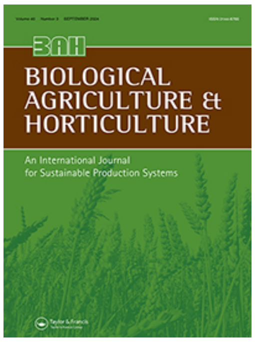

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RESEARCH ARTICLE

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The use of mineral dynamised high dilutions for natural plant biostimulation; effects on plant growth, crop production, fruit quality, pest and disease incidence in agroecological strawberry cultivation

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ABSTRACT
Strawberries (*Fragaria* x *ananassa)* are one of the most important fruit crops worldwide. Most of the strawberries that are produced today rely on the use of pesticides and fungicides, leading the crop to be listed as one of the most contaminated by contentious inputs. Plant biostimulants are used as a plant health strategy focused on stimulating plant growth and development with a low residual impact. The objective of this study was to assess the plant biostimulation effects of strawberry plants treated with mineral dynamised high dilutions (DHDs): *Sulphur* 12CH, *Phosphorus* 12CH, *Kali carbonicun* 12CH, *Calcarea carbonica* 12CH, *Silicea terra* 12CH, *Natrum muriaticum* 12CH and *Mercurius solubilis* 12 CH, distilled water 12CH and distilled water (control). The experiment was undertaken under greenhouse conditions at the University of Santa Catarina State (UDESC), in Lages, Santa Catarina (SC), Brazil in 2019 and was repeated in 2021 at the Federal University of Santa Catarina (UFSC), in Curitibanos, SC. The experiment used a randomised block design, following double-blind treatment application. Data were analysed by ANOVA and when significant ($p \leq 0.05$) by Dunnett's test. The results showed that plants treated with DHDs of *Sulphur* 12CH, *Phosphorus* 12CH and *Kali carbonicum* 12CH increased plant growth and crop yield and were less affected by leaf spot disease (*Mycosphaerella fragariae*). Plants treated with *Silicea terra* 12CH, or with *Calcarea carbonica* 12CH showed increased development of the root system. The results obtained suggested that mineral DHDs could be used as plant biostimulants to support agroecological strawberry production.

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Introduction

Global importance of strawberry cropping systems

The strawberry (*Fragaria x ananassa)* is one of the most important fruit crops in the world (Antunes et al. 2020). This status reflects the socio-economic relevance of the crop for both

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human nutrition and trade at different scales (Simpson 2018). The fruit not only has an attractive, vivid colour, inviting aroma and sweet taste, but also has an abundance of vitamin C and antioxidants that offer health benefits to the consumer. In particular, fruit consumption has been reported to contribute to the preservation of bones, teeth, gums, and blood vessels (Giampieri et al. 2017). Strawberry cropping systems also have a significant social impact, as the sector creates employment for a large number of workers at all production scales (Antunes and Peres 2013). In particular, the crop represents an important source of income for family farmers, as production aggregates value even under small scale conditions (Fagherazzi et al. 2021). These attributes have rendered strawberry cultivation a favourable position in the fruticulture sector, in both local and global markets (Kumar et al. 2018). Although the strawberry market encompasses primarily fresh fruit, an additional strong demand exists in the processing industry for products like ice cream, jams, jellies, dried snacks, syrups, teas, juices and cosmetics (Jeong et al. 2016).

Native to South America, strawberries are grown commercially in 76 countries across more than 522,527 ha. The cultivated area, according to Simpson (2018), increased by 40% from 2013 to 2018 and in 2020 exports of strawberries totalled 2.6 billion USD. Farming enterprises dedicated to strawberry cultivation have an average size of 0.5 ha to 2.5 ha, however, larger areas of monoculture cultivation can exceed 15 ha or more (Antunes et al. 2020). In terms of fruit production, average strawberry yields range from 15 t ha^{-1} to 51 t ha^{-1}, while annual fruit consumption ranges from 1.5 kg to 5 kg $person^{-1}$ (Simpson 2018).

Contentious inputs used in conventional strawberry crop systems

Most of the strawberries farmed today are industrially cultivated with a reliance on the use of fossil fuels, pesticides, herbicides, fungicides and soluble fertilisers (Sójka et al. 2015). The use of pesticides in the management of the crop is particularly concerning (Jardim and Caldas 2012), leading the fruit to be listed among the most contaminated by pesticide residues worldwide (Jardim and Caldas 2012; Sójka et al. 2015; Fraga 2020). In conventional systems, management practices include an average use of 45 pesticide-sprays per year or season (Gonçalves et al. 2016; Song et al. 2020). For instance, in the United States, the main global strawberry producer, the crop is the fourth-highest pesticide user among all Californian crops, using of over 40 kg ha^{-1} $year^{-1}$ (CADPR 2014).

Over recent decades, research has shown that pesticide residues, including those found in strawberries, can negatively affect both human and environmental health (Blair et al. 2015). Evidence links the ingestion of pesticides to short- and long-term health problems, including neurological deterioration, reproductive and fertility issues, learning and developmental disabilities, cancer, endocrine disruption, and metabolic disorders (Arcury and Quandt 2003; Li et al. 2011; Bombardi 2017; Zhang et al. 2018; Rani et al. 2021). This deleterious contamination by pesticide residues is not exclusively a problem of the fresh fruit; evidence shows that the dietary risk to consumers from conventional strawberry by-products such as jams is equally comparable (Mahmood et al. 2016). The growth of pesticide residues through the trophic levels increases the risk of poisoning via the food chain and water supplies (Saxton 2015).

Therefore, there are both environmental and agricultural needs to search for more sustainable alternatives through the use of eco-friendly methods as well as through exploring the salutogenic approaches in agriculture and food systems (Antonovsky 1996; Gliessman 2006). This is recognised as a core path for transformative developments in sustainable agriculture (Pimbert 2017).

The use of dynamised high dilutions (DHDs) in agriculture: an agroecological biostimulation approach

Amongst the suite of agroecological methods to promote health in agriculture, the use of homoeopathic preparations can play a significant role in increasing farm sustainability (Cordoba

Correoso et al. 2022). The term dynamised high dilution (DHD) defines any solution that has been subjected to the technique of sequential dilutions and succussions, whereby the solution is shaken vigorously between each dilution. This potentisation or dynamisation process is derived from the homoeopathic pharmacotechnic (Brasil 2011a).

According to Deboni et al. (2021) and Boff et al. (2021), the utilisation of dynamised high dilutions (DHD) in agriculture has the potential to enhance an organism's innate capacity for growth, development, and resilience to environmental stressors in the farmed environment. Taking a systems approach (Bertalanffy 1971), this method was first developed to treat humans, and over time started to be used to treat animals, and more recently plants. In 2004, the use of the homoeopathic methodology (Hahnemann 2001) was certified by UNESCO as an effective social and therapeutic approach, that has the potential to solve health problems linked in particular to ecology and environment (Kohler and Negrão 2018). The use of homoeopathy in agriculture and the application of dynamised high dilutions is approved within organic and biodynamic standards in agriculture in Brazil (Brasil 2011b; MAPA 2014), and in other countries the method is approved in accordance with regional or private company organic certification regulations (USDA 2010; EU 2014).

The research on the potential of DHDs has been explored across multiple disciplines including biology, physics, agriculture, veterinary studies, ecology and sociology (Rey 2003; Roy et al. 2005; Giesel et al. 2017; Russo et al. 2018; Abasolo-Pacheco et al. 2020). Applying homoeopathic preparations requires a different rational than for technologies that are solely focused on maximising production factors (Moreno 2017; Boff et al. 2021). This is because these preparations function to restore the homoeostasis of living organisms, stimulating natural morphophysiological processes in plants and animals (Andrade and Casali 2011). This can be termed the salutogenic approach (Antonovsky 1996) and is concerned with building resilience and vitality. The use of DHDs in agriculture offers such a salutogenic approach to enhance the performance of food growing systems, meaning that its epistemology focuses on understanding the means of health promotion of the organism rather than the means of disease control. This sociological, therapeutic and ecological change in the perspective (Kohler and Negrão 2018) exemplifies how the use of DHDs can contribute to the management of agroecosystems, particularly through biostimulation of both the organism and the agroecosystem that supports the establishment of its homoeostasis (Giesel et al. 2017).

