Chapter

Agricultural Homoeopathy: A New Insight into Organics

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Abstract

At present, agricultural homoeopathy is being increasingly implemented worldwide to mitigate the negative effects caused by the indiscriminate use of chemical products in conventional agricultural practices. It is a viable alternative to improve organic agriculture, since homoeopathic medicines are innocuous substances with a capability to activate measurable response mechanisms when used in plants, animals and humans. Experimental research results allow us to conclude in this chapter that agricultural homoeopathy is able to stimulate favourable biological and even genetic responses in plants (basil *Ocimum basilicum* L., bean *Phaseolus vulgaris* L., cucumber *Cucumis sativus* L., tomato *Solanum lycopersicum* L.), which shows a novelty insight for organic agriculture.

Keywords: agricultural homoeopathy, organic production

1. Introduction

According to the four principles of organic agriculture, health, ecology, care and fairness, all materials used as fertiliser to control pests must be innocuous for people, animals and environment. Those principles are mainly focused to respecting and emulating tha natural biological cycles in order to leave for future generations the same possibility of using natural resources of the planet. All materials or supplies must come preferably from the same farm to avoid introducing external risk agents and to reduce costs [1]. In this context, homoeopathy should be an alternative resource to help crop plants improve their production under several biotic and abiotic stress conditions.

Homoeopathy is a branch of universal medicine based on the 'principle of the like' (*Similia Similibus Curentur* = Like treats Like) and in minimum doses. It means that a substance in a massive dose generates pathological symptomatology;

symptomatology, has the possibility of cure it, if applied in the minimum doses obtained by dilution and intense agitation, in other words, homoeopathic succussion [2]. Homoeopathy is derived from a Hippocratic concept developed by the German physician, Samuel Hahnemann (1755–1843), with serially diluted medicines (1:9, 1:99, 1:999 and others) in water-ethanol vehicle alternating dilution with succussion [3]. Through this process, medicinal products were obtained, called potencies, dilutions or dynamisations, in decimal, centesimal or thousands, and others [4].

The starting point in obtaining a certain boosting curative capacity is a concentrate or mother tincture (MT), which is an alcoholic extract prepared from plants, animals, minerals and even inert materials as nanoparticulate metals [4]. Despite its high dilution, it is possible to detect molecules or nanoparticles of the 'ponderable active principle' (MT) in dynamisations, even in high centesimal dilutions (12CH, 30CH, 200CH) although according to Avogadro's theory, the dynamisation 12CH should have a single molecule of the original substance contained in the MT [5].

The principle of similarity should be understood as the parallelism between the toxic effect of a substance and its therapeutic use for the treatment of similar, but not necessarily identical, symptomatology. Some drugs used for the treatment of depression, anxiety and panic can induce panic and depressive symptoms in a healthy person when they have been homoeopathically energised by means of a dilution-succussion process [6]. The most paradigmatic and controversial aspect of homoeopathy is that these serial dilutions have a measurable effect even when they are given to an animal or a plant in infinitesimal doses, which leave no possibility of suggestion and placebo effect.

Formerly, the somewhat ethereal or subjective concept of 'vital force' was handled, which has fallen into disuse because it has been found that a large part of the effects of homoeopathic medicine are mediated by cells of the immune system [7, 8]. This is the case of ultradilutions of Aspirin® that have a platelet pro-aggregating effect, just opposite to the effects of aspirin in a ponderable dose [9], and whose action would be mediated by the COX-2 coenzyme [10].

Animal model research has its advantages, and possibly the most robust, reproduced and tested research topic in the world is the effect of thyroxine on the induction of frog metamorphosis. The effect of thyroxine in massive dose is just the opposite to the effect of the same product in homoeopathic dose [11], which has been proven by different authors in different frog species [12]. In veterinary medicine, homoeopathy is used, among others, for the treatment of foetal death in pigs (Day, 1984); control of mastitis in cattle [13] and sheep (Day, 1986); and elimination of ticks in bovines [14], salmonellosis in chickens [15] and gastroenteritis in dogs [16]. In aquaculture systems, it can be administered in food or added directly to culture water [17] to promote resistance to pathogens in stress situations and a better postinfection recovery [2, 18]. Freshwater fish, when raised in low stress conditions, have greater survival, production potential and meat quality [19]. Other relevant research topics are gonadic development and sexual maturation [20], stress response [21], physiological and neuroendocrine changes [20, 22], and growth/survival [20]. Recently published results [23, 24] have shown that aquacultural homoeopathy is able to produce measurable biological effects in marine molluscs, shrimp and fish, which trigger physiological, humoral, genomic, metagenomic and transcriptomic responses [17, 25, 26] to recover the internal homoeostasis of the treated individual, which is a synonym of health.

In the plant model, the most replicated study is the protective effect of homoeopathically ultradiluted arsenic versus the effect of arsenic itself in ponderable doses (pre- and post-treatment) on maize seeds [27]. Positive results have been achieved in prevention and control of plague organisms in the *Solanum quitoense* Lam [28] crop, in germination and initial growth of *Hancornia speciosa* Gomes [29] and in remediation of soils affected by heavy metals [30]. The homoeopathic medicine *Natrum muriaticum* has been used successfully to increase resistance to salt stress in *Solanum lycopersicum* [31]. Those previous and other published results, suggest that aquacultural and agricultural homoeopathy are viable alternatives from the economic, ecological and social points of view to contribute to the environment and extinguish the harmful footprint left on the planet by the indiscriminate use of various toxic agrochemicals [32].

