93	86.00	1.075	81.87	25.36

Basil trial

Basil plants grown under 25% NEREA+W and 50% NEREA+W were significantly taller than Promix+F and 25% zeolite+F. The fresh weight per pot was significantly higher under 25% NEREA+F compared to 25% zeolite+F (**Figure 6**), indicating natural zeolite is not the appropriate substrate component for greenhouse production. The nutrients from the fertilizer may have charged and been held in the natural zeolite, instead of being used by the plants. Also, the pH of natural zeolite was higher compared to NEREA substrates (**Table 2**), posing potential limitations for plant nutrient uptake. Although not statistically significantly different, basil grown under 25% NEREA+W still had an averaged 12.4%-33.5% increase compared to all other treatments. Plants grown under 100% zeolite had 18.2%-26.6% higher chlorophyll content index compared to all other treatments. Compared to Pro-mix plants that were fertigated, plants grown under 25% NEREA with only fresh water had a 31% higher fresh weight, and were 25% taller with the same level of chlorophyll content. However, it is worth noting that the plants grown with Pro-mix were more uniform than all other NEREA and zeolite treatments.

Plants grown under 25% NEREA+W had comparable nutrient contents compared to Promix+F, except a decrease in copper content. Additional fertilizer (25% NEREA+F) further increased zinc and iron contents in the leaf tissue while it decreased manganese content. Plants grown under 100% NEREA+W had substantially lower zinc, manganese and boron contents compared to other NEREA treatments (**Table 6 and 7**).

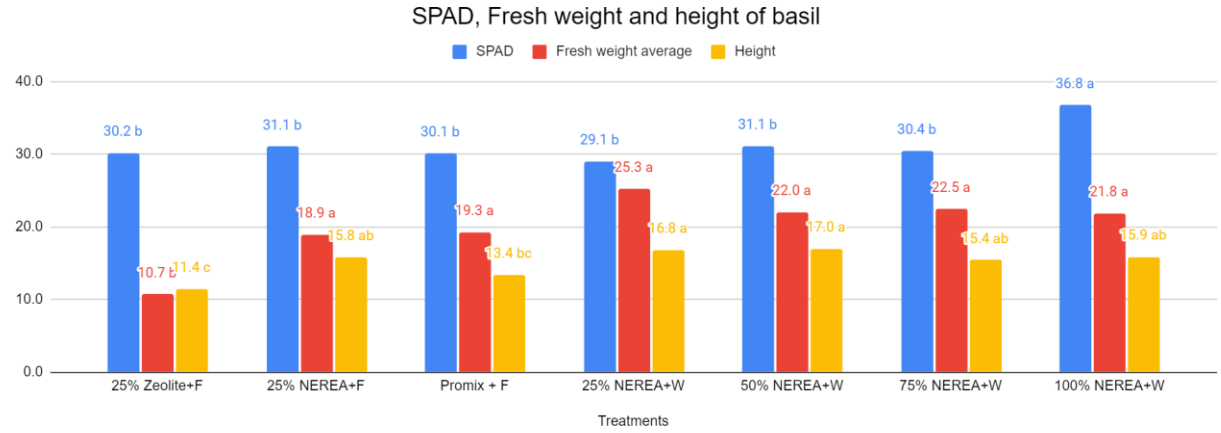


Figure 6. Average fresh weight, height, and chlorophyll content of basil plants under each treatment when harvested.

Table 6. Macronutrients content (%) of basil leaves grown under different growing substrates (2 samples from each treatment).

Treatments	N%	P%	K%	Mg%	Ca%	S%
25% Promix+F	5.81	1.08	7.25	0.565	2.4	0.42
25% NEREA+F	5.585	1.615	6.875	0.795	2.14	0.375
25% NEREA+W	5.255	1.26	6.62	0.715	2.045	0.355
50% NEREA+W	5.59	1.3	6.5	0.69	2.125	0.385
75% NEREA+W	5.92	1.32	6.07	0.595	2.105	0.38
100% NEAREA+W	4.53	1.11	5.18	0.345	1.415	0.415
Reference range	(3.25-5)	(0.4-0.85)	(5.5-8)	(0.4-0.85)	(1.25-2.5)	(0.35-0.75)

Table 7. Micronutrients content (ppm) of basil leaves grown under different growing substrates (2 samples from each treatment).