A number of empirical studies build evidence of the positive impact of DHDs for the management of crop diseases. Deboni et al. (2021) demonstrated that DHDs of *Silicea terra* 12CH and *Sulphur* 12CH stimulated the production of biochemical resistance elicitors in beans, thus strengthening the plant's vigour and natural defence system. In a study on peas (*Pisum sativum*), DHDs increased seed germination, seedling development and photosynthetic activity (Panda et al. 2013). Pulido et al. (2017), observed that DHDs of *Sulphur* 12CH increased broccoli shoot mass from 23% to 37%, and in the potency 60CH it increased root mass by 59%. Another study by Domingues et al. (2019) verified the biostimulation effect on yerba mate (*Ilex paraguariensis* A. St.-Hill.) with DHDs supporting the regrowth of the plants after pruning. Other studies observed that DHDs of *Calcarea carbonica*, *Silicea terra* and *Sulphur* influenced the biostimulation effect through the increase in elicitors of peroxidase, catalase, kinase, β-1-3-glucanase and phytoalexins, thereby inducing the biochemical defence mechanism of bean plants (Oliveira et al. 2014). A similar effect was observed in in vitro studies, where DHD of *Natrum muriaticum* 5CH inhibited the growth of the fungus *Aspergillus niger* by 66% (Gama et al. 2015). In field trials, bean plants treated with *Sulfur* at 12 and 30CH, and *Propolis* 12CH reduced the disease progression curve from 66% down to 49% (Toledo et al. 2015). Another interesting observation highlighted by Moreno (2017) was that the DHDs have a small residual impact, hence they can support the phasing out of contentious inputs in agriculture in order to avoid polluting or contaminating food systems. Other research evidence on the use of DHDs for improving yields include in beans (*Phaseolus vulgaris*) (Pulido et al. 2014), rice (*Oryza* *sativa*) (Verdi et al. 2020), and other horticultural plants (Teixeira and Smtpg 2017). However, and not withstanding this small number of promising findings, DHDs are scarcely used in agriculture.

Theories underpinning the mechanisms of DHDs

The paucity of incidences of application of DHDs in agriculture, as noted above, is influenced by the lack of understanding of their operating mechanisms and the corresponding, anomalous theoretical bases that underpin these mechanisms. This holds true even among the agroecological and organic scientific sectors. So, whilst the objective of this paper is to present results of empirical trials, it acknowledges the relevance and importance of providing an overview of the suggested theoretical foundations. These theories explore post-materialism, complexity and systems concepts and non-linear models in relation to agriculture (Capra and Luisi 2014; Boff et al. 2021).

A popular, common argument used to explain away the efficacy of DHDs is the placebo effect. When dynamising a solution via the serial method of agitations and dilutions, the degree of dilution may exceed Avogadro's constant and according to classical biochemistry this ought to limit the action of the preparation (Chikramane et al. 2010), and this fuels the argument that a placebo effect is involved when a successful result is achieved (Mulet 2018). However, empirically based, scientific research shows otherwise. Despite the alleged absence of chemical molecules of the original active ingredients, the biological effects on plants (Ücker et al. 2020), animals and humans (Rupp et al. 2012; Weiermayer et al. 2022), have been repeatedly observed. Furthermore, the use of double-blind animal and plant trials have eliminated the possibility of a placebo effect (Bonamin 2014; Jäger et al. 2015). Therefore, the bio-stimulation effect observed in biological systems allows for the hypothesis that the curative property of the homoeopathic preparation may be preserved in the DHDs (Rey 2003; Roy et al. 2005; Bonamin 2008; Russo et al. 2018). If this is the case, the active properties of the remedies may be preserved and reliably identifiable, matching the characteristics of the original starting material. Supporting this hypothesis, Chikramane et al. (2010) confirmed the presence of nano-colloidal structures in the form of clusters in the DHDs' structure using electron microscopy and emission spectroscopy. These nanobubbles contained gaseous inclusions of oxygen, nitrogen and carbon dioxide in addition to silica and trace elements of the starting material of the DHDs. In this way, the water molecules may serve as nucleation centres, amplifying the formation of supramolecular structures and organising the solution (Demangeat 2015).

As described by Bellavite et al. (2014), water has in its nature strong coherence properties and a unique electrochemical structure (Cartwright 2020), and these can be influenced by the interactive phenomenon of coherence, epitaxy (Bell and Schwartz 2013) and weak electromagnetic fields (Konovalov 2015; Lobyshev and Lobyshev 2019). These events happen at the atomic level when a template structure is transferred to a liquid without any material transfer. This process is often used in the semiconductor industry to produce thin layers of semiconducting solutions for use in electronic devices (Bellavite et al. 2014). Therefore, during the dynamisation process, an information pattern is created within the DHD via epitaxy and cavitation, whereby the transferring of the electrochemical information into the DHD occurs. This hypothesis around the information pattern contained in the DHDs has been explored in studies that use resonance and crystallography as analytical methods. For instance, Zanco (2016) used biophotonic techniques to measure DHDs' bioelectric signals. Similarly, Kokornaczyk et al. (2014) employed the use of crystallography to identify differences in the potencies of DHDs. Rey (2003) on the other hand also verified that DHDs have specific electromagnetic fields across the dynamisation process, identifying them through electronic magnetic patterns measured with thermoluminescence.

There is no unanimous agreement over the causes of the observable effects of dynamised high dilutions in biological systems. However, some plausible arguments can be made when integrating multidisciplinary biology and wave-nature theories (Sheldrake 2009; Lambert et al., 2013; Nauenberg 2016; McFadden and AL-Khalili 2016). As verified by Rey (2003), Chikramane et al. (2010) and Kokornaczyk et al. (2014), by possessing an electromagnetic pattern, the DHD is able to configure itself as an information carrier. Information pattern is a term with many meanings, and is interpreted in information theory, as proposed by Brillouin (2013) and Capra and Luisi (2014), as where information is related to a stimulus. As with any pattern represented by a wavelength, frequency, pixel etc., the quality exists of a message passing from the sender, in this case the DHD, to the recipients, which in this case are biological systems. This concept assumes neither accuracy nor parts that directly communicate, nor the involvement of someone capable of understanding this relationship (Brillouin 2013).

In this case, nature and its complex biological, cognitive and autopoietic living systems (Maturana and Varela 1991) must be considered as capable of receiving and performing diverse forms and patterns of stimuli, processes or information patterns (Capra and Luisi 2014). Some authors, such as J and J (2016), argue that some of the very fundamental interconnections between biological systems and their environments happen via a quantum dynamics effect. As far back as 1988, studies have used mathematical models to explore quantum dynamics in relation to water molecules (Del Giudice et al. 1988). The quantum dynamics is explained by Engel et al. (2007) as that energy and the information it contains can be transferred by resonance or electromagnetism and turned into a response in the form of a stimulus. Other authors studying communication processes call these information patterns as signifiers of the ecosystem (Bastide and Lagache 1997, 1998). Such an understanding can be pragmatically applied: information cannot be destroyed and can be transmitted through a wide range of processes (Engel et al. 2007; Haramein 2013; McFadden and AL-Khalili 2016). Through these intricate and complex investigations that attempt to understand the relationship between quantum dynamics and ecosystems, the underpinnings of DHDs are also being revealed.

Overall, there are few robust, open access studies exploring the use of DHDs in strawberry crop systems and there is a need for more research on the topic to understand the potential and limitations of the method. Therefore, this study investigated the effects of using DHDs as natural plant biostimulants on strawberry plants, evaluating a series of agronomic, physiological and morphological aspects of the plant including crop production, plant growth and development, fruit quality and crop pest and disease.

Materials and methods

This study was developed by the University of Santa Catarina State (UDESC), Brazil, with writing support from Coventry University, United Kingdom. The experiment was undertaken in 2019 at the Centre of Agricultural Sciences (CAV) in Lages, Santa Catarina (SC) and was repeated in 2021 at the Federal University of Santa Catarina (UFSC), in Curitibanos, SC, Brazil. The experiments were performed in a controlled greenhouse environment from June to November in each of the two years, during which the experiment was evaluated fortnightly over a 24-week period. All the materials and inputs used in the experiment were approved under the regulations for organic cultivation standards (Brasil 2011b). During the experiment and apart from the application of DHD treatments, the plants did not receive any extra nutritional supplementation or inputs for the management of pests and diseases.