The homoeopathic medical therapy establishes a principle that there are no illnesses but sick people and that every disease is only the reflection of a disturbance of the dynamic balance between the organism and biotic and abiotic elements of their environments. It is officially recognised as an alternative, holistic or integrative medicine therapy in various countries of the world where medical professionals are also trained at bachelor, master and doctoral degrees. Particularly in Mexico, the practice of homoeopathy was authorised by a presidential decree on July 31, 1895, and its study and practice are now officially recognised in the General Health Law (2015), and only health professionals can prescribe homoeopathic medicines. Homoeopathic medicines must have an official code and registration, so they differ from other products that are not medicines but herbs for infusion or herbal remedies. In countries such as Brazil, there are homoeopathic medicines for exclusive use in veterinary medicine and aquaculture of marine and freshwater fishes registered with the Ministry of Agriculture [17, 24].

Homoeopathy is an emerging holistic therapy whose application continues to grow throughout the world and is gaining ground thanks to rigorous scientific research in human, animal and plant models. Due to new discoveries in the field of quantum physics, it has even been proposed to rename homoeopathy as 'adaptive network nanomedicine' [33]. As a counterpart, the official medicine known as 'allopathy' is derived from a Galenic concept: the 'principle of opposites' based on the application of massive doses of various chemotherapeutic agents. These drugs, generically synthetic, are officially classified as antimicrobial, antiviral, anti-inflammatory, antispasmodic, antihistamine, anti-fever, etc., which, although they can alleviate, eliminate, or conceal symptoms, invariably have side effects and contraindications. Because it can affect health, pharmaceutical laboratories must communicate and describe these risks to the consumer on the label of the drug, following legal provisions.

This chapter describes recent research results about the use of homoeopathic medicines (HOM) and homoeopathic treatments (HOM treatments) in vegetable model. For the purposes of this chapter, commercial homoeopathic medicines for human use have been utilised in the form of liquid hydro-alcoholic dynamisations (Similia® Laboratories, Mexico) and injectable aqueous dynamisations (Rubio Pharma®, Mexico). They were considered 'stock dynamisations' from which the respective 'study dynamisations' were obtained through a serial process of decimal or centesimal dilution-succussion. The following HOM treatments were used for plants in controlled laboratory conditions and semicontrolled conditions in the field, as dynamisations of *Natrum muriaticum* (NaM), *Silicea terra* (SiT), *Magnesia phosphorica* (MaP), *Arsenicum album* (ArA), *Zincum phosphoricum* (ZiP) and *Phosphoric acid* (PhA) and *Magnesium metallicum* (MgM) (Provider: Similia®, CDMX, México) and *Magnesium Manganum phosphoricum* (MaMnP) (Provider: Rubiopharma®). These and other nosode-type HOM products have been designed by CIBNOR, which is processing the respective industrial property titles (Office for industrial protection and technology transfer, OTT-CEPAT/CIBNOR, www.cibnor.gob.mx). Distilled water (DW) was used as control treatments. HOM treatments and controls are used to soak seeds and sprinkle leaf area or added directly to substrate for crop species. It is very important to consider that homoeopathy could be used in fulfilling the organic agriculture principles. Nowadays authors of this chapter are studying the effects and substance homoeopathy in organic agriculture principles.

2. Effect of homoeopathy on plant species

2.1 Promotion of plant growth health, nutrition and performance

The application of homoeopathy in agriculture is known as agricultural homoeopathy, which offers an ecologically and economically viable model with the potential to reduce the use of agrochemicals in world agriculture. Homoeopathy in plants contributes to the improvement of internal processes to optimise their growth and development [34]. Scientifically proven results in crops have validated their ability to modify the physiological response of the plant, abundance of foliage and amount of fruit [30]. Recently the homoeopathic medicines *Sulphur*, *Silicea terra* and *Nux vomica* have been assessed on different plants of commercial interest, including corn [35], while the use of other HOM treatments is recommended, such as *Calcarea carbonica*, *Carbo vegetabilis* and *Magnesia carbonica*, because their active ingredients can provoke favourable responses in plants [34].

2.1.1 Germination and emergence of cucumber (Cucumis sativus) and tomato plants (Solanum lycopersicum)

Horticultural fruit crops are of great interest in agricultural industry due to their high consumption worldwide either fresh or canned, so it is important to increase their production. At the Universidad Técnica Estatal de Quevedo (UTEQ, Ecuador, FOCICYT Project), the effect of HOM treatments on tomato and cucumber was studied during germination and emergence from certified seeds of tomato and cucumber (Floradade and Marketmore varieties, respectively). The bioassays were carried out in the experimental area of the Plant Biotechnology Laboratory (germination) and in 'La María' farm (emergence) at UTEQ.

