High dilution preparations for organic production system of broccoli

Preparados en altas diluciones en el manejo de brócoli bajo sistema de producción orgánica

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ABSTRACT

The aim of this study was to evaluate the influence of high dilution preparations on seedlings growth and production of broccoli under organic system. Three experiments were conducted in a greenhouse with completely randomized design. Two others experiments were conducted under field conditions, and the experimental design was randomized in blocks. Both designs were done with four replications and nine treatments. The treatments consisted of high dilution preparations of *Arnica montana*, *Silicea terra*, *Carbo vegetabilis* and *Sulphur* at 6CH or 30CH, water was used as control. In greenhouse, plant height, root length and stem diameter of seedlings were evaluated, along with the fresh and dry mass of shoot and root. In field, fresh and dry mass of inflorescences, plant height and stem diameter were evaluated. *Silicea terra* at 30CH increased the stem diameter, root length and dry mass of shoot and root in the broccoli seedlings. *Sulphur* at 6CH increased the fresh and dry mass of broccoli inflorescences. *Silicea terra* at 6CH, *Carbo vegetabilis,* and *Sulphur* at 30CH increased the plant height in field experiments. This suggests that high dilution preparations can be utilized to increase the biomass production and others desirable characteristics of broccoli crop under organic system.

Key words: *Brassica oleraceae* var. *italica*, agroecology, agrohomeopathy, seedling production, sustainable agriculture.

RESUMEN

El objetivo de este estudio fue evaluar la influencia de preparados en altas diluciones en el crecimiento de plántulas y producción de brócoli en sistema orgánico. Fueron realizados tres experimentos en diseño completamente al azar (invernadero) y dos experimentos en diseño de bloques completos al azar (campo), todos con cuatro repeticiones y nueve tratamientos. Los tratamientos fueron preparados de *Arnica montana*, *Silicea terra*, *Carbo vegetabilis* y *Sulphur* en 6CH o 30CH, como testigo fue utilizado agua. En invernadero fueron evaluadas: altura de planta, longitud de raíz, diámetro de tallo, masa fresca y seca de la parte aérea y de la raíz. En campo fueron evaluadas: masa fresca y seca de inflorescencias, altura de plantas y diámetro de tallo. *Silicea terra* 30CH aumentó en las plántulas el diámetro de tallo, longitud de raíz, masa seca de la parte aérea y de la raíz. *Sulphur* 6CH incrementó masa fresca y seca de las inflorescencias. *Silicea terra* 6CH, *Carbo vegetabilis* 30CH y *Sulphur* 30CH aumentaron la altura de las plantas en experimentos de campo. Esto sugiere que preparados en altas diluciones pueden ser utilizados para aumentar la producción de biomasa y otras características deseables en el cultivo orgánico del brócoli.

Palabras claves: *Brassica oleraceae* var. *italica*, agroecología, agrohomeopatía, producción de plántulas, agricultura sustentable.

Introduction

Vegetables of *Brassica* are consumed fresh (*in natura*) or mildly cooked, and are important components of diets around the world (Baenas *et al*., 2012). The broccoli (*Brassica oleraceae* L. var. *italica*) also stands out in special meal compositions due to its high nutritional value, being rich in calcium, folic acid, selenium, potassium, vitamins A and C (Jahangir *et al*., 2009). anticarcinogenic properties, bowel cleansing by the presence of glucosinolates and photochemical products that offer an extra protection against heart disease (Rosa and Rodrigues, 2001; Keck and Finley, 2004; Baenas *et al*., 2012). The conventional production of broccoli based in agrochemical use contrasts with their nutraceutic properties, given the residues of pesticides and the high content of nitrates/nitrites not metabolized in the plant tissue. Furthermore, production systems with low inputs provide greater food security for rural families and are socially and environmentally more sustainable (Altieri, 2002).