Experimental design

The agronomic trials used a randomised block design (RBD) following a double-blinded treatment application, with nine treatments and nine repetitions totalling 81 experimental units (Supplemental Figure S1). The experimental unit consisted of one strawberry (*Fragaria x ananassa*) plant allocated in a 3.6 l plastic pot filled with growing media. In the greenhouse, the pots were placed above ground on metal tables and the pots were spaced 15 cm apart from each other. The composition of the growing media used in this study is approved by organic farming standards (Brasil 2011b) and comprised 25% cow manure, 50% local soil (Lages, Santa

Catarina, Brazil, Humic Cambisol; Embrapa 2006), 10% bio-charcoal produced by Ipê® commercial ecological plant charcoal (Ipê, Rio Grande do Sul, Brazil), 5% rice flour sourced by Malleti® commercial organic rice flour (Lages, Santa Catarina, Brazil), 1% commercial organic brown sugar brand Guimaraes®(Porto Alegre, Rio Grande do Sul), 5% expanded vermiculite (medium grain size) and 4% organic commercial plant compost produced by Tecnomax® (Coronel Freitas, Santa Catarina, Brazil). The solid mixture was homogenised (350 l of growing substrate) and then moisturised with 20 l of tap water. The growing medium matured for 15 days, monitoring the temperature monitored and revolving the mixture manually once a day during this period. The basic characteristics of the medium were analysed by the Laboratory of Soil Analysis (LAS) in the Department of Soil and Natural Resources (DSRN) at CAV/UDESC (Supplemental Table S1). No other nutritional inputs or supplemental nutrition was given to the plants in the experiment.

In the greenhouse, the strawberry plants were irrigated daily with an automatic irrigation system using local dripping type nipple, with an irrigation volume of 350 ml of water plant^{-1}. The greenhouse temperature was controlled automatically and maintained at 27 C° with the help of an enforced ventilation system. The strawberry cultivar Pircinque was used in the experiment. This cultivar was chosen as it has good adaptation to southern region of Brazil, as well as being widely used by local organic strawberry farmers. The plants were grown from bare rooted runners (vegetatively propagated plants) obtained from the certified nursery Pasa, in the city of Farroupilha-Rio Grande do Sul, Brazil. The uniformity was ensured by pruning the bare rooted runners through a cross section, leaving them 8 cm in length. The runners were then categorised in 9 blocks according to degree of shoot vigour: 3 blocks with high vigour runners (6–9 mm), 3 blocks with medium vigour runners (3–6 mm) and 3 blocks with low vigour runners (1–3 mm). The blocks were distributed in the greenhouse in a randomised fashion. All runners were transplanted on the same day.

Selection of DHD treatments

The DHDs were manufactured and coded by the Laboratory of Plant Health and Homoeopathy at the Agricultural Research and Extension Agency of Santa Catarina State (EPAGRI) according to the standards of the Brazilian Homeopathic Pharmacopoeia (Brasil 2011a) and the recommendations for fundamental research on homoeopathy (Stock-Schröer et al. 2009). The DHDs were dynamised using a Hahnemannian mechanical arm model Denise 10–50 from AUTIC©, Campinas/BR, with semicircular vertical movement, an angle of 55°, radius of 300 mm, and a cycle of 100 strokes in 33 seconds. The DHDs and control were made on the same day and stored at room temperature protected from light until use. The DHDs were coded by the EPAGRI lab technician and then handed blind to the researcher ready to be used in the experiment fortnightly. The application of the DHDs and assessment of crop attributes were thus performed blind. The codes were not made known to the researcher until after the documentation of the results.

The treatments were applied by mixing 1 ml of the DHD with 49 ml of distilled water, and this solution treatment was applied every 15 days directly to the plants using a graduated container, each time after the experiment was evaluated. The dynamised high dilutions tested in this study were all mineral based, and they were: *Calcarea carbonica* 12CH (calcium carbonate; Calc 12CH), *Kali carbonicum* 12CH (potassium carbonate; Kali 12CH), *Mercurius solubilis* 12CH (metallic mercury; Merc 12CH), *Natrun muriaticun* 12CH (sodium chloride; Natu 12CH), *Phosphorus* 12CH (elemental phosphorus; Phos 12CH), *Sulphur* 12CH (elemental sulphur; Sulp 12CH), *Silicea terra* 12CH (silicon oxide; Sili 12CH), potentised distilled water 12CH (HD 12CH) and distilled water as the control. The distiled water used for the control as well as for the potentised dilutions came from the same source. The treatments and potencies were selected based on results of previous studies testing biostimulant effects on plants using DHDs, as discussed in the introduction (Kaviraj 2018; Boff et al. 2021).

Agronomic parameters assessed of the strawberry plants

Fruit production: yield per plant and fruit weight

Fruit production was assessed by recording the fruit yield as weight of harvested fruit per plant (g plant^{-1}), calculating the average weight of fruit per harvest occasion and the total production during the season (Supplemental Figure S2). The fruits were harvested 3 times per week, from August to November, when ripe (visual criteria of 75% of the fruit skins were red). After harvest, the fruit were weighed using a precision digital electronic scale (0.0001 g), model GE1302 Sartorius® and classified according to market standards for fresh fruit destinations (> 12 g individual fruit^{-1}) and for processing destinations (< 12 g individual fruit^{-1}). On each harvest occasion, the juice of the fruit was extracted and frozen in order to take sugar and acidity measurements.

Plant growth and morphophysiological development

Plant growth and architecture were assessed every 15 days from the day of transplanting until the end of the experiment. Plant height was measured with a millimetric ruler from the crown base to the canopy, and the number of green and dead leaves were counted. The stolons were marked with identification tags and the quantity produced were counted across the season from the day of transplanting to the end of the experiment. The flowers were marked with identification markers, and the number of viable and dead flowers were assessed across the season. Chlorophyll content of the leaves was measured using the equipment SPAD® 502 PLUS, Konica Minolta® (Ozaka, Japan), taking measurements from the three topmost leaves from the canopy, which were marked and renewed every 15 days.

At the end of the experimental period, the canopy area and root system area were analysed using the digital Scanner EPSON Expression 11000XL®, using the software WinRHIZO® and WinLeaf® (LC4800-II). The roots and leaves were cleaned before weighing for fresh and dry mass. The roots were first soaked in water to loosen the soil and then gently rinsed manually. The root system was assessed for length (cm), root volume (cm^3), average root diameter (mm), root projected area (cm^2) and surface area (cm^2). After that the roots and leaves were dried in an air forced chamber (60 Cº) for 72 hours. The crown diameter was measured using a digital caliper Starret® 799. For more details, please refer to Supplemental Figures S2 and S3.

Fruit quality

The fruit quality assessment considered the colour of the fruit epidermis (luminosity (L), the chroma (C) and the hue angle (hº)) which were evaluated with a Konica Minolta® CR 400 colorimeter (Osaka, Japan) in the equatorial region on opposite sides of each fruit. L expressed luminosity on a scale ranging from 0, corresponding to black, to 100 corresponding to white. C expressed colour saturation. The h° defined the basic colour, where 0° = red, 90° = yellow and 180° = green.

The textural attributes, comprising the forces for pulp penetration and peel rupture, were determined in the equatorial region of the fruits, using a TAXT-Plus® texturometer (Stable Micro Systems Ltd., United Kingdom), measured in Newton (N). Pulp penetration was performed on two opposite sides of the fruit, using a 2 mm diameter PS2 tip without removing the epidermis. The tip was introduced to a depth of 8 mm, with pre-test, test and post-test speeds of 1 mm and 10 mm s^{-1}, respectively. Further, the SS (ºBrix) content was determined with the aid of a digital refractometer ATAGO®, using the extracted juice from the fruit samples. The juice was also used to measure the pH with a Fisherbrand™ accumet™ FE150 Benchtop pH Metre. For more detail, please refer to Supplemental Figure S3.

Phytosanitary assessment

The phytosanitary assessment in this study focused on the most important associated organisms for strawberry culture: leaf spot disease (*Mycosphaerella fragariae (*Tul) Lindau) and spider mite

(*Tetranychus urticae* Koch). The phytosanitary status was assessed fortnightly, always before the treatments had been applied. The leaf spot disease incidence and severity on strawberry leaves were assessed with the aid of the diagrammatic scale proposed by Brugnara and Colli (2014) evaluating the average (%) of leaf lesions per plant. For the mites, the diagrammatic scale proposed by Iwassaki et al. (2008) counted the number of adult insects present on each plant (Supplemental Figure S4).