For germination and emergence, a completely randomised design was applied with a 2 axis bifactorial arrangement where factor A was dynamisations (7CH and 13CH) and B was homoeopathic medicines. HOM treatments T1 (NaM 7CH), T2 (SiT 7CH), T3 (MaP 7CH), T4 (ArA 7CH), T5 (NaM 13CH), T6 (SiT 13CH), T7 (MaP 13CH) and T8 (ArA 13CH) were applied in cucumber and T1 (NaM 7CH), T2 (SiT 7CH), T3 (ZiP 7CH), T4 (PhA 7CH), T5 (NaM 13CH), T6 (SiT 13CH), T7 (ZiP 13CH) and T8 (PhA 13CH) in tomato. For both species, distilled water (DW) was applied as a control. Each experimental treatments included six repetitions, each one with 30 seeds. The seed were previously disinfected and washed and then submerged for 20 min in each of the corresponding HOM treatments. The germination tests were carried out in sterilised Petri dishes (150 × 15 mm) placing filter paper as a substrate on the dish bottom. In each dish, 5 ml of the corresponding HOM treatment was initially added, and humidity of the paper substrate kept adding distilled water daily. The germination tests were carried out under controlled conditions with a 12:12 h photoperiod and 27 ± 1°C temperature. The seeds were considered germinated when the radicle is measured around 2 mm in length. To evaluate emergence, the seeds were also previously submerged in the HOM treatments and then planted in 200-well polystyrene trays with commercial substrate (Novarbo®). The seeds were considered emerged when the seedling broke the surface and emerged through the substrate.

Germination was recorded daily, determining the final percentage at 24 h in cucumber and 7 days in tomato. Emergence was also recorded daily, and the final percentage was determined at 4 days for cucumber and at 15 days for tomato. The germination and emergence rates were calculated using Maguire's equation [36]: ($M = n_1/t_1 + n_2/t_2 + \dots n_{30}/t_n$ (1)), where $n_1, n_2, \dots n_{30}$ are the number of seeds germinated and emerged at times $t_1, t_2, \dots t_n$. From each treatment/repetition, ten

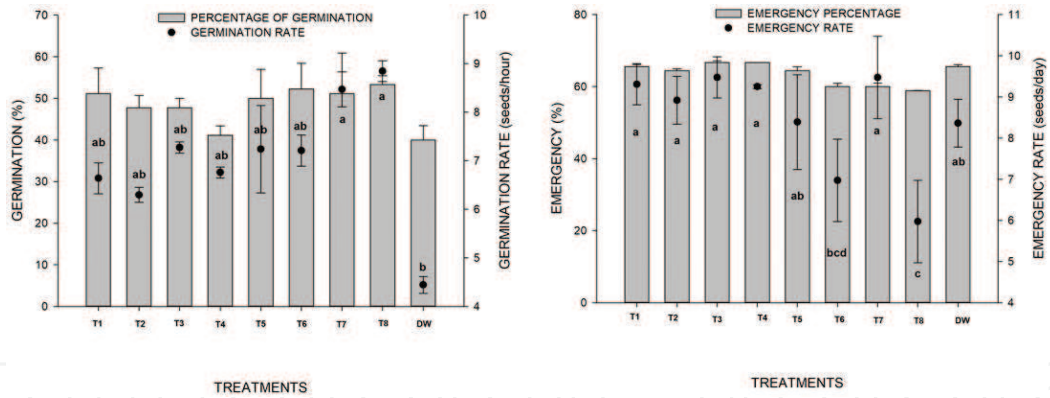

Figure 1.
Effect of HOM treatments on cucumber (Cucumis sativus) germination (% and rate; left) and emergence (% and rate; right); average values with different literals correspond to statistically different treatments ($p \leq 0.05$).

random seedlings were taken for measurement of the morphometric variables at 14 days after emergence. After separating the tissues (radicle, stem and leaves), the length of the stem from the base to the apical part was measured with a graduated ruler (mm). *Idem*, after having washed the roots with drinking water and finally with distilled water, the length of the radicle was measured from the base of the stem where root hairs start up to where the main root ends. To determine fresh and dry biomass of the aerial part (stems + leaves) and radicle, each one was weighed separately in analytical balance (Mettler Toledo®, model AG204). Then aerial part and radicle were placed separately in paper bags in a drying oven (80°C) during 72 h until complete dehydration and then weighed in the same analytical balance. The data were expressed in grams of plant material (fresh or dry).

Test 1. In cucumber the results revealed that germination rate and percentage reached the highest value with T8 (**Figure 1**) with 53% in comparison to the control (40%). The highest germination rate (9 seeds/h) was also obtained with this HOM treatment, compared to control (4 seeds/h). The emergence percentage was similar for T1 and for the control (65%), which was lower than in other treatments. The emergence rate for T1 was higher than the other treatments and without difference with the control (**Figure 1**). During germination, the plants treated with T7 reached the longest stem length (6.5 cm) without statistical difference from other HOM treatments: T1, T2, T4, T5, T6 and T8. In those cases, significant statistical differences were found with the control (4.5 cm). The longest radicle was obtained with T7 (10.9 cm), with respect to the control (8.4 cm). Regarding biomass, the aerial part with the highest fresh biomass was recorded with T2 (0.18 g), which was statistically different from the control (0.12 g). The plants treated with T8 had the highest fresh radicle biomass (0.08 g), compared to the control (0.06 g). Also with T8 treatment, the highest dry radicle biomass (0.0032 g) was recorded with respect to the control (0.0018 g). During the emergence stage using T3, the longest stem length (12.3 cm) was reached, compared to control (9 cm). The greatest radicle length (6.0 cm) was obtained with T4, and no statistical difference was recorded with the rest of the treatments (5.0–5.9 cm). The highest yield in fresh biomass production of the aerial part was obtained with T2 (0.79 g) with respect to the control (0.47 g). Seedlings with the highest fresh radicle biomass (0.12 g) were obtained with T7, showing statistical differences compared to other experimental interactions (0.04–0.07 g). Finally, the highest radicle dry weight (0.0049 g) was attained with HOM treatment T5, which was statistically higher than that of the control (0.0017 g).