Treatments	Zn	Mn	Cu	Fe	B
25% Promix+F	103.105	51.025	8.965	140.865	23.875
25% NEREA+F	120.765	145.19	3.13	187.91	24.305
25% NEREA+W	88.575	259.515	3.75	130.755	29.82
50% NEREA+W	79.735	275.82	4.56	140.115	27.015
75% NEREA+W	65.065	255.1	6.285	142.5	21.7
100% NEAREA+W	31.625	142.845	6.705	161.79	12.695
Reference range	(30-50)	(25-200)	(8-30)	(60-200)	(25-50)

Lettuce trial

Lettuce seeds had the highest germination rate under 100% NEREA+W in both trays and pots. 25% NEREA+W resulted in the highest fresh weight per plant among all treatments, which averaged at 102g and was 33% higher compared to plants grown with Pro-mix and irrigated fertilizer. This was also 41% and 74% higher compared to 100% NEREA+W and 25% zeolite+F (**Figure 7**). It is worth mentioning that the fresh weight of 25% NEREA+W was significantly higher for plants directly sowed in pots compared to plants that were propagated in trays (**Figure 8**), which can be taken into consideration to reduce labor cost. The plant size, including height and width, were not significantly different among 25% NEREA+F, 50% NEREA+F, 75% NEREA+F and Pro-mix. Plants grown under high percentages of NEREA (e.g., 75% and 100%) had higher chlorophyll content index than plants grown in Promix+F, 25% NEREA+F or 25% zeolite+F (**Figure 9**).

Leaf tissue analyses indicated that NEREA substrate mixtures, even at 25% with fresh water, could provide all necessary nutrients plants need throughout the growth period of 45 days.

However, lettuce grown under NEREA substrates might have excessive levels of sodium, manganese and iron (**Figure 10**).

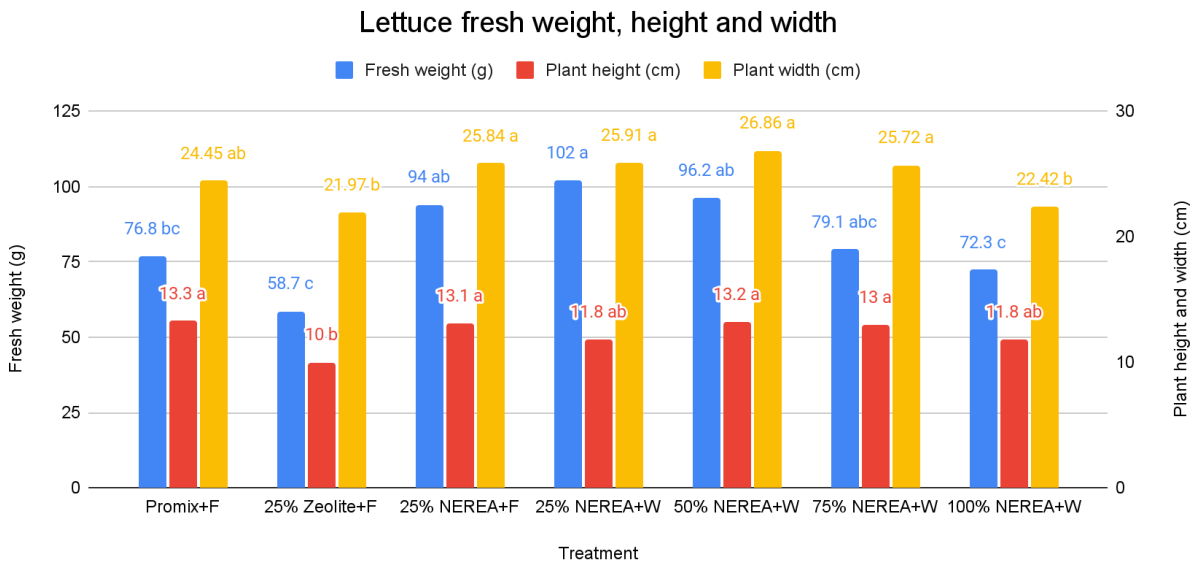


Figure 7. Fresh weight, height and width of lettuce grown plants under each treatment when harvested.

