Original Article

A pilot study of the influence of *Natrum muriaticum* 6cH and 30cH in a standardized culture of *Phaseolus vulgaris* L.

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ABSTRACT

The use of highly diluted and agitated solutions is widespread. Its use extends to all living beings including plants, acting effectively in the latter's primary and secondary metabolism. Aims: this is a pilot study designed to assess the action of *Natrum muriaticum* in dilutions 6 CH and 30 CH in comparison to the action of 5.0% NaCl solution administered separately to a population of *Phaseolus vulgaris* L. (common bean). Materials and methods: it was determined the relative growth rate (RGR) of the bean population treated for 6 weeks and subdivided into 4 groups (5 vases each): P1 (control) treated with 30% alcohol solution only; P2, treated with aqueous 5.0% NaCl solution; P3, treated with *Nat-m* 6 CH; P4, treated with *Nat-m* 30 CH. Results: it was seen an increase in the salinity of the soil that caused the inhibition of the development of the bean population P2. In addition, the use of *Nat-m* promoted a significant increase in vegetable growth, chiefly in dilution 6 CH (P3), causing a significant increase in the RGR of the bean population. Conclusion: high dilutions of Nat-m showed to be efficient to stimulate the growth of common bean.

Keywords: High dilutions, *Phaseolus vulgaris* L, Relative growth rate, *Natrum muriaticum*.

1. Introduction

The use of highly diluted and agitated solutions is widespread and efficient in the treatment of human beings. In time, the use of these preparations was extended to all living beings, as well as soil and water.

In plants, it is believed that high dilutions act on the metabolism, resulting in the increased formation of secondary metabolic products related to the mechanisms of defense of plants [1,2] or in the decreased formation of these products [3]. In some cases, these preparations may influence the primary plant metabolism provoking a response in their growth and vitality. For this reason, farmers from various locations in Brazil and from other countries have been using these solutions in plants, achieving positive results regarding resistance to parasites and diseases, unfavorable physical conditions, efflorescence, breaking seed dormancy and production of healthy seedlings [4].

Diverse preparations have been used in plants, including *Arnica montana, Sulphur, Nux vomica, Natrum muriaticum, Phosphorus, Thuja occidentalis, Carbo vegetabilis, Calcarea carbonica, Medohrrinum, Staphisagria, Mercurius solubilis, Kalium iodatum,* and others [5].

Natrum muriaticum improves plant acclimatization in locations that are inappropriate for growth and development. It is also indicated for plants that are under stress due to drought and frost. This medication is prepared from sea salt and employed according to the principle of similarity and minimum doses. From the perspective of the principle of similarity, a high salinity of the soil, mainly caused by sodium salts, sodium chloride (NaCl) in particular, has a damaging effect on plants [6].

According to Greenway and Munns [7], plants exposed to high salinity levels usually respond by osmoregulation inside the cell due to an increase in ion absorption. Plant adaptation to these conditions may depend on an increased amount of specific organic solutes or an ability to prevent the accumulation of salts in the cytoplasm. Studies suggest that high salt contents induce alterations in protein metabolism (hydrolysis) [6]. According to Távora [8], the most remarkable effect of salinity in plants, besides the alteration of the osmotic potential, is toxicity and a lack of balance in nutrient absorption, provoking a generalized decrease in plant growth.

The response of plants to salinity conditions is related to the expression of various genes [9] and each species has a tolerance level to salt that depends on the concentration and nature of the dissolved salts, climatic factors, water absorption and vegetable nutrition [10].

The present pilot study was aimed at assessing the action of *Natrum muriaticum* at 6 CH and 30 CH potencies, compared to the action of a 5.0% NaCl when separately administered to *Phaseolus vulgaris* L. (common bean) populations.

Materials and Methods

Phaseolus vulgaris L. was chosen due to easy cultivation and short life cycle. Furthermore, bean culture is considered to be very sensitive to salinity [11, 12].

For planting, seeds from a same lot were used to reduce genetic variability and thus produce a more uniform result. The experiment was conducted in a greenhouse at *Pontifícia Universidade Católica de Campinas*, in Campinas, São Paulo, Brazil. The climate of the city is classified as tropical according to altitude, with an average annual temperature of 22.3ºC.