Food production systems on ecological bases are a trend that is consolidated worldwide. There is a growing initiative on the part of farmers, farmers associations and researchers to improve agriculture toward a non-contamination of sustainable systems (Warner, 2006). Innovative technologies

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for low cost and less environmental impact will be required to grant efficiency to healthy food systems. In this sense, the sanitary management of vegetable crops considerate technologies of low environmental impact and free of toxic waste, such as the homeopathic high dilutions (Betti *et al*., 2009; Boff, 2013). Several authors have documented the efficacy of high dilutions homeopathic preparations to manage vegetable crops. Bonato and Silva (2003) identified that *Sulphur* at 5CH and 12CH dynamizations increased the fresh and dry mass of shoot and root in plants of radish (*Raphanus sativus* L.). Rossi *et al*. (2006) obtained increases in plant height and root length of lettuce seedlings with application of *Carbo vegetabilis* at 6CH, 100CH and 200CH. Grisa *et al*. (2007) found an increase of shoot dry mass in lettuce treated with *Arnica montana* at 6CH and 12CH dynamizations. The aim of this study was to evaluate the influence of high dilution preparations on the quality of broccoli seedlings and the impact of its production under organic field systems.

Materials and methods

Localization of experiments

Experiments were carried out at the Experimental Station of Lages/Agricultural Research and Rural Extension Company of Santa Catarina State - EPAGRI (Area 1), and in a farm located in the Pedras Brancas Community (Area 2), Lages (27°48' S and 50°19' W; 931 m a.s.l.), Santa Catarina State, Brazil, during the crop cycles of 2011 and 2012. The predominant climate in the region consists of an average temperature of 22°C in the warmest month, and in winter from 6 to 8°C. The total annual rainfall varies from 1,360 to 1,600 mm and the soils are classified as "cambissolo humico alico" (Embrapa, 2013).

High dilution preparations

High dilution preparations were obtained from the Laboratory of Homeopathy and Plant Health, Lages Experimental Station of EPAGRI, following the methodology described in the Brazilian Homeopathic Pharmacopoeia (2011). High dilutions of *Arnica montana*, *Carbo vegetabilis*, *Silicea terra,* and *Sulphur* were made from a matrix of 5CH dynamization, using a part of the matrix in 99 parts of 5% alcohol and followed by 100 times succussion with the help of mechanical arm (Autic®, Denise Model 10-50), to achieve a 6CH dynamization; and so forward until reaching the desired 30CH dynamization.

Seedling production in greenhouse experiments

Three experiments were conducted in a greenhouse using a completely randomized design with four replicates from 30/09/2011 to 01/10/2012. The treatments were *Arnica montana*, *Silicea terra*, *Carbo vegetabilis,* and *Sulphur* at 6CH and 30CH, and water as the control plot. The experimental plot consisted of 20 plants in the middle of 64 seedlings in expanded polystyrene trays with capacity of 128 cells. Piracicaba precocious hybrid of broccoli was used for all experiments. Organic substrate was composed of cattle manure (composted for six months), soil, vermiculite, and rock phosphate, at volume rate of 3:1:1:0.2, respectively. The density of substrate material was 0.7 kg L^{-1} of cattle manure; 1.0 of soil; 0.08 of vermiculite and 2.8 of rock phosphate. The materials were mixed and dried. The substrate was then transferred into tray cells. Two seeds per cell were placed at 0.5 cm depth. Thinning was done a week after germination, leaving one plant per cell. The irrigation of the seedlings was done daily by micro sprinklers during three minutes. Whenever necessary the irrigation was repeated. The spray treatments were applied every four days on the seedlings until transplantation, with total of seven applications. Trays were isolated from each other at the time of spraying. The applications were performed using manual sprayer Vonder® with capacity for 1.5 L. Each homeopathic preparation was used at a rate of 10 mL L^{-1} of water and homogenized with ten shakes. Double-blind technique was used for conducting experiments, where neither the assessor nor the applicator knew the nature of each treatment. Homeopathic preparations only revealed after the statistical analysis.

The evaluations were performed after 70% of seedlings reach the phenological stage V_0, characterized by seedlings with three to four leaves and 10 to 12 cm height (Jaramillo and Diaz, 2006). Then the seedlings were harvested and the plant height (a), determined by the distance between the stem base to the apex of the newest leaf using a standard ruler; the root length (b) was measured with a standard ruler, and the stem diameter (c) was measured with a digital caliper. The fresh and dry mass of shoot and root were evaluated with the assistance of a digital scale. For the evaluation of dry mass of shoot and root, the fractions were wrapped separately in Kraft paper bags, and taken for drying in an oven with forced air at 65°C, until the weight was stabilized.

Broccoli production under field conditions

Two experiments were conducted in the field from 13/12/2011 to 17/04/2012 at the Experimental Station of EPAGRI (Area 1) and from 14/05/2012 to 17/09/2012 at a family farm (Area 2). Both experiments were located in Lages (27°48' S and 50°19' W; 931 m a.s.l.), Santa Catarina State, Brazil (Tab. 1). Piracicaba hybrid of broccoli was the variety used for all experiments.