Statistical analyses

To verify the effect of the DHDs on crop production, plant growth, development of the root system, fruit quality, and pest and disease incidence and severity, the statistical analyses were performed using the environment R® software (R Core Team 2021), using the classical variance analysis model easyanova package, DBC and Dunnett test with 5% of significance level. The assumptions of normality and homogeneity of variance were verified by Bartlett's and Shapiro-Wilk tests, respectively. In cases where at least one of them was not met, the transformation proposed by Box-Cox was used (Venables and Ripley 2002). The data from the two years were analysed separately to facilitate the visualisation of the results obtained in both years (2019 and 2021).

Results and discussion

Crop production: fruit yield and individual weight

The results from the fruit production assessments are presented in the Tables 1 and 2. In 2019, plants treated with Kali 12CH (889.86 g plant^{-1}), Sulp 12CH (854.87 g plant^{-1}) and Phos 12CH (817.73 g plant^{-1}) had increased total yields per plant compared to the control treatment (517.10 g plant^{-1}), particularly for fresh market fruits (> 12 g individual fruit^{-1}) (Table 1). The total yield data from 2021 confirmed the positive influence of the treatments Kali 12CH (771.30 g), Sulp 12CH (1042.34 g plant^{-1}) and Phos 12CH (694.53 g plant^{-1}) compared to the control (205.61 g plant^{-1})

Table 1. Fruit production of strawberry plants treated with high dynamised dilutions (DHD) in Lages, SC/BR, 2019.

DHD	Yield (g plant^{-1} harvest^{-1})	Total production (g plant^{-1})	Fruit (nº plant^{-1} harvest^{-1})	Total fruit production (nº plant^{-1})
Fresh market fruit				
Kali 12CH	98.87 a	889.86 a	6.77 a	61 a
Sulp 12CH	95.00 a	854.87 a	6.77 a	61 a
Merc 12CH	70.56 b	632.05 b	4.66 a	42 b
Phos 12CH	90.86 a	817.73 a	6.55 a	59 a
Natu 12CH	68.03 b	612.32 b	4.44 b	40 b
Calc 12CH	65.50 b	589.50 b	4.44 b	40 b
Sili 12CH	60.23 b	542.07 b	4.55 b	41 b
HD 12CH	58.61 b	527.65 b	4.33 b	39 b
Control	57.45 b	517.10 b	3.44 b	31 b
Processing fruit				
Kali 12CH	33.71	303.40 b	6.11	55
Sulp 12CH	35.82	322.40 a	6.00	54
Merc 12CH	30.46	274.20 b	5.77	49
Phos 12CH	32.27	290.49 b	6.11	55
Natu 12CH	23.01	207.16 b	6.00	51
Calc 12CH	26.00	234.06 b	5.77	50
Sili 12CH	24.64	221.80 b	6.00	52
HD 12CH	23.89	215.05 b	5.00	45
Control	56.10	291.80 b	5.11	46

Notes: Means in columns followed by the same letter do not differ according to Dunnett's test ($p < 0.05$); no following letters = no significant differences. DHDs: Kali = *Kali carbonicum* 12CH; Sulp = *Sulphur* 12CH; Merc = *Mercurius solubilis* 12CH; Phos = *Phosphorus* 12CH; Natu = *Natrum muriaticum* 12CH; Calc = *Calcarea carbonica* 12CH; Sili = *Silicea terra* 12CH; HD = Dynamised distilled water 12CH; Control = Distilled water. 12CH = Potency, Centesimal Hahnemannian. The fresh market fruit (> 12 g fruit^{-1}) and processing fruit (< 12 g fruit^{-1}) are categories of market classification for the crop; Yield = average yield per harvest; Total = total yield from successive harvests. 81 experimental units (plants/pots) per trial.

Table 2. Fruit production of strawberry plants treated with dynamised high dilutions (DHD) in Curitibanos, SC/BR, 2021.

DHD	Yield (g plant^{-1} harvest^{-1})	Total production (g plant^{-1})	Fruit (nº plant^{-1} harvest^{-1})	Total fruit production (nº plant^{-1})
Fresh market fruit				
Kali 12CH	85.70 a	771.30 a	7.33 a	66 a
Sulp 12CH	115.82 a	1042.34 a	8.33 a	75 a
Merc 12CH	58.21 b	523.85 b	3.67 b	33 b
Phos 12CH	77.17 a	694.53 a	4.67 a	42 a
Natu 12CH	42.78 b	385.02 b	2.44 b	22 b
Calc 12CH	55.99 b	503.88 b	3.56 b	32 b
Sili 12CH	50.94 b	458.43 b	2.89 b	26 b
HD 12CH	31.57 b	284.15 b	2.11 b	19 b
Control	22.84 b	205.61 b	1.26 b	11 b
Processing fruit				
Kali 12CH	19.72 a	177.47 a	2.78	25 a
Sulp 12CH	21.71 a	195.42 a	3.33	30 a
Merc 12CH	14.71 b	132.36 a	1.78	16 b
Phos 12CH	19.94 a	179.42 a	3.22	29 a
Natu 12CH	16.60 b	149.37 a	1.33	12 b
Calc 12CH	17.72 b	159.50 a	2.13	19 b
Sili 12CH	11.29 b	101.64 a	1.11	11 b
HD 12CH	9.76 b	87.87 b	2.12	18 b
Control	8.66 b	77.93 b	2.44	22 a

Notes: Means in columns followed by the same letter do not differ according to Dunnett's test ($p < 0.05$); no following letters = no significant differences. DHDs: Kali = *Kali carbonicum* 12CH; Sulp = *Sulphur* 12CH; Merc = *Mercurius solubilis* 12CH; Phos = *Phosphorus* 12CH; Natu = *Natrum muriaticum* 12CH; Calc = *Calcarea carbonica* 12CH; Sili = *Silicea terra* 12CH; HD = Dynamised Distilled Water 12CH; Control = Distilled water. 12CH = Potency, Centesimal Hahnemannian. The fresh market fruit (> 12 g fruit^{-1}) and processing fruit (< 12 g fruit^{-1}) are categories of market classification for the crop; Yield = average yield per harvest; Total = total yield from successive harvests. 81 experimental units (plants/pots) per trial.

(Table 2). In 2019, there were no significant differences between the treatments regarding the total production yields of processing fruit production (fruit < 12 g), except from for Sulph 12CH where the total production was higher than that in the other treatments. However in 2021, the treatments Kali 12CH (177.47 g plant^{-1}), Sulp 12CH (195.4 g plant^{-1}), Phos 12CH (179.42 g plant^{-1}), Merc 12CH (132.36 g plant^{-1}), Natu 12CH (149.37 g plant^{-1}) and Calc 12CH (159.50 g plant^{-1}) all produced higher total yields of processing fruit per plant when compared to the control treatment (77.93 g plant^{-1}) (Table 2).

The biostimulation effect promoted by the dynamised high dilutions made from minerals such as Kali 12CH (K), Sulp 12CH (S), and Phos 12CH (P) could be correlated to the elements that these preparations were made from as well as to their morphophysiological role in plant development: K regulates the translocation of water and nutrients in the plant, helping in carbohydrate transport and storage, S is fundamental in metabolic processes and protein production, which influences fruit development, and P is responsible for storing and transferring ATP (Taiz et al. 2017). The results regarding the size and category of the fruits produced are also important for the growers, as larger fruits (>12 g) make harvesting and packaging a faster process as well as having greater market value (Fagherazzi et al. 2021).

Kaviraj (2018) and Boff et al. (2021) explained that the DHDs have a biostimulation effect that enhances the plant's natural responses and its capacity to assimilate nutrients and thus to improve its agronomic performance. The results observed for the fruit yield and weight supported this perspective. The potential impact of this increment in yield components by using DHDs can be illustrated by comparing it with productivity parameters from China and U.S.A., the two biggest strawberry producers, which estimate a production per plant ranging from 0.5 kg to 1.3 kg per plant per annual cycle (Simpson 2018). This is an interesting reflection as in this study reported here the production results were obtained without the use of any additional supplementary source of feeding or fertilisation, apart from the nutrients present in the growing medium in which the plants were cultivated. In other words, DHDs supported the plants in achieving their natural productive capacity. These results represented the outcomes of a two-year experiment under a controlled environment, and therefore it was recommended to repeat the study over more treatment cycles and under field conditions.