The populations of beans were treated for 6 weeks, from September to November 2009. Each of the 4 populations was composed of 25 plants in 5 vases, with 5 plants in each vase. Such numbers were justified by culture standardization with seed selection and cultivation in a greenhouse decreasing potential interferences. Moreover, the results were analyzed by paired t test and this configuration was sufficient to perform the study. The first population (P1), named control group, was treated only with water added to 5 drops of 30% ethanol. The second population (P2) was treated with an aqueous solution of 5.0% sodium chloride. The third population (P3) was treated with homeopathic preparation *Natrum muriaticum* 6 CH, and the fourth population (P4) was treated with *Natrum muriaticum* 30 CH.

Highly diluted solutions were prepared according to the Brazilian Homeopathic Pharmacopeia – 2nd Edition [13] and thus the 6 cH and 30 cH dilutions were prepared in 30% ethanol.

The solutions were administered to the 4 populations for a total period of 6 weeks, 5 days a week. In the control population (P1), each vase received 5 drops of 30% alcohol solution diluted in 50ml of water. P2 was treated with 50 ml of an aqueous solution of a 5.0% sodium chloride (each vase). P3 received *Natrum muriaticum* 6 cH and P4 received *Natrum muriaticum* 30 cH. For both groups, 5 drops of the respective high dilution were diluted in 50ml of water and administered to each vase. None of the populations had been previously intoxicated with sodium chloride solution and therefore P2 was used as positive control.

Each week an individual plant from each population of *Phaseolus vulgaris* L. was randomly removed from each vase and submitted to drying in a sterilizer at a temperature of 100 to 105 ºC until a constant mass was obtained (for approximately 1 hour). Subsequently, the samples were cooled in a desiccator for time sufficient to achieve room temperature. After drying, the samples were weighed on an analytical balance. The values obtained were used for calculation of the relative growth rate (RGR), which represents the relation between the assimilative efficiency of vegetable leaves and foliage (number of leaves/plant and leaf size) of the plant itself, used to observe mean growth velocity throughout the observation time period [14]. This parameter is calculated [15] by the ratio between the natural logarithm of the total dry mass obtained from two successive samplings (P_2 and P_1) and time interval (t_2 and t_1) between these two samplings, therefore

$$\mathrm{RGR} = \frac{\ln P_2 - ln\, P_1}{(t_2 - t_1)}$$

After calculation of the RGR, the results were statistically treated by analysis of variance, ANOVA, with repeated measures, and the difference between groups was analyzed by paired t test, which compared 2 populations at a time.

In addition to determining the RGR during the experimental period, characteristics such as leaf depigmentation and desiccation were observed and recorded. However, no attempt was made to quantify these observations. All experimental procedures were conducted in a double blind manner.

Results

In agreement with reports in the literature [6, 15], the plant population that received a 5.0% NaCl solution manifested signs of intoxication, as shown in figures 1 and 2.

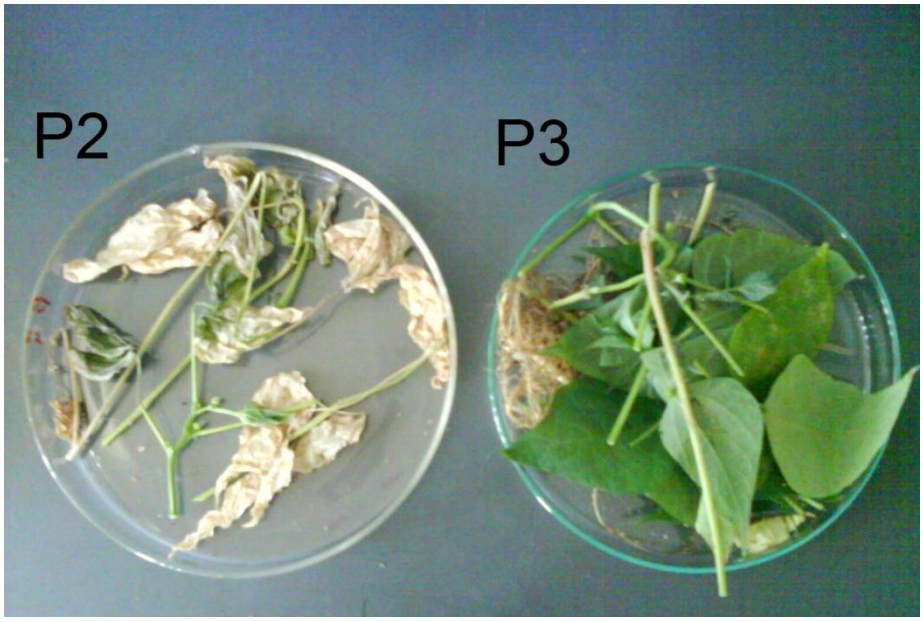

Figure 1**:** Petri dishes containing individual plants from population P2 (5.0% NaCl) and population P3 (*Natrum muriaticum* at 6cH), respectively, collected on the fifth week of treatment, before drying, showing a significant difference between the growth and development of those groups.