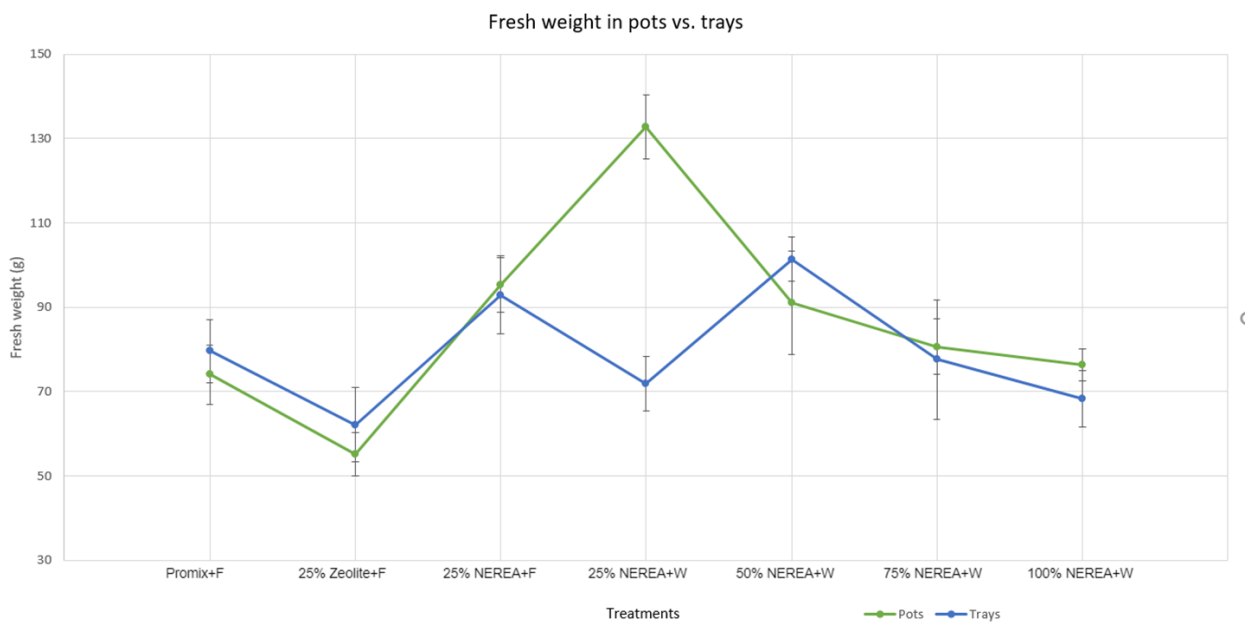


Figure 8. Fresh weight of lettuce grown directly in pots compared to transplanted under different substrate treatments.

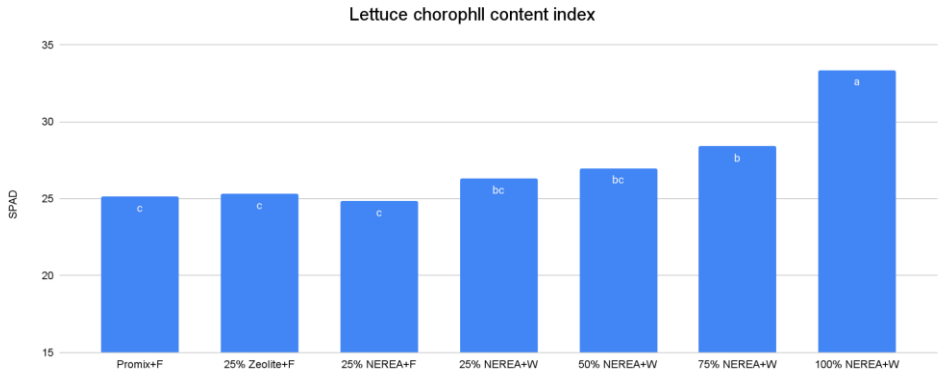
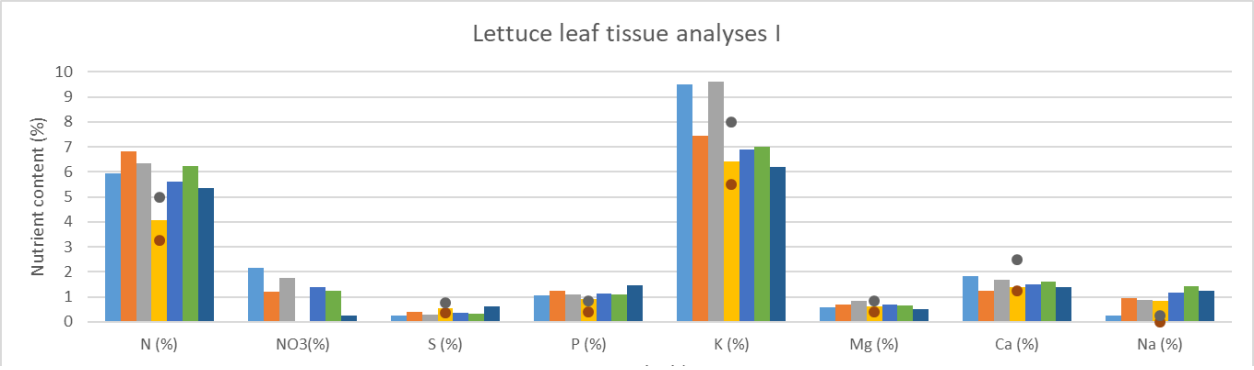