Plant growth

The plant growth parameters comprising plant height, plant mass (fresh and dry), foliar area, and foliar production (dead and viable leaves) are presented in Tables 3 and 4. In 2019, plant height was positively influenced by the treatment Sulp 12CH, which stimulated the tallest plant growth (22.67 cm), followed closely by Kali 12CH (22.32 cm), Phos 12CH (22.36 cm) and Sili 12CH (22 cm). The differences in height between these four treatments were not significant, but all four treatments were significantly taller than the control (18.16 cm). In 2021, the plants presented similar heights (±21 cm), but at this time no significant differences were observed. For the other architectural parameters: fresh mass, dry mass, foliar area and number of dead leaves, significant differences were observed when compared to the control treatment. Plants treated with Sulp 12CH had the highest fresh mass (900.87 g), dry mass (46.11 g) and foliar area (35.07 cm^2).

Table 3. Plant growth assessments of strawberries plants treated with dynamised high dilutions (DHD) in Lages, SC/BR, 2019.

DHD	Plant height (cm)	Fresh mass (g plant^{-1})	Dry mass (g plant^{-1})	Foliar area (cm^2)	Viable leaves (nº plant^{-1})	Dead leaves (nº plant^{-1})
Kali 12CH	22.32 a	623.07	20.52	28.50	24.00	10.41
Sulp 12CH	22.67 a	642.47	20.00	28.76	28.77	11.00
Merc 12CH	20.85 b	559.58	20.80	21.71	20.88	11.00
Phos 12CH	22.36 a	655.75	20.64	29.71	25.33	12.62
Natu 12CH	19.47 b	500.84	18.07	21.97	23.44	19.21
Calc 12CH	20.16 b	418.52	18.71	19.68	23.11	14.42
Sili 12CH	22.00 a	637.34	21.01	29.92	26.00	12.63
HD 12CH	20.56 b	443.22	19.51	19.30	23.11	11.01
Control	18.16 b	525.65	18.38	19.54	23.77	17.41

Notes: Means in columns followed by the same letter do not differ according to Dunnett's test ($p < 0.05$); no following letters = no significant differences. DHDs: Kali = *Kali carbonicum* 12CH; Sulp = *Sulphur* 12CH; Merc = *Mercurius solubilis* 12CH; Phos = *Phosphorus* 12CH; Natu = *Natrum muriaticum* 12CH; Calc = *Calcarea carbonica* 12CH; Sili = *Silicea terra* 12CH; HD = Dynamised distilled water 12CH; Control = Distilled water. 12CH = Potency, Centesimal Hahnemannian. Fresh mass = whole plant; Dry mass = whole plant; Viable leaf= average number of leaves plant^{-1}; Dead leaves = average number of dead leaves accumulated during the crop cycle. 81 experimental units (plants/pots) per trial.

Table 4. Plant growth assessments of strawberries plants treated with dynamised high dilutions (DHD) in Curitibanos, SC/BR, 2021.

DHD	Plant height (cm)	Fresh mass (g plant^{-1})	Dry mass (g plant^{-1})	Foliar area (cm^2)	Viable leaves (nº plant^{-1})	Dead leaves (nº plant^{-1})
Kali 12CH	23.44	754.67 a	39.38 a	30.23 a	34.12	2.00 b
Sulp 12CH	20.17	900.87 a	46.11 a	35.80 a	38.73	1.89 b
Merc 12CH	21.55	599.11 a	36.40 a	22.08 a	30.14	2.78 a
Phos 12CH	22.40	795.78 a	31.40 a	27.16 a	35.07	1.78 b
Natu 12CH	21.47	473.53 b	21.63 b	18.33 b	33.81	3.33 a
Calc 12CH	21.90	558.14 a	34.19 a	22.98 a	33.28	2.00 b
Sili 12CH	22.12	470.41 b	21.98 b	17.29 b	36.12	6.63 a
HD 12CH	20.02	393.14 b	19.56 b	15.44 b	33.14	5.22 a
Control	19.56	374.95 b	17.89 b	12.51 b	33.87	4.33 a

Notes: Means in columns followed by the same letter do not differ according to Dunnett's test ($p < 0.05$); no following letters = no significant differences. DHDs: Kali = *Kali carbonicum* 12CH; Sulp = *Sulphur* 12CH; Merc = *Mercurius solubilis* 12CH; Phos = *Phosphorus* 12CH; Natu = *Natrum muriaticum* 12CH; Calc = *Calcarea carbonica* 12CH; Sili = *Silicea terra* 12CH; HD = Dynamised Distilled Water 12CH; Control = Distilled water. 12CH = Potency, Centesimal Hahnemannian. Fresh mass = whole plant; Dry mass = whole plant; Viable leaves = average number of leaves plants^{-1}; Dead leaves = average number of dead leaves accumulated during the crop cycle. 81 experimental units (plants/pots) per trial.

These results supported those obtained by Betti et al. (2009), who also verified increased plant height for strawberry plants treated with *Sulphur* 12CH. Similarly, Abasolo-Pacheco et al. (2020), using *Silicea terra* 12CH, and *Phosphorus* 12CH, recorded increased plant height in cucumber (*Cucumis sativus L.*), turnip (*Brassica napus L.*) and Modolon et al. (2012) in tomato (*Solanum lycopersicum*). In addition, *Sulphur* 12CH stimulated plant growth in radish (*Raphanus sativus L.*), where Bonato and Silva (2003) also observed increases in fresh and dry matter mass and leaf area. However, contrasting results were found by Nunes et al. (2021) when testing *Kali carbonicum* 12CH, *Sulphur* 12CH, and *Phosphorus* 12CH, whereby the authors observed no increases in plant height or fresh mass in St John's Wort (*Hypericum perforatum*) plants.

The plant growth data suggested that DHDs could be used as a management practice to help crops optimise their natural development. For instance, increased plant height could be advantageous for strawberry crops to intercept more light, helping them to maintain sufficient levels of photosynthetic activity and influencing production and growth as well as the plants' ability to compete with spontaneous weeds (Taiz et al. 2017).

These results also suggested that different biostimulation responses might be observed depending on the interface between crop x DHD x potency. In other words, the same DHDs can have different effects on different crops. Furthermore, the results from Pulido et al. (2017) indicated positive effects of the DHD *Sulphur* in terms of increasing plant height, dry and fresh mass in broccoli (*Brassica oleracea* var. *italica)*, the authors testing the potency 30CH rather than the 12CH as used in this current study. In this sense, it is possible that the same DHDs can have different stimulation responses depending on the potency used as well as the studied crop.

Morphophysiological development

The attributes of morphophysiological development are presented in Tables 5 and 6. Overall, in 2019, plants treated with Sulp 12CH (6.68 cm) developed the largest crown diameter when compared with the control treatment (4.58 cm). Similarly, plants treated with Phos 12CH also developed larger crowns (6.66 cm) (Table 5). In 2021, Sulp 12CH (6.69 cm), Phos 12CH (5.42 cm) and Kali 12 CH (5.21 cm) once again stimulated plants with larger crown diameters when compared to the control treatment (3.63 cm) (Table 6). This was regarded as important results, as strawberry plants with bigger crown diameters are known to be taller and more productive plants (Fagherazzi et al. 2021). This result helped to explain the plant growth and production results presented in the previous sections.

According to Cocco et al. (2015), strawberry plants that have larger crown diameters have more energy reserves in the form of starch. As a consequence, such plants are more likely to have a higher

Table 5. Morphophysiological parameters of strawberry plants treated with dynamised high dilutions (DHD) in Lages, SC/BR, 2019.