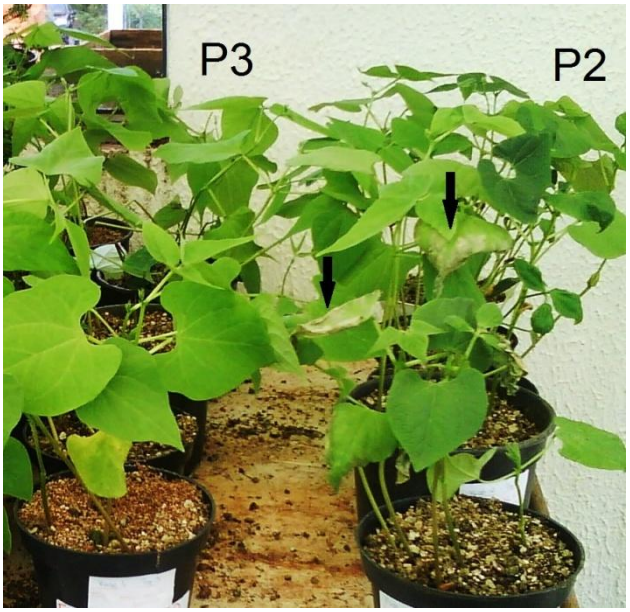

Figure 2: Population P3 (*Natrum muriaticum* at 6cH) and population P2 (5.0% NaCl), respectively. It may be observed that leaves referring to individuals from population P2 showed signs of toxicity (arrows).