Figure 9. Chlorophyll content index of lettuce grown plants under each substrate treatment.



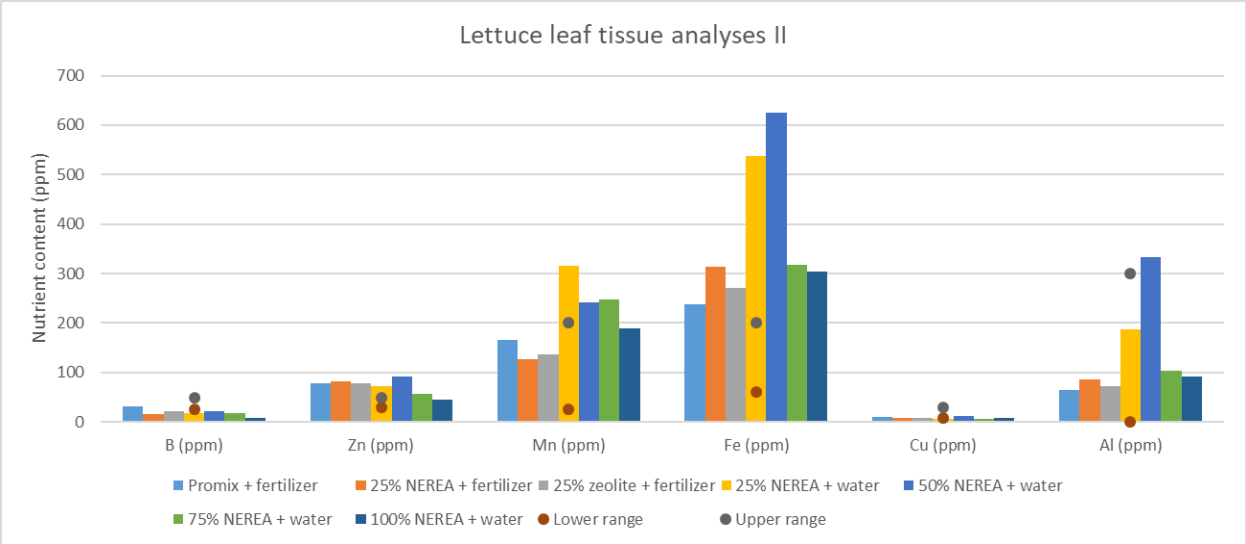


Figure 10. Lettuce leaf tissue analyses (I and II) under each substrate treatment.

Summary

The success of the 25% NEREA mixtures throughout our trials suggested a benefit to using NEREA as a substitute for perlite in peat moss based substrate mixtures. The findings

demonstrate that 25% NEREA provided all the plant essential nutrients required for the growth of basil and lettuce. Considering that basil and lettuce plants grown in a 25% NEREA mixture with only fresh water had comparable if not higher fresh weight compared to Pro-mix, replacing perlite with NEREA could potentially save the cost for fertilizer while improving the crop yield and quality at the same time. Although 100% NEREA promoted chlorophyll content index in both basil and lettuce, higher percentages of NEREA are generally not recommended as they increase water consumption and pot weight significantly, and could compromise plant growth metrics.