DHD	Stolon (nº plant^{-1})	Viable flowers (nº plant^{-1} assessment^{-1})	Dead flowers (nº plant^{-1} assessment^{-1})	SPAD (unit)	Crown diameter (cm)
Kali 12CH	1.00 b	2.00	0.16 b	53.06 b	5.26 b
Sulp 12CH	5.00 a	2.00	0.17 b	54.57 a	6.68 a
Merc 12CH	1.00 b	1.33	0.17 b	53.17 b	5.68 b
Phos 12CH	5.20 a	1.33	0.28 b	54.71 a	6.66 a
Natu 12CH	1.44 b	1.22	0.33 a	51.70 b	5.66 b
Calc 12CH	1.33 b	2.11	0.17 b	51.96 b	5.50 b
Sili 12CH	1.00 b	2.11	0.16 b	54.69 a	5.95 b
HD 12CH	1.00 b	2.00	0.20 b	51.70 b	5.57 b
Control	1.00 b	1.22	0.16 b	52.24 b	4.58 b

Notes: Means in columns followed by the same letter do not differ according to Dunnett's test ($p < 0.05$); no following letters = no significant differences. DHDs: Kali = *Kali carbonicum* 12CH; Sulp = *Sulphur* 12CH; Merc = *Mercurius solubilis* 12CH; Phos = *Phosphorus* 12CH; Natu = *Natrum muriaticum* 12CH; Calc = *Calcarea carbonica* 12CH; Sili = *Silicea terra* 12CH; HD = Dynamised distilled water 12CH; Control = Distilled water. 12CH = Potency, Centesimal Hahnemannian. Stolon = total production plant^{-1}; SPAD = chlorophyll concentration in SPAD units. 81 experimental units (plants/pots) per trial.

Table 6. Morphophysiological parameters of strawberry plants treated with high dynamised dilutions (DHD) in Curitibanos, SC/BR, 2021.

DHD	Stolon (nº plant^{-1})	Viable flowers (nº plant^{-1} assessment^{-1})	Dead flowers (nº plant^{-1} assessment^{-1})	SPAD (unit)	Crown diameter (cm)
Kali 12CH	1.22 b	4.00	0.11 b	50.15 a	5.21 a
Sulp 12CH	4.45 a	4.77	0.22 b	55.67 a	6.69 a
Merc 12CH	1.44 b	4.56	0.11 b	43.22 b	4.04 b
Phos 12CH	1.33 b	4.78	0.11 b	44.31 b	5.42 a
Natu 12CH	1.11 b	2.78	1.12 a	41.10 b	3.93 b
Calc 12CH	1.11 b	4.33	0.10 b	41.36 b	3.81 b
Sili 12CH	1.44 b	4.11	0.33 b	44.39 b	3.86 b
HD 12CH	1.60 b	4.10	1.13 a	41.71 b	3.19 b
Control	1.00 b	3.56	1.78 a	42.54 b	3.63 b

Notes: Means in columns followed by the same letter do not differ according to Dunnett's test ($p < 0.05$); no following letters = no significant differences. DHDs: Kali = *Kali carbonicum* 12CH; Sulp = *Sulphur* 12CH; Merc = *Mercurius solubilis* 12CH; Phos = *Phosphorus* 12CH; Natu = *Natrum muriaticum* 12CH; Calc = *Calcarea carbonica* 12CH; Sili = *Silicea terra* 12CH; HD = Dynamised distilled water 12CH; Control = Distilled water. 12CH = Potency, Centesimal Hahnemannian. Stolon= total production plant^{-1}; SPAD = chlorophyll concentration in SPAD units. 81 experimental units (plants/pots) per trial.

number of buds capable of differentiating into flowers, resulting in increased numbers of fruits per plant. The results observed in this study suggested that the DHDs Sulp 12CH, Phos 12CH and Kali 12CH stimulated crown development in strawberry plants, probably due to the regulation of the morphophysiological activities of strawberry plants (Taiz et al. 2017), which accumulated more carbohydrates in the crown, leading to larger plants, increasing the plant's biomass and the capacity to mobilise reserves for the fruit set (Antunes and Peres 2013).

The biostimulation effect on crown diameter helped to explain the stolon production data. The stolon is a two-node axillary shoot, with a main function to support natural clonal multiplication capacity (Martins de Lima et al. 2021). The production of stolons play an important role on improving nursery production. In 2019, plants treated with Sulp 12CH (5 plant^{-1}) and Phos 12CH (5 plant^{-1}) produced more stolons compared with the control treatments (1 plant^{-1}). In 2021, the plants treated with Sulp 12CH (4 plant^{-1}) also were stimulated in terms of stolon production, however, they produced fewer stolons when compared to the 2019 results.

The results of differences in crown diameter and stolon production are relevant for examining the SPAD results in relation to chlorophyll density. A higher chlorophyll content of the leaves was observed in plants that had larger crown diameters and heights. This set of morphophysiological attributes were also noted by Massetani and Neri (2016), who observed that taller plants tended to intercept more light photons, resulting in higher chlorophyll content in the leaves than in smaller plants. In the same sense, plants with larger crown diameters are an indicator that photosynthetic activity is performing well, as the photo-assimilates are stored in that structure (Antunes and Peres 2013).

In 2019, plants treated with Sulp 12CH (54.57), Phos 12CH (54.71) and Sili 12CH (54.69) had higher SPAD units when compared to the control treatment (52.24) (Table 5). Interestingly, in 2021 strawberry plants treated with Sulp 12CH (55.67) had again a higher content of chlorophyll in their leaves compared with plants in the control (42.54), as did Kali 12CH (50.15) Table 6). The connection between the SPAD reading and the leaf's chlorophyll content in strawberry plants has been widely studied (Martínez et al. 2017). The chlorophyll content is related to the nitrogen concentration in the leaf, which is fundamental to the photosynthetic apparatus, reflecting the plant's capacity to produce carbohydrates (Taiz et al. 2017).

These morphophysiological characteristics help the plants to be more productive and have a reliable source of energy in case of stress. For instance, Fagherazzi et al. (2021) explained that strawberry plants with bigger crown diameters (over 5 cm) were more productive and more efficiently overcame environmental stress such as periods of drought conditions. In addition, increasing stolon production is an important feature because it can help strawberry growers to produce more of their own planting material, thus minimising their production costs. This would have an important impact in countries such as Brazil, where around 300 million strawberry plants are needed annually to meet the demand of the growers (Savini et al. 2008; Fagherazzi et al. 2021). Overall, the results verified in this study suggested that dynamised high dilutions of Sulp 12CH, Phos 12CH and Kali 12CH supported strawberry plants by stimulating positive morphophysiological features.

Root system development

Root development and architecture parameters are presented in Tables 7 and 8. It is well understood that the root system plays a vital role regarding water and nutrient intake as well as with the ecological associations of the plant with the soil biological community (Massetani and Neri 2016). In 2019, strawberry plants treated with Sulp 12CH (0.71 mm), Calc 12CH (0.70 mm) and Sili 12CH (0.75 mm) developed roots with larger diameters compared to plants in the control (0.63 mm) (Table 7). In addition, Calc 12CH increased the root volume (47.51 cm^3) compared to the control (36.49 cm^3). There were no differences regarding the length of the roots (82.28 cm^2), root projected area (37.60 cm^2), or surface (12.17 cm^2) (Table 7). In 2021, the majority of the DHD treatments

Table 7. Architecture and development of strawberry root systems treated with high dynamised dilutions (DHD), Lages, SC/BR, 2019.

DHD	Root length (cm)	Root volume (cm^3)	Average diameter (mm)	Root projected area (cm^2)	Surface (cm^2)
Kali 12CH	80.80	39.56 b	0.65 b	38.02	11.94
Sulp 12CH	78.76	45.45 b	0.71 a	37.03	13.41
Merc 12CH	91.56	37.32 b	0.66 b	41.94	12.17
Phos 12CH	75.90	29.52 b	0.66 b	33.47	10.51
Natu 12CH	73.79	29.50 b	0.66 b	36.53	11.89
Calc 12CH	88.40	47.51 a	0.70 a	39.12	12.51
Sili 12CH	87.98	43.13 b	0.75 a	43.93	13.80
HD 12CH	83.61	39.38 b	0.68 b	35.37	12.59
Control	79.72	36.49 b	0.63 b	34.19	10.74

Notes: Means in columns followed by the same letter do not differ according to Dunnett's test ($p < 0.05$); no following letters = no significant differences. DHDs: Kali = *Kali carbonicum* 12CH; Sulp = *Sulphur* 12CH; Merc = *Mercurius solubilis* 12CH; Phos = *Phosphorus* 12CH; Natu = *Natrum muriaticum* 12CH; Calc = *Calcarea carbonica* 12CH; Sili = *Silicea terra* 12CH; HD = Dynamised distilled water 12CH; Control = Distilled water. 12CH = Potency, Centesimal Hahnemannian. 81 experimental units (plants/pots) per trial.