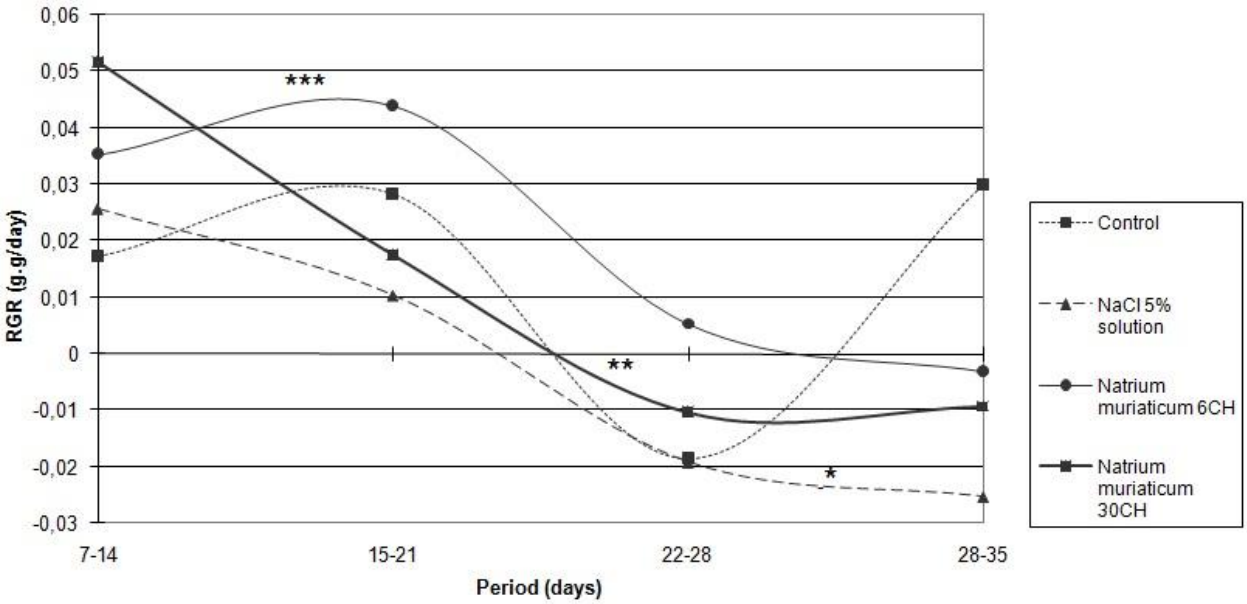

Figure 3: Graphic representation of the evolution of Relative Growth Rate (RGR) of four bean populations (control, NaCl 5.0%, *Natrum muriaticum* at 6 cH and *Natrum muriaticum* at 30 cH) during 42 days of experiment. The results were analyzed by Analysis of Variance (ANOVA with repeated measures) and there was a significant difference between groups ($p<0.05$). Two populations were compared simultaneously by the paired t test. There was a significant difference (***) ($p<0.05$) in relation to the control population (P1) and a 5.0% NaCl population (P2), (**) a significant difference ($p<0.05$) in relation to a 5.0% NaCl population (P2) and (*) a significant difference ($p<0.05$) in comparison to the control population (P1).

In this population (P2), premature plant development of a yellowish color occurred, lower development of green mass (expressed by the RGR) and significant leaf desiccation were obsersed. In contrast, the population that received *Nat-m* 6 cH showed visibly greater development of the green mass compared to the control populations and those treated with *Nat-m* 30 cH. Furthermore, the same population preserved the greenish pigment for a longer period of time. Characteristics of the plant population that received *Nat-m* 30 cH were very similar to those observed in the control population, i.e. pattern of pigmentation, development of green mass and leaf desiccation were similar in both groups. These observations were confirmed after analyzing the results of the Relative Growth Rate (figure 3), in which there was significant difference among the 4 groups analyzed by ANOVA and significant difference between P3 (*Nat-m* 6 cH) and P1 (control group) when the paired t test was applied.

Discussion

Highly diluted and agitated solutions have been employed with success in human beings, animals, plants, soil and even microorganisms [16]. In various vegetable studies, experimentation has proved that vegetables are sensitive to these solutions, while are exempt from an important interfering factor: the placebo effect. The data obtained thus becomes more concrete, since it deals with the measurement of physiological and not psychological alterations.

In agriculture, highly diluted solutions may be used for the control of pests, diseases, improving culture productivity and natural plant defense. They may also be used directly on soils with excellent results [5].

It is important to highlight the advantages of experiments in vegetables similar to ours. There is a great diversity in research: studies can vary from perennial cultures to cultures of a very short cycle. It is possible to study large plant populations. Furthermore, some plants propagate non-sexually, meaning that it is easy to do research with genetically identical clone individuals [16].

The present study corroborates previous reports, confirming the effectiveness of high dilutions in vegetables, more specifically in beans (*Phaseolus vulgaris* L*),* in which a positive influence of dilutions 6 CH and 30 CH has been observed. The hypothesis formulated to carry out this experimental model was based on the well-known immediate effects of salinity on vegetables, e.g. physiological drought, originating in a decrease in osmotic potential, nutritional imbalance, due to a high ionic concentration, inhibiting the absorption of other nutrients [15].

The results obtained confirmed the hypothesis that the addition of 5.0% NaCl to the culture globally affected the growth of the *Phaseolus vulgaris* L. population evaluated, mainly in terms of individual height, formation of new leaves, yellowish leaves and completely necrotized leaves. In contrast, populations treated with *Natrum muriaticum* 6 cH and 30 cH responded to treatment in a positive manner and did not present signs of toxicity during growth. A better development occurred throughout the 6 weeks of treatment with *Natrum muriaticum*. *Natrum muriaticum* 6 cH presented higher RGR when compared to the control group, unquestionably demonstrating a significant difference. Dilution 6 cH proved to be more effective than dilution 30 cH, which failed to provoke significant results when compared to the control group.

To justify the lack of a significant effect of dilution 30 cH, it is suggested that this solution might have produced mild pathogenetic effects. This is a phenomenon observed in clinical practice, when the use of inadequate dilutions may cause the appearance of symptoms characteristic of the primary action of a drug. It is evident that this dilution did not have the same effects as those caused by the 5.0% NaCl solution. However, it is an indication of a borderline dilution. Dilutions above this concentration could possibly have an inhibitory effect rather than a stimulating action on vegetable growth.