TABLE 1. Chemical composition of soil for the experimental areas in Lages-SC, Brazil.

Experimental area	Clay ($m\ v^{-1}$)	pH-water (1:1)	O.M. ($m\ v^{-1}$)	Ca ($cmolc\ L^{-1}$)	Mg ($cmolc\ L^{-1}$)	Al ($cmolc\ L^{-1}$)	P ($mg\ L^{-1}$)	K ($mg\ L^{-1}$)
Area 1	38	5.3	3.0	5.9	2.0	0.3	18.5	213
Area 2	32	5.5	3.7	6.6	2.5	0.0	32.4	171

The seedling production was done in expanded polystyrene trays with 128 cell capacity. The trays were washed with water and disinfected with sodium hypochlorite at 2.5% of active chlorine, in the proportion of 5 mL L^{-1} of water. The substrate was composed of cattle manure, soil, vermiculite and phosphate, at rate of 3:1:1:0.2 in volume. One year before transplanting, soil tillage was performed with plowing and disking, followed by fertilization with green manure (oats + vetch). The fertilization was performed with 2.5 L m^{-2} of compost [cattle manure: C (%) = 48; N (% dry matter) = 2.3; P_2O_5 (kg m^{-2}) = 4.1; K_2O (kg m^{-2}) = 3.8; Ca (kg m^{-2}) = 3.0], and phosphate in the proportion of 0.15 kg m^{-2}. Transplantation was done when seedlings reached the phenological stage V_0 (Jaramillo and Diaz, 2006), where seedlings had three to four leaves and a height of 10 to 12 cm.

Treatments were of high dilution preparations of *Arnica montana*, *Silicea terra*, *Carbo vegetabilis,* and *Sulphur* at 6CH and 30CH. Pure water was used as control. The experimental design was organized in completely randomized blocks with four replicates. Each plot consisted of 10 plants transplanted in double line.

The irrigation was conducted according to the requirements of the broccoli crop and weeding was done by manual methods. One seedling was planted per hole with the spacing of 0.4 m between plants and 0.6 m between rows, resulting in a density of 41.500 plants per hectare.

The application of spray treatments was applied using backpack sprayer Guarany® with capacity for 5 L. The frequency of applications was every fifteen days, starting seven days after the transplant until harvest (for a total of four applications). Each homeopathic preparation was dosed in a proportion of 10 mL per liter of water, followed by ten shakes. Double-blind technique was used for spray the treatments, where neither the appraiser nor the applicator knew the nature of each treatment, until after the statistical analysis was done.

At harvest the evaluated variables were: (a) plant height, determined with a measuring tape, from ground level up to the apical part and (b) stem diameter, measured at one cm from the ground with the assistance of digital caliper. External leaves of broccoli plants were removed and the fresh mass of the inflorescences was later determined with a digital scale, measured in grams. The dry mass of the inflorescences was obtained by packaging in Kraft paper bags and placing in a drying oven with forced air at a temperature of 65°C, until the weight stabilized.

Statistical analysis

Data were statistically analyzed with R version 3.1.2 (R Core Team, 2014), using analysis of variance (ANOVA). Means significantly were compared by Tukey test ($P \leq 0.05$).

Results and discussion

Effect of high dilution preparations on seedling production

High dilution preparations of *Silicea terra* at 30CH and *Arnica montana* at 6CH increase of dry mass of shoot in the broccoli seedlings, when compared to the water control in at least one experiment (Tab. 2). Bonfim *et al.* (2008) also using of *Arnica montana* at 3CH, 6CH and 12CH found an increase of dry mass in rosemary (*Rosmarinus officinalis* L.) and royal sage shoots (*Lippia alba* (Mill.) N.E. Br.). Also Grisa *et al.* (2007) found an increase of dry mass in lettuce (*Lactuca sativa* L.) shoots with *Arnica montana* dynamizations at 6CH and 12CH. Further, Almeida (2002) observed an increase of 40% of fresh mass of basil inflorescences (*Ocimum basilicum* L.) when exposed to *Silicea terra* at 30CH.