Test 2. In tomato, no significant differences were recorded regarding germination rate and percentage. However, with HOM treatment T2, the highest rate and percentage of emergence were obtained (3 and 26%), unlike the control group

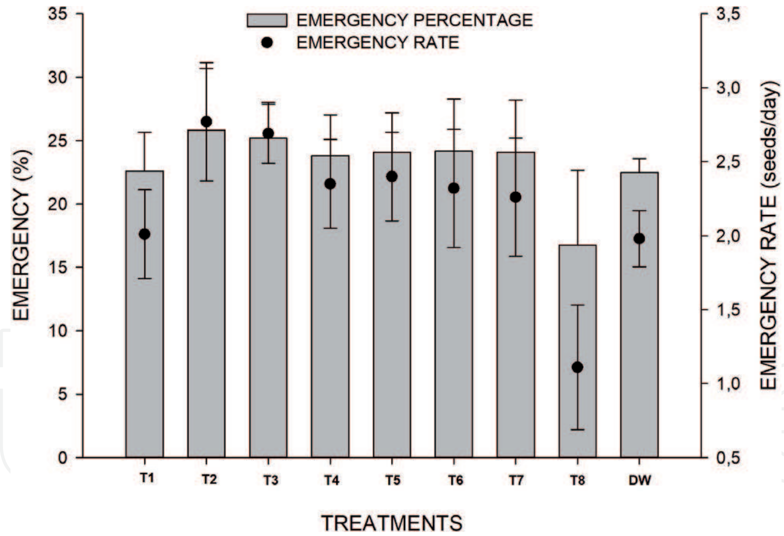

Figure 2.
Effect of HOM treatments on tomato (Solanum lycopersicum) emergence (% and rate).

(≤2 and ≤23%, respectively). In general, most of the HOM treatments had a stimulating effect during the emergence stage (**Figure 2**) since significant differences were recorded in the morphometric variables evaluated. During germination, the length of stem (SL) was greater using T3 (5.5 cm) with respect to the control (4.3 cm). With HOM treatment T2, the highest root development was obtained with a length of 8.2 cm compared to the control (5.6 cm). Significant differences were observed in dry biomass of stem and root obtaining higher dry stem biomass with T3 and T5 (0.01 g) than that obtained with control and other HOM treatments (0.0027 g). When T1 was applied, a higher dry root biomass (0.002 g) was observed with respect to control (0.0005 g), and other HOM treatments (≤0.0013 g) were assessed. During the emergence stage, significant differences were found regarding stem length with HOM treatment T2 (6.6 cm), and with T3 a similar growth was obtained (5.9 cm). The length of the stem was smaller with the rest of the treatments (≤5.8 cm) but, even so, higher than the control (4.6 cm). Regarding root length, no significant differences were found; however, the seedlings treated with T4 and T7 had the highest root growth (4.5 and 4.6 cm, respectively) beyond the control (3.3 cm). Finally, with regard to biomass, no significant differences were observed between HOM treatments and the control.

2.1.2 Initial plant growth of common bean (Phaseolus vulgaris L.) variety Quivicán plants

The common bean (*Phaseolus vulgaris* L.) is one of the edible legumes of greater consumption worldwide, providing an important source of proteins, vitamins and minerals to the diet of populations in developing countries in the Americas [37]. For this reason, research development has focused on improving crop response variables to increase their agricultural production. Thus, the objective of this study was to assess homoeopathic medicines to promote the overall performance and productivity of *P. vulgaris* L. (white-tinted Quivicán variety) during the stages of initial plant growth in pots with substrate, within cultivation houses with shadow mesh at Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, Mexico.

Test 1. Certified seeds of this Quivicán variety (Seed Company, Villa Clara, Cuba) were used; a completely randomised design was used with three HOM treatments, T1 (MgM-31CH), T2 (MgMnP-3CH) and T3 (MgM-31CH + MgMnP-3CH), and a control with distilled water (DW), each one with four replicates. This

Treatments	Morphological parameters										
	SL (cm)	RL (cm)	FRB (mg)	FLB (mg)	FSB (mg)	DRB (mg)	DLB (mg)	DSB (mg)	FA (cm^2)	SD (mm)	L No.
T1	13.4^{ab}	$20.6a^{b}$	12.25^{ab}	13.75^{b}	6.25^{b}	0.71^{b}	1.43^{b}	0.67^{a}	476.15^{b}	2.97^{b}	5.75^{a}
T2	13.4^{ab}	20.05^{ab}	8.88^{b}	14.75^{b}	7.0^{b}	0.58^{b}	1.56^{b}	0.72^{a}	495.14^{ba}	2.92^{b}	5.75^{a}
T3	15.4^{5a}	27.97^{a}	23.75^{a}	21.12^{a}	8.2^{a}	1.52^{a}	2.05^{a}	0.85^{a}	629.37^{a}	3.07^{a}	6.25^{a}
DW	10.5^{b}	21.47^{b}	11.12^{b}	7.87^{c}	3.87^{c}	1.04^{ab}	0.72^{c}	0.36^{b}	283.83^{c}	2.2^{c}	3.75^{b}

Average values with different literals in the same column differ statistically (Tukey HSD, $p \leq 0.05$)

Table 1.
Effect of HOM treatments on morphometric parameters during growth stage of the common bean (Phaseolus vulgaris L.) variety Quivicán.

experiment was developed to evaluate initial growth phase. Seeds were planted (five replications per treatment) in plastic pots (3 seeds/pot) with 5 kg of commercial substrate (Sogemix PM®). Emergence evaluation was done daily; when 50% + 1 of the seeds emerged, 1 ml of the respective HOM treatment and water for the control (NT) was applied around the stems of the plants on alternate days. After 35 days, we proceeded to measure stem length (SL), root length (RL), fresh biomass (g) of root, stem and leaves (FRB, FSB, FLB), foliar area (FA) (cm^2), stem diameter (SD) (mm) and number of leaves (L No). The yields corresponding to dry biomass (DRB, DLB, DSB) were also measured. The most relevant result of the study was highly favourable and statistically significant effect of HOM treatment T3 with respect to the untreated control group (**Table 1**).