These results corroborate those obtained by Carvalho [17], who observed the effect of *Natrum muriaticum* 2cH in artemisia plants (*Tanacetum parthenium* (L.) Sch. Bip.) considered healthy, where it increased the proline content of leaves, and in the same plants submitted to water deficiency where it presented an immediate reduction in leaf proline content. These results also corroborate those obtained by Grisa et al. [18], who observed an increase in the dry matter weight of cultivated lettuce treated with *Arnica montana* 6 cH and 12 cH. The same result was not found when dilution 30 cH was used. Furthermore, Luis and Moreno [19] studied the effect of a highly diluted solution based on *Calcarea* 30 cH on the vegetative growth of scallions, and observed that *Calcarea fluorica* 30 cH increased the production of fresh weight by approximately 45% compared to the control group.

A different result was obtained by Carvalho *et al* [4] in the cultivation of artemisia treated with *Arnica montana* in high dilutions, in which the height and fresh mass of the plants did not suffer changes after the application of these solutions, and observed that the content of parthenolide decreased, especially with dilutions 3 cH and 5 cH.

From the results obtained in the experimental model defined for the present study, it was concluded that the use of *Natrum muriaticum* in high dilutions promoted a significant increase in vegetable growth, especially in dilution 6 cH, which caused a significant increase in the RGR of the bean population.

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Influência de *Natrum muriaticum* 6 cH e 30 cH em cultura padronizada de *Phaseolus vulgaris* L.: estudo piloto.

RESUMO

O emprego de soluções dinamizadas em altas diluições é amplo e se estende a todos os seres vivos, inclusive aos vegetais, atuando efetivamente nos metabolismos primário e secundário. Este estudo piloto tem como objetivo verificar a ação do *Natrum muriaticum* 6 CH e 30 CH comparando-as à ação da solução de NaCl 5.0% quando administradas separadamente em populações de *Phaseolus vulgaris* (feijão). A avaliação foi realizada através da determinação da Taxa de Crescimento Relativo de populações de *feijão* tratadas por seis semanas e subdivididas em 4 grupos (com 5 vasos cada): P1, grupo controle, foi tratado somente com solução de álcool 30%; P2, tratado com solução aquosa de NaCl a 5,0%; P3, tratado com *Natrum muriaticum* 6 CH; e P4, tratado com *Natrum muriaticum* 30 CH. Os resultados obtidos mostraram que o aumento

da salinidade no solo causa inibição no desenvolvimento da população de feijão (P2) e que a utilização da solução de *Natrum muriaticum* promoveu o aumento significativo do crescimento vegetal, sobretudo com relação à potência 6 CH (P3), que causou significante aumento na TCR da população de feijão, evidenciando a eficiência deste método em vegetais.

Palavras chave: *Natrum mutiaticum, Phaseolus vulgaris*, Taxa de Crescimento Relativo, alta diluição.

Influencia de Natrum mur 6cH y 30 cH en la cultura estandarizada de Phaseolus vulgaris L.: un estudio piloto

RESUMEN

El uso de soluciones dinamizadas es amplio y se extiende a todos los seres vivos, incluidas las plantas, actuando de manera efectiva en el metabolismo primario y secundario. Este estudio piloto tiene por objetivo verificar la acción de Natrum mur 6 cH y 30 cH comparándolos con la acción de NaCl al 5,0% cuando se administra por separado en las poblaciones de Phaseolus vulgaris (poroto). La evaluación se realizó mediante la determinación de la tasa de crecimiento relativa de las poblaciones de poroto tratados durante seis semanas y divididos en 4 grupos (con 5 recipientes cada una): P1, el grupo control, fue tratado únicamente con una solución de alcohol 30%, P2, tratados con una solución acuosa de NaCl 5,0%, P3, tratados con Natrum mur 6 cH, y P4, tratado con Natrum mur 30 cH. Los resultados mostraron que el aumento de la salinidad en el suelo pueden inhibir el desarrollo de la población de porotos (P2), y que el uso de la solución de Natrum mur promovió un aumento significativo en el crecimiento de las plantas, particularmente en relación a la potencia 6 cH (P3), lo que provocó un aumento significativo en TCR de la población de porotos, que muestran la eficacia de este método en las plantas.

Palabras clave: Natrun muriaticum, Phaseolus vulgaris, tasa de crecimiento relativo, alta dilución.

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