The benefits of irrigating the 25% NEREA and 75% peat moss mixtures with water versus fertilizer varied between species. The results indicate that 25% NEREA + F was the most successful treatment in the gerbera trial in terms of days to flower and flower diameter, while being comparable with Pro-mix in terms of flowers per plant, leaves per plant and chlorophyll content. Further, 25% NEREA+W did not perform as well throughout the gerbera trial, and had a significantly smaller average flower diameter than both Pro-mix and 25% NEREA+F. However, when looking at both the lettuce and basil trials, 25% NEREA+W performed comparably well if not better than the Pro-mix+F and 25% NEREA+F treatments, especially during seedling stage. This difference when looking species to species could be related to basil and lettuce being more vegetative growth focused crops, while in the gerbera trial performance was heavily determined by flower growth. The results indicate that 25% NEREA+W mixture could be a productive substrate for leafy green crop production, while allowing growers to reduce fertilizer inputs. However, certain mineral nutrient contents, like sodium, could be potentially too high and need to be considered when making recommendations on use.

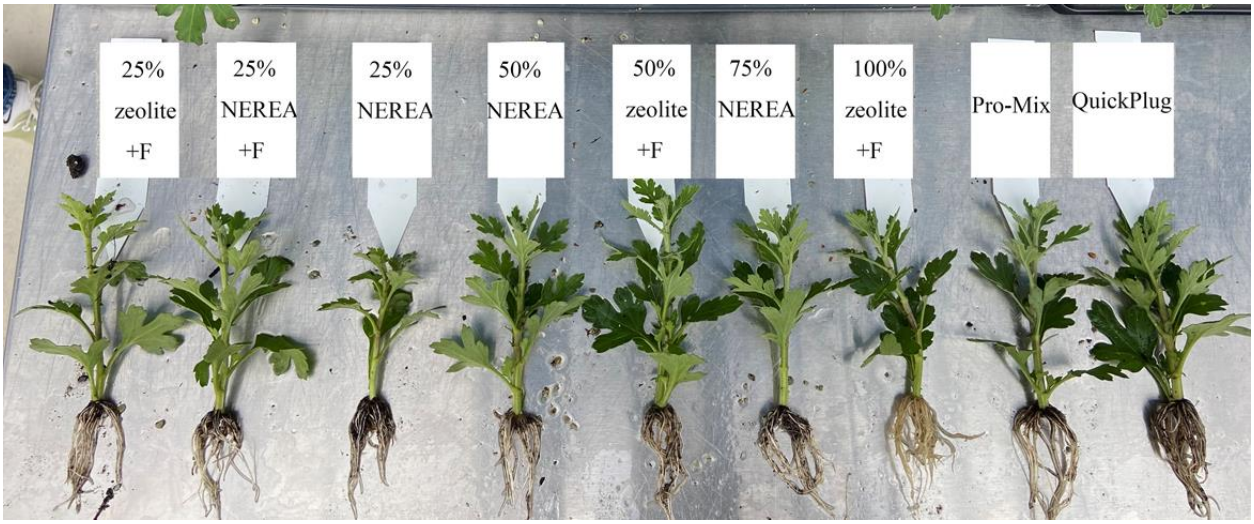
Natural zeolite was generally not recommended for greenhouse plant production, indicated by a lower flowering rate, fresh weight and nutrient content in various crops. Soluble fertilizer could have charged the zeolite, therefore becoming unavailable for plant growth and development.

When comparing lettuce plants sown directly in pots versus plants sown in trays and transplanted, the average fresh weights were not significantly different across all treatments except for 25% NEREA+W, which had a significantly higher average fresh weight for plants started in pots versus trays. This suggests that when growing lettuce in NEREA and peat moss mixtures, or Pro-mix, it may not be worth the labour to start lettuce in trays and then transplant them to pots, especially considering the increased weight of the NEREA mixtures.