The cause-effect results in the plant model demonstrated a synergistic effect of the homoeopathic medicines included in HOM treatment T3, which evidently could not have been a placebo effect because it clearly favoured the growth of *P. vulgaris* variety Quivicán. Magnesium and manganese were components of T3; both are essential for the growth of any living cell and necessary secondary macronutrients for plant growth and development. Around 75% of foliar magnesium was involved in protein synthesis, and 15–20% of total magnesium was associated with pigments, a constituent element of the chlorophyll molecule and extremely important in photosynthesis [38]. Magnesium acts mainly as a cofactor of several enzymes involved in photosynthetic carbon fixation and also in basic metabolism [39]. Both magnesium and manganese play an important role in plant general nutrition and enhance or reinforce their resistance to diseases [40]. The results obtained in this study suggest that agricultural homoeopathy has application in the cultivation of the common bean (*Phaseolus vulgaris* L.) variety Quivicán, during the stages of initial plant growth.

2.2 Attenuation of the effects of abiotic stress

Currently, numerous studies have focused on elucidating the negative effects of abiotic stress on agricultural crops. Saline stress associated with high temperatures and solar irradiation is the most important environmental process that stops cultivated plant growth, development and survival, decreasing productivity [41]. Worldwide, millions of hectares have a high degree of aridity and the presence of salts [42]. In Mexico, arid and semiarid regions constitute more than 50% of the national territory [43], so the study of these factors is important due to the negative impact they have on the agricultural sector. Salinity has environmental, social and economic consequences because sustainability and yield of the cultivated species decrease in the affected areas. Salinity affects plant metabolism and growth, causing a decrease in biomass production [44]. Global losses due to salt stress are estimated at 12 billion dollars per year and affect a fifth of the farmland [45]. Among the main harmful effects, decrease in water absorption, ion assimilation that can cause toxicity and nutritional imbalance and physiological changes, such as the reduction of the photosynthetic rate due to a lower leaf area, which reduces crop viability [46] are included.

Several alternatives have been studied to mitigate the effect of salinity in agriculture, such as genetic improvement, selection of tolerant varieties and physical treatments to seeds to induce tolerance or agrochemicals to stimulate plant growth. In these sense, HOM treatments imply the application of ultra-diluted substances, which are an eco-friendly and economic variant that could be used effectively in any condition and circumstance [47]. Unfortunately, this organic-like alternative has been little studied worldwide.

2.2.1 Salinity stress by NaCl in the bean crop (Phaseolus vulgaris L.)

The common bean *Phaseolus vulgaris* L. is a key product in world food security [48], but the adverse environmental conditions, mainly drought and salinity in soils, affect their general performance reducing productivity and harvest [49]. Saline stress occurs due to high concentrations of sodium (Na^+) and chlorine (Cl^-) that seriously alter the plant metabolism, affecting its growth and development [50]. Water deficit is an osmotic stressing agent, specifically associated with salinisation, which reduces the rate of fixation of carbon dioxide (CO2) and affects processes associated with photosynthesis [51]. Abscisic acid (ABA) is a key hormone that regulates the responses of plants to abiotic stress [52] and initiates the activation of stomatal closure when facing salt stress. When it happens, CO2 levels decrease and consequently photosynthesis, causing oxidative stress [53]. The objective of this study was to evaluate HOM treatment NaM (7CH) as attenuator of the harmful effect of salinity induced by NaCl application. The experiment was developed at CIBNOR to assess the initial growth stage of the species and study the expression of the genes associated with physiological response of *P. vulgaris* against HOM treatments.

A completely randomised design was applied with HOM treatment T1 (NaM 7CH) and control (DW) each one with five replicates and two concentrations of NaCl (0 and 75 mM). Seeds of the variety white-tinted Quivicán (Empresa de Semillas, Villa Clara, Cuba) were used.

Test 1. The seeds were disinfected, then imbibed for 30 min in HOM treatment NaM 7CH or in DW (control treatment), planted in 5 kg plastic pots (3 seeds/pot) with commercial substrate (Sogemix PM®) and grown for 35 days. Once the seeds germinated and the plants emerged, NaM 7CH or DW (15 ml) was applied on alternate days near the stem of each plant. The addition of NaCl began 15 days after germination and is applied gradually until reaching 75 mM to avoid osmotic shock.

The photosynthesis rate (A, $\mu mol\ m^{-2}\ s^{-1}$) was measured with a LCpro-SD portable computer with a wide-blade camera (ADC, Hoddesdon, Herts, United Kingdom). Three measurements were made during the fourth week of the trial, on healthy leaves and on completely sunny days. At the begining of frlowering, a sample was taken to perform the expression analysis of the 9-cis-epoxycarotenoid gene (PvNCDE1) [54]. Total RNA isolation, cDNA synthesis and real-time PCR amplification were performed following the methodology reported by Morelos et al. [55], and the relative expression of PvNCDE1 was estimated following the model proposed by Hellemans et al. [56]. One-way ANOVA followed by Tukey's exact test was performed using STATISTICA 8.0 (StatSoft, Inc.). A p-value <0.05 was considered significant.