Table 8. Architecture and development of strawberry root systems treated with high-dynamised dilutions (DHD), Curitibanos, SC/BR, 2021.

DHD	Root length (cm)	Root volume (cm^3)	Average diameter (mm)	Root projected area (cm^2)	Surface (cm^2)
Kali 12CH	72.42 a	76.71 a	0.65 b	26.43 a	28.40 a
Sulp 12CH	85.63 a	94.56 a	0.71 a	33.64 a	31.95 a
Merc 12CH	64.38 a	60.03 b	0.66 b	22.10 a	25.97 a
Phos 12CH	65.49 a	60.97 b	0.66 b	22.47 a	26.12 a
Natu 12CH	57.62 b	69.67 a	0.66 b	22.42 a	26.06 a
Calc 12CH	69.47 a	76.17 a	0.70 a	25.12 a	28.28 a
Sili 12CH	61.35 a	64.42 a	0.75 a	22.45 a	26.19 a
HD 12CH	40.17 b	44.01 b	0.68 b	14.82 b	12.59 b
Control	35.60 b	29.76 b	0.63 b	11.41 b	18.67 b

Notes: Means in columns followed by the same letter do not differ according to Dunnett's test ($p < 0.05$); no following letters = no significant differences. DHDs: Kali = *Kali carbonicum* 12CH; Sulp = *Sulphur* 12CH; Merc = *Mercurius solubilis* 12CH; Phos = *Phosphorus* 12CH; Natu = *Natrum muriaticum* 12CH; Calc = *Calcarea carbonica* 12CH; Sili = *Silicea terra* 12CH; HD = Dynamised distilled water 12CH; Control = Distilled water. 12CH = Potency, Centesimal Hahnemannian. 81 experimental units (plants/pots) per trial.

increased the strawberry root system architecture and development when compared to the control treatment. Noticeably, plants treated with Sulp 12CH presented longer roots (85.63 cm), larger volumes (94.56 cm^3), with a wider root projection area (33.64 cm^2) and surface area (31.95 cm^2) (Table 8). These results were supported by a series of similar findings from other studies. Data from a systematic review on plant bioassay by Jäger et al. (2015) reported positive results on the development of plant root systems with 12CH *Phosphorus*, *Silicea terra* and *Kali carbonicum*, being the potency with best response. Other studies also attest to a positive influence of DHD of *Sulphur* on different crops and potencies, suggesting this as a biostimulator for root system development (Bonato and Silva 2003; Pulido et al. 2014, 2017). From all the mineral DHDs tested in their studies, only one, *Natrum muriaticum*, showed no positive effects in relation to root development. In contrast, a study made by Lensi et al. (2010) on root development in beans (*Phaseolus vulgaris* L) using *Natrum muriaticum* showed positive growth with the potency 6CH.

These results have important practical implications for strawberry cropping system management. In particular, the shock caused by transplanting the young plants from the nurseries to the field can potentially compromise the viability of crop development (Fagherazzi et al. 2021). Transplant shock symptoms manifest as leaf wilt, alteration of metabolic processes, cessation of growth and death (Trebbi et al. 2014). Therefore, overcoming transplanting shock via stimulating root growth may be crucial for restoring growth and development within a short period of time. The results of this study show that strawberry plants treated with DHDs may have overcome this critical transplant stage, leading to the development of a robust root system. Similar results were verified by Bonato and Silva (2003) who evidenced an increase in the root development of radish (*Raphanus sativus*) using *Sulphur* 12CH, and by Pulido et al. (2017) who found an increase in the root system and the number of viable seedlings of cabbage (*Brassica oleracea var. capitata*) using *Sulphur* 6CH and *Silicea terra* 30CH. On the other hand, Bonfim et al. (2010) obtained no response in root growth parameters when using *Calcarea carbonica* 12CH. However, Bonfim et al. (2010) used a single application of the DHD when treating lettuce (*Lactuca sativa* L.) whereas in this present study the DHDs treatments were applied fortnightly over 24 weeks. The results from this study suggested that a more frequent application could potentially stimulate the plants more effectively.

Other authors studied the effect of DHD biostimulation on root system development from a metabolic standpoint. Panda et al. (2013) and Deboni et al. (2021) explained that DHDs accelerated the plant's metabolism, leading to a higher response in terms of auxin production, promoting root growth through the polar transportation coupled with gibberellin functioning in root cells. In roots, auxins increased root hair length, diameter and consequently volume (Taiz et al. 2017). In this sense, the efficiency of the roots to obtain water and nutrients is partially determined by the root system architecture and this therefore plays a significant role in determining the successful growth of the crop (Fagherazzi et al. 2021).

Fruit characteristics

The results for the fruit characteristics are shown in Tables 9 and 10. In the 2019 cycle, the influence of the DHDs on fruit quality could not be verified statistically for any of the characteristics assessed (Table 9). In the 2021 cycle, however, the fruit from plants treated with DHDs had significantly firmer pulp when compared with the control (1.62 N) (Table 10).

Regarding the acidity of the fruits, in 2021, Kali 12CH (6.29) and Sili 12CH (6.23) produced fruits with a more alkaline pH in comparison with the control treatment (5.55). Furthermore, plants treated with Sulp 12CH (6.42), Phos 12CH (6.18) and Calc 12CH (7.76) obtained fruits with the highest brix levels when compared to the control (5.16°) (Table 10). These results helped to build the still scarce data about the effects of DHDs on fruit quality characteristics. Other research that mentions positive effects on fruit quality is that of Sakalauskienė et al. (2011) who observed firmer pulp in tomatoes (Solanum lycopersicum) when using a DHD of *Ocimum basilicum* L 30CH and *Silicea terra* 30CH.

Table 9. Fruit quality parameters from strawberry plants treated with high dynamised dilutions (DHD), Lages, SC/BR, 2019.

DHD	SS	TA	PF	C	Colour L	hº
Kali 12CH	6.77	5.55	1.83	45.67	33.34	1.69
Sulp 12CH	7.50	5.54	1.89	47.42	33.81	1.71
Merc 12CH	6.63	5.58	1.72	49.27	33.68	1.69
Phos 12CH	6.33	5.62	1.61	47.06	32.72	1.69
Natu 12CH	7.60	5.62	1.39	47.91	33.72	1.69
Calc 12CH	7.13	5.56	1.84	50.12	34.45	1.71
Sili 12CH	6.68	5.59	1.61	45.53	32.51	1.69
HD 12CH	6.75	5.61	1.94	43.89	32.32	1.69
Control	7.65	5.54	1.71	45.91	33.49	1.70

Notes: Means in columns followed by the same letter do not differ according to Dunnett's test ($p < 0.05$); no letters = no significant differences. DHDs: Kali = *Kali carbonicum* 12CH; Sulp = *Sulphur* 12CH; Merc = *Mercurius solubilis* 12CH; Phos = *Phosphorus* 12CH; Natu = *Natrum muriaticum* 12CH; Calc = *Calcarea carbonica* 12CH; Sili = *Silicea terra* 12CH; HD= Dynamised Distilled Water 12CH; Control = Distilled water. 12CH = Potency, Centesimal Hahnemannian. SS = Brixº; TA = Titratable acidity; PF = Pulp firmness, the strength necessary to break fruit epidermis in Newton (N); Colour: L expresses luminosity on a scale ranging from 0, corresponding to black, to 100 corresponding to white; C expresses colour saturation, from a scale ranging from 0 to 100%; h° defines the basic colour, where 0° = red, 90° = yellow and 180° = green. 81 experimental units (plants/pots) per trial.

Table 10. Fruit quality parameters from strawberry plants treated with high dynamised dilutions (DHD), Curitibanos, SC/BR, 2021.