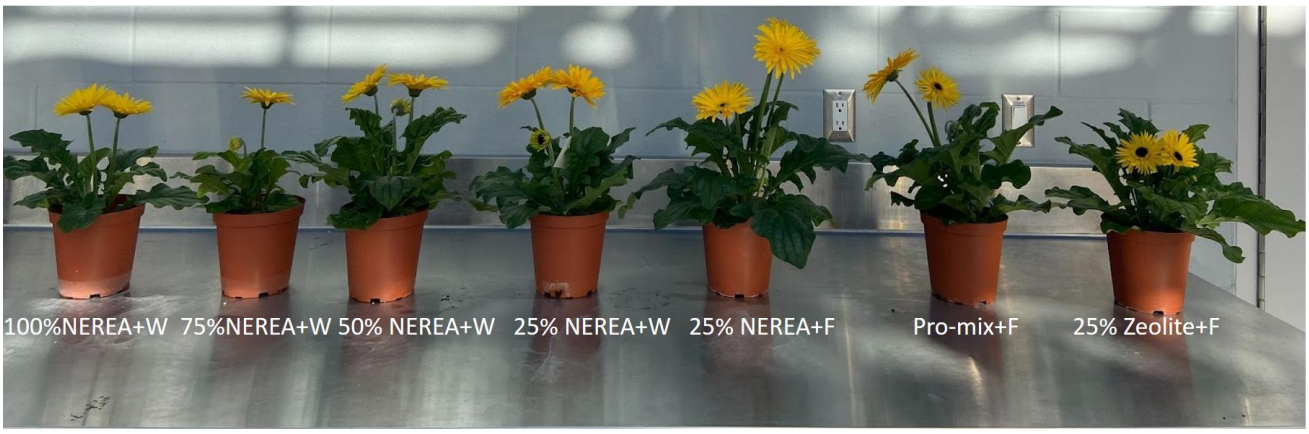
Overall, 25% NEREA and 75% peat moss, with or without fertilizer depending on species, can be used for potted plant production in greenhouses with the potential of promoting yield and quality, as well as shortening the crop cycle. The capability of recharging and reusing zeolite after the crop cycle should also be considered, which could potentially improve input use efficiency, and decrease energy consumption and greenhouse gas emissions related to horticultural practices.

Appendix. Photos monitored during the trials

I. Chrysanthemum rooted under different substrate mixtures after 10 days

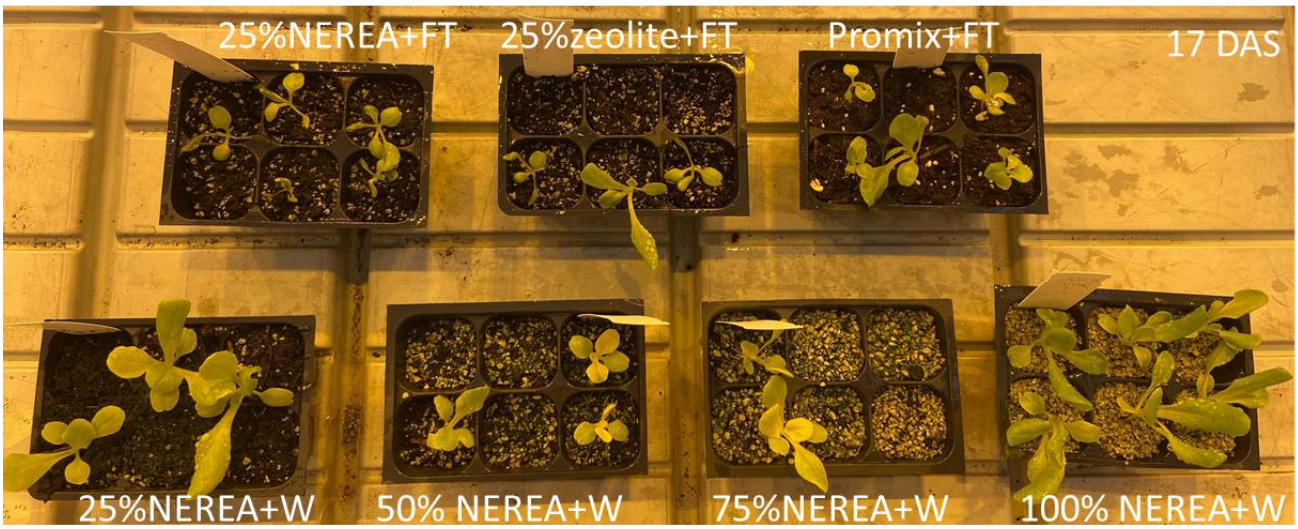


II. Gerbera grown under different substrate mixtures at harvest (49 DAT)



III. Basil grown under different substrate mixtures 17 days and 31 days after sowing

IIII. Lettuce grown under different substrate mixtures 17, 33 and 45 days after sowing.



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