At the end of the study, photosynthesis showed a considerable decrease in the control (DW) when the plants underwent saline stress (**Figure 3**). In these stressed plants, stomatal closure was produced by ABA, generating a direct blockage of photosynthesis due to the limited uptake of CO2, an indispensable substrate for the Calvin cycle. When it happens, a blockage of the photo phase occurs due to the null

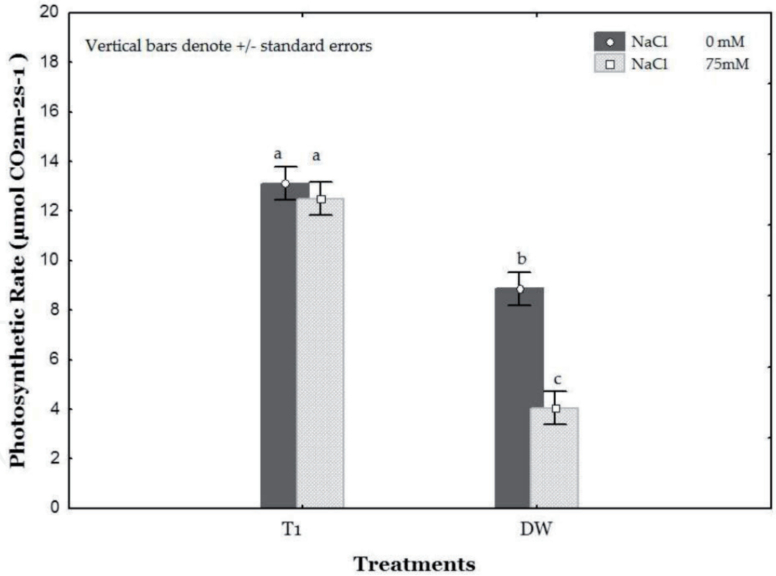

Figure 3.
Photosynthetic rate is recorded in Phaseolus vulgaris treated and non-treated with NaM-7CH and subjected to 0 and 75 mM NaCl. Average values with different literals in the same treatment or control differ statistically ($p \leq 0.05$).

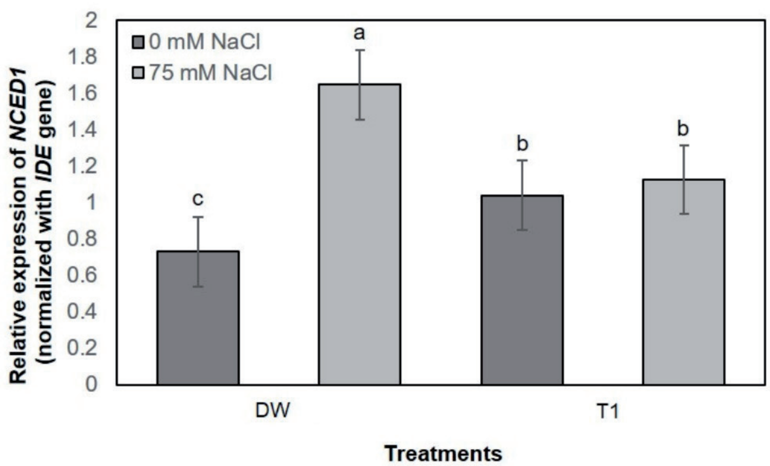

Figure 4.
Expression of the pvNCED1 gene of HOM-treated (NaM-7CH) bean Phaseolus vulgaris L., exposed to NaCl stress. Average values with different literals correspond to statistically different treatments ($p \leq 0.05$).

demand of the reducing power and ATP, resulting in oxygen production with consequent damage to the chloroplast membranes and their subsequent disintegration [57]. Contrary to the above, in HOM-treated plants that were subjected in parallel to salt stress, photosynthesis increased and no significant difference ($p > 0.05$) was observed with respect to plants not subjected to salt stress by the addition of NaCl. This result suggests that plants receiving HOM treatment did not become stressed, despite having been exposed to saline conditions (**Figure 3**).

The 9-cis-epoxycarotenoid gene (NCED) is known to be overexpressed under drought stress conditions in *P. vulgaris* bean, and it is considered fundamental for the regulation of ABA biosynthesis [58]. The increase of ABA under stress conditions causes a change in gene expression and adaptive physiological responses of plants [59]. As a result of this study, the relative expression of the pvNCED1 gene (**Figure 4**) was higher ($p < 0.05$) in the plants without HOM treatment of the control group (DW) than that were exposed to 75 mM of NaCl with respect to those not exposed to NaCl.

The response of the HOM-treated plants did not show significant differences ($p > 0.05$) under normal conditions or in high salinity (75 mM NaCl), which means that the plants receiving homoeopathy were not stressed despite also receiving 75 mM NaCl since a lower expression of the pvNCED1 gene was found in the gene expression study. This result confirms a cause-effect relationship of T1 and the impossibility of a placebo effect in a plant model investigation where there is no possibility of suggestion of the treated individual.

2.2.2 Salinity stress by NaCl in basil (Ocimum basilicum L.)

Basil is an important aromatic species for its use as flavouring and dry and fresh seasoning in the food industry; as stimulant, antispasmodic and antialopecic in pharmacy; and as aromatising cosmetics in the perfume industry [60]. The growing interest of consumers for products of natural origin stimulates the market of aromatic plants, making them a viable option for the organic agricultural sector and the possibility of exporting them fresh or processed in extracts, essences and oils used in culinary industries, cosmetics and pharmaceuticals [61]. This work aimed to assess the effect of NaM as an attenuator of NaCl stress in plant photosynthesis and biomass production of two varieties of basil, grown under hydroponic system.