DHD	SS	TA	PF	C	Colour L	hº
Kali 12CH	6.08 b	6.29 a	2.69 a	42.49	39.54	1.79
Sulp 12CH	6.42 a	6.11 b	2.75 a	41.63	39.62	1.61
Merc 12CH	5.87 b	5.38 b	2.79 a	42.35	39.70	1.77
Phos 12CH	6.18 a	5.71 b	2.65 a	43.13	39.80	1.79
Natu 12CH	5.85 b	5.63 b	2.28 a	43.51	40.09	1.84
Calc 12CH	7.76 a	5.94 b	2.34 a	42.50	40.08	1.75
Sili 12CH	5.91 b	6.23 a	2.68 a	43.24	40.49	1.89
HD 12CH	5.18 b	5.34 b	1.51 b	42.47	38.40	1.79
Control	5.16 b	5.55 b	1.62 b	42.43	37.93	1.75

Notes: Means in columns followed by the same letter do not differ according to Dunnett's test ($p < 0.05$); no letters = no significant differences. DHDs: Kali = *Kali carbonicum* 12CH; Sulp = *Sulphur* 12CH; Merc = *Mercurius solubilis* 12CH; Phos = *Phosphorus* 12CH; Natu = *Natrum muriaticum* 12CH; Calc = *Calcarea carbonica* 12CH; Sili = *Silicea terra* 12CH; HD= Dynamised distilled water 12CH; Control = Distilled water. 12CH = Potency, Centesimal Hahnemannian. SS = Brixº; TA = Titratable acidity; PF= Pulp firmness, the strength necessary to break fruit epidermis in Newton (N); Colour: L expresses luminosity on a scale ranging from 0, corresponding to black, to 100 corresponding to white; C expresses colour saturation, from a scale ranging from 0 to 100%; h° defines the basic colour, where 0° = red, 90° = yellow and 180° = green. 81 experimental units (plants/pots) per trial.

Incidence and severity of pests and diseases

Tables 11 and 12 show the results regarding the incidence and severity of pests and diseases. In the 2019 cycle, plants treated with Sulp 12CH (1.76) and Sili 12CH (1.69) were less affected by leaf spot disease (*Mycosphaerella fragariae (*Tul) Lindau) when compared to the control (2.33), as measured by disease incidence (Table 11), but there were no significant differences regarding disease severity. In the same way, no differences were observed regarding incidence and severity of spider mite (*Tetranychus urticae* Koch) (Table 11). In the 2021 cycle, all strawberry plants treated with the DHDs were less affected by leaf spot disease (disease incidence) when compared to the control (Table 12). Once again Sulp 12CH (1.67) and Sili 12CH (1.69) obtained lowest disease scores compared with the control (5.22) (Table 12). There were no differences concerning the disease severity. In addition, plants treated with DHDs were less frequently attacked by spider mite. Noticeably, Sulp 12CH obtained the lowest scores in relation to disease incidence (0.78) and severity (1.01) when compared with the control (3.56 and 5.67 respectively) (Table 12). This could be linked to some extent to the plant`s physiology, as silicon signalises the strengthening of the cell wall of leaves and stems, making plants more upright and increasing the area of exposure to the sun. In the

Table 11. Incidence and severity of leaf spot (*mycosphaerella fragariae (*Tul) Lindau) and spider mite (*tetranychus urticae* koch) in strawberry plants treated with high dynamised dilutions (DHDs), Lages, SC/BR, 2019.

DHD	DI	DS	PI	PS
Kali 12CH	2.31 a	0.98	2.49	2.06
Sulp 12CH	1.76 b	1.06	2.13	1.77
Merc 12CH	2.30 a	1.02	3.20	2.65
Phos 12CH	2.32 a	0.98	2.48	2.06
Natu 12CH	2.22 a	0.97	3.20	2.65
Calc 12CH	2.30 a	1.01	2.84	2.06
Sili 12CH	1.69 b	1.25	3.20	2.65
HD 12CH	2.31 a	1.03	2.84	2.35
Control	2.33 a	0.98	2.49	2.06

Notes: Means in columns followed by the same letter do not differ according to Dunnett's test ($p < 0.05$); no letters = no significant differences. DHDs: Kali = *Kali carbonicum* 12CH; Sulp = *Sulphur* 12CH; Merc = *Mercurius solubilis* 12CH; Phos = *Phosphorus* 12CH; Natu = *Natrum muriaticum* 12CH; Calc = *Calcarea carbonica* 12CH; Sili = *Silicea terra* 12CH; HD= Dynamised distilled water 12CH; Control = Distilled water. 12CH = Potency, Centesimal Hahnemannian. DI = disease incidence; DS = foliar disease severity; PI = foliar pest incidence; PS = foliar pest severity. 81 experimental units (plants/pots) per trial.

Table 12. Incidence and severity leaf spot (*mycosphaerella fragariae (*Tul) Lindau) and spider mite (*tetranychus urticae* koch) in strawberry plants treated with high dynamised dilutions (DHDs), Curitibanos, SC/BR, 2021.

DHD	DI	DS	PI	PS
Kali 12CH	2.78 b	3.00 ns	1.13 b	1.10 b
Sulp 12CH	1.67 b	2.56 ns	0.78 b	1.01 b
Merc 12CH	3.11 b	3.11 ns	1.11 b	1.56 b
Phos 12CH	2.22 b	2.11 ns	1.28 b	1.33 b
Natu 12CH	2.56 b	2.78 ns	1.33 b	2.67 b
Calc 12CH	2.33 b	1.67 ns	1.56 b	2.06 b
Sili 12CH	1.69 b	2.89 ns	1.33 b	1.89 b
HD 12CH	4.56 a	4.78 ns	3.22 a	3.22 a
Control	5.22 a	9.44 ns	3.56 a	5.67 a

Notes: Means in columns followed by the same letter do not differ according to Dunnett's test ($p < 0.05$); no letters = no significant differences. DHDs: Kali = *Kali carbonicum* 12CH; Sulp = *Sulphur* 12CH; Merc = *Mercurius solubilis* 12CH; Phos = *Phosphorus* 12CH; Natu = *Natrum muriaticum* 12CH; Calc = *Calcarea carbonica* 12CH; Sili = *Silicea terra* 12CH; HD = Dynamised distilled water 12CH; Control = Distilled water. 12CH = Potency, Centesimal Hahnemannian. DI = disease incidence; DS = foliar disease severity; PI = foliar pest incidence; PS = foliar pest severity. 81 experimental units (plants/pots) per trial.

case of sulphur, this element stimulates the synthesis of phenolic compounds, flavonoids and alkaloids which are used as plant defence compounds, many of them with the hormonal action of stimulating plant growth and development, as a strategy of plant defence responses under conditions of biotic and abiotic stress (Taiz et al. 2017).

Based on the discussion relating to plant growth and development presented in the previous sections of this study, the data suggested that the DHDs of Sili 12CH and Sulp 12CH stimulated plant growth and vigour, thus playing a role in the natural resistance of the plants to environmental stress caused by pests and diseases (Antunes et al. 2020). Fagherazzi et al. (2021) explained that plant vigour is a fundamental factor to explain the natural susceptibility of disease occurrence in strawberry plants. Specifically, regarding the stimulus by DHDs,Deboni et al. (2021) observed in their study with beans (*Phaseolus vulgaris L.*) that DHD of *Sulphur* has the potential to induce biochemical defence mechanisms in the plant, acting as resistance elicitors. However, in this study no biochemical analyses were done, and therefore the reasoning in relation to this stance is more constrained.

Conclusions

The results of this research undertaken in greenhouse conditions provided evidence that dynamised high dilutions (DHDs) can perform positively as agroecological plant biostimulants for strawberry crops (*Fragaria × ananassa*). The results suggested that mineral DHDs of *Sulphur* 12CH, *Kali carbonicum* 12CH and *Phosphorus* 12CH increased the yield, plant height, crown diameter, leaf chlorophyll content and production of stolons in the strawberry plants. In addition, DHDs of *Silicea terra* 12CH and *Calcarea carbonica* 12CH had positive influence on the development and architecture of the strawberry root system, particularly the volume of the roots as well as the root diameter and surface. The potency 12CH used in this study showed positive results for plant biostimulation and can be used as a reference for other studies testing DHDs in other crops. However, many variables still remain to be tested, including different potencies and lengths and frequencies of treatment. For future studies, treatments which obtained positive results should be tested over more cycles under field conditions to expand the data set and comparisons. The results of this study presented an innovative method to be used and tested by growers and researchers globally, particularly for those interested on investigating plant health and sustainable alternatives for the crop cultivation. Furthermore, the use of DHDs as plant biostimulants supported the strawberry plants to achieve appropriate crop vitality and resilience, increased yields and promoted natural plant development. The use of DHDs offers a health centred approach to agriculture focusing on ecological principles and low residual impact methods for the management of strawberry cropping systems with potential application to other crops.

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