Test 2. This experiment was developed at CIBNOR, and seedlings were obtained from certified organic seeds of basil *O. basilicum* L., Emily and Napoletano varieties (Vis Seed Company, USA). A completely randomised experimental design with factorial arrangement (2A × 2B × 3C) was used, considering the Napoletano and Emily as factor A, the concentration of NaCl (0 and 75 mM) as factor B and HOM treatments NaM 7CH and NaM 13CH as factor C. The study included a total of 12 treatments each with 4 replications. The bioassay was carried out in expanded polyurethane boxes of 69 × 38.5 × 25 cm and 38 L capacity, gauged with potable water (electrical conductivity 0.22 dS m^{-1}). Six plastic pots (150 ml) with an experimental plant inside were fixed above each box letting the roots inside the box with liquid-enriched media pass through 1 inch holes. The plants received a nutritious solution adapted for basil according to Samperio [62].

The application of the HOM treatments and control (DW) began after a period of acclimatisation 7 days after transplant, spraying the aerial part of the plants with 150 ml $plant^{-1}$ on alternate days. After 15 days, the application of saline treatments began gradually to avoid osmotic shock until a concentration of 75 mM was reached.

The photosynthetic rate (A, μmol m^{-2} s^{-1}) was measured with (IRGA) LCpro-SD portable photosynthesis system equipment with a broad-leaf leaf chamber (ADC, Hoddesdon, Herts, UK) in a completely turgid and healthy leaf on completely sunny days (three measurements, 1 week before cutting); 45 days after transplant, the leaf area was determined (LA, cm^2) by integrating leaf area metre (Li-Cor®, model-LI-3000A, series PAM 1701) and biomass using fresh weight of the aerial part (BFAP, g); an analytical balance was used (Mettler Toledo®, model AG204).

The cause-effect results of this study revealed significant differences between varieties × NaCl × HOM treatment for A ($F_{2,60}$ = 4.14, $p \leq 0.020$), FA ($F_{2,36}$ = 2.87; $p \leq 0.01$) and BFAP ($F_{2,36}$ = 11.1; $p \leq 0.0001$). Napoletano showed highest fresh weight of aerial part without the addition of NaCl and the HOM treatment NaM 7CH (**Table 2**). When both basil varieties were subjected to 75 mM NaCl, the photosynthetic rate decreased 53.6 and 63.6% with respect to the control in Napoletano and Emily, respectively. However, this result was reversed with the application of HOM treatments (NaM 7CH or NaM 13CH) in all variables and both varieties. At the end of the study, increases greater than 50% were obtained with respect to the plants that received 75 mM NaCl, but they did not receive a 'similar' HOM treatment.

These results suggest that *O. basilicum* plants naturally and positively responded to saline stress with both HOM treatments without the possibility of a placebo effect, both at cellular level and in the tissues as a whole, increasing growth. According to Zhu [63], stress agents offer environmental signals that are perceived and recognised by plants,

Varieties	NaCl (mM)	*NaM*	A (μmol m^{-2} s^{-1})	BFAP (g)	FA (cm^2)
Napoletano	0	0	15.46^{bc}	174.6^{b}	4768.9^{a}
	0	7CH	22.7^{a}	190.9^{a}	5013.1^{a}
	0	13CH	15.68^{bc}	164.0^{c}	4304.6^{b}
	75	0	7.17^{g}	61.7^{h}	846.3^{g}
	75	7CH	17.02^{b}	140.7^{e}	3060.9^{d}
	75	13CH	13.15^{de}	110.7^{f}	2471.9^{e}
Emily	0	0	12.68^{ef}	143.2^{de}	3389.2^{cd}
	0	7CH	15.39^{bcd}	150.2^{d}	3501.9^{c}
	0	13CH	12.55^{ef}	142.4^{de}	3133^{cd}
	75	0	4.62^{i}	57.2^{h}	747.5^{g}
	75	7CH	13.47^{cde}	108.2^{f}	2195.4^{ef}
	75	13CH	10.69^{f}	93.4^{g}	1951.5^{f}

Average values with different literals in the same column differ statistically (Tukey HSD, $p \leq 0.05$)

Table 2.
Effect of the interaction of varieties × NaCl × NaM in the variables evaluated in two varieties of basil (O. basilicum) subjected to saline stress (NaCl).

transduced into cells and re-transmitted, which generate a cascade of biochemical, physiological and genetic responses that allow the plant to adapt to change when it is gradual, but this response depends on each species and variety. It is possible to hypothesise that the presence of magnesium nanoparticles initially bioavailable in sea salt (NaM origin) has contributed to the formation of chlorophyll molecules and photosynthesis, which is the main process of plant biomass production. Additionally, Magnesium has a predominant role in enzymatic activity related to carbohydrate metabolism [64]. The results of this study suggest that agricultural homoeopathy besides being an organic-like treatment can increase general overall performance and productivity of *O. basilicum*, strengthening its ability to tolerate saline stress conditions without the need of using agrochemicals.

2.3 Attenuation of the effects of biotic stress

With the indiscriminate use of pesticides in agriculture, the resistance of pathogenic microorganisms to the chemicals used has increased, which has negatively affected the environment and therefore the consumption of products with high levels of toxicity. Therefore, technological alternatives have been generated to replace conventional ones. Recently the application of safe products has increased in agriculture, many of which are framed in novel agricultural homoeopathy. Agricultural homoeopathy is an alternative for agricultural farmers, compatible with traditional, organic, ecological, biodynamic and even conventional agriculture, capable of influencing the biological processes of plants by controlling health problems caused by fungi, viruses and bacteria. It contributes to pest control and influences crop growth and development [34].

Test 1. The experiment was developed at UTEQ, Ecuador, to assess in vitro activity of homoeopathic medicines against *Fusarium oxysporum* f. sp. lycopersici. One of the most important diseases affecting tomato crop is vascular wilt caused by *Fusarium oxysporum* f. sp. lycopersici (Fol) (Sacc.) Snyder and Hansen [65]. The control of fungal diseases in agriculture is controlled by agrochemicals including toxic pesticides whose excessive and indiscriminate use has caused the decrease or loss of the fertile soil layer and death of microorganisms in soil. Additionally, the pathogens have developed resistance to the active ingredient of the agrochemical that generally has a high cost [34]. Therefore, it is necessary to search for ecological and less polluting alternatives for the control of pests and diseases in agriculture, ensuring the safety and future of the agro-food industry. The objective of this study was to evaluate the in vitro effect of homoeopathic medicines on the pathogenic fungus *Fusarium oxysporum* f. sp. lycopersici, highly damaging various agricultural crops [66].

An experimental design which is completely randomised was applied with six homoeopathic medicines, each in two dynamisations (7 CH and 13 CH, Similia® CDMX, Mexico): T1 (MaP 7CH), T2 (ZiP 7CH), T3 (PhA 7CH), T4 (SiT 7CH), T5 (NaM 7CH), T6 (ArA 7CH), T7 (MaP 13CH), T8 (ZiP 13CH), T9 (PhA 13CH), T10 (SiT 13CH), T11 (NaM 13CH) and T12 (ArA 13CH) and one control (DW) to measure antifungal activity against *F. oxysporum*, using the method of the poisoned medium [67]. The diameter of the mycelium was measured daily, and the percentage of growth inhibition was determined by the formula [%inhibition = mycelial growth of the control − mycelial growth of the treatment/mycelial growth of the control × 100].

No significant differences were observed in radial growth of the phytopathogenic fungus in the HOM treatments with respect to the untreated control (NT). On the other hand, significant differences were observed between HOM treatments and their dynamisations (7 CH and 13 CH) with respect to NT. The dynamisation 13 CH increased the percentage of inhibition of the phytopathogenic fungus. These results confirmed the variability of the response induced by homoeopathic medicines in plant model, whose response depends on the dynamisation used [68]. These results are in agreement with those reported by Narváez-Martínez et al. [28] who used a homoeopathic treatment developed from a pathogen (nosode); when they applied different dynamics in tomato *Solanum quitoense* Lam, they found different effects against a pest caused by *Neoleucinodes elegantalis*. HOM treatments T2, T7 and T12 offered a greater inhibition percentage against the pathogen (70, 65 and 51%, respectively). These results agree with Tichavsky [69], who stated that *Phosphorus* homoeopathic medicine helped to control diseases caused by fungi, and two of these treatments contained phosphorus. According to Casali et al. [70], this result was due to the production of secondary metabolites (essential oils). However, during this study, HOM treatments T3 and T9 favoured the growth and reproduction of the fungus. Our results coincide with Damin et al. [71] who evaluated nine homoeopathic medicines against the pathogen *Metarhizium anisopliae* and obtained stimulation in the production of conidia by this fungus. It is necessary to conceptualise that homoeopathy acts on living beings; therefore, fungus can also be favoured with a HOM treatment.

The attained results revealed that the HOM treatments showed activity against *F. oxysporum*, highlighting *Zincum phosphoricum* and *Magnesium phosphoricum*. These cause-effect results in the plant model demonstrated that a placebo effect is not only absent in homoeopathic medicine but also supports agricultural homoeopathy. These results contribute to search for alternatives to control diseases caused by this phytopathogen in tomato plants, by using effective, innocuous and more eco-friendly tools to substitute the use of agrochemicals.

3. Conclusions

A lot of experimental scientific results related with the use of homoeopathic medicines in plants, besides new results and insights discussed along this chapter, eliminate the principal argument of homoeopathy detractors. Actually, they cannot sustain their arguments in the sense that the suggestion and the placebo effect are the only mechanisms of action of this old, and at the same time new, alternative for organic agriculture.

As shown throughout the text, there is sufficient scientific evidence that homoeopathy strengthens the energy and vitality of plants even under conditions of abiotic stress and promotes a dynamic balance of the plant with soil, water and the environment. Homoeopathic dilutions from different origins can be applied by soaking seeds and spraying leaves or directly to the soil or substrate. These ultra-diluted and innocuous treatments have the ability to initiate cascade responses, promoting favourable physiological reactions in the plant, with a systemic approach against the symptoms associated with an infection or a stressor. This is possible because, as in any living organism, plants have a genetic memory, which is continuously enriched. The response of plants treated with homoeopathy is natural, lasting and without the negative side effects that some agrochemicals have, which can even accumulate in their tissues and affect the safety of the harvested product, which makes it unfit for human consumption. Undoubtedly, homoeopathy has great potential, not only in human public health and aquaculture and veterinary production but also in organic, ecological and sustainable agriculture, which will be essential for the future development of humanity.

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