Dry mass of root in broccoli seedlings treated with *Silicea terra* at dynamization of 30CH was higher than the water control plots. The preparations of *Sulphur* 6CH, *Arnica montana* 6CH and *Carbo vegetabilis* 30CH increased the dry mass of root in broccoli, in at least one of the three experiments (Tab. 2).

Root length of broccoli seedlings was affected by the use of high dilution preparations, being that *Sulphur* 6CH and *Silicea terra* 30CH promoted greater root length compared to the water control in at least two of the three experiments (Tab. 3). The root system is a determining factor in the quality of the seedlings, and seedlings with poor root systems have growth difficulty in field conditions (Krasowski and Owens, 2000; Davis and Jacobs, 2005).

TABLE 2. Dry mass of shoot and root in seedlings of Piracicaba precoce hybrid of broccoli treated with high dilution preparations.

Preparations	Shoot dry mass (g plant^{-1})			Root dry mass (g plant^{-1})		
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
Arnica montana 6CH	0.108±0.007 ab	0.119±0.006 a	0.122±0.009 a	0.034±0.002 abc	0.043±0.003 ab	0.060±0.006 a
Arnica montana 30CH	0.105±0.009 ab	0.114±0.001 a	0.106±0.005 ab	0.028±0.001 c	0.044±0.004 ab	0.033±0.001 b
Carbo vegetabilis 6CH	0.082±0.009 b	0.106±0.006 a	0.097±0.002 ab	0.030±0.002 bc	0.034±0.001 b	0.032±0.001 b
Carbo vegetabilis 30CH	0.100±0.008 ab	0.118±0.009 a	0.118±0.007 ab	0.046±0.007 ab	0.035±0.001 ab	0.053±0.004 a
Silicea terra 6CH	0.105±0.006 ab	0.111±0.002 a	0.109±0.005 ab	0.030±0.003 bc	0.036±0.002 ab	0.033±0.002 ab
Silicea terra 30CH	0.137±0.006 a	0.117±0.004 a	0.120±0.006 ab	0.049±0.003 a	0.046±0.001 a	0.055±0.008 a
Sulphur 6CH	0.111±0.004 ab	0.105±0.007 a	0.113±0.006 ab	0.048±0.003 a	0.044±0.003 ab	0.047±0.001 ab
Sulphur 30CH	0.110±0.003 ab	0.116±0.003 a	0.111±0.006 ab	0.040±0.004 abc	0.046±0.003 ab	0.048±0.002 ab
Water	0.111±0.009 ab	0.109±0.006 a	0.094±0.004 b	0.028±0.004 c	0.036±0.001 ab	0.033±0.003 ab
CV (%)	14.8	10.0	10.7	19.8	12.4	17.7

Means ± S.E. followed by the same letter in the column did not differ significantly from each other by Tukey test ($P \leq 0.05$). Exp1 = Sep-Oct (2011); Exp2 = Feb-Mar (2012); Exp3 = Aug-Oct (2012).

Preparations of *Silicea terra* at a 30CH dynamization and *Arnica montana* at 6CH increased the stem diameter of broccoli seedlings when compared to the water control in two of three experiments. *Sulphur* at 6CH also increased this characteristic in one of three experiments (Tab. 3).

There were no significant differences between the treatments in the three experiments concerning the height of broccoli seedlings (Tab. 3). These results suggest that the 6CH and 30CH dynamics are more appropriate for promoting the root strengthening, but not manifested in the height of the seedling. If the root system is strong, it is possible that this characteristic is observed in the post-transplant, as it can be measured in field experiments. According to Bonato and Silva (2003), who observed different effects of high dilutions in different organs of plants. The same authors suggest that the action of high dilutions in plants should always be considered in its full cycle.

Effect of high dilution preparations on the production of broccoli in field

Piracicaba hybrid of broccoli plants conducted at area 2, when treated with *Sulphur* 6CH increased the fresh and dry mass of the inflorescences, compared to water control. Though this result was not confirmed in the conditions of area 1 (Tab. 4). Preparations of *Sulphur* at 6CH increased the production and the dry mass of cabbage (Pulido *et al.*, 2014), and the fresh and dry mass of mint plants (Bonato *et al.*, 2009). Toledo *et al.* (2015) also observed that *Sulphur* (6CH, 12CH, 30CH, and 60CH) increased the fresh mass of shoot and (30CH) root of tomato plants.

Preparations of *Silicea terra* at 6CH, *Carbo vegetabilis,* and *Sulphur* at 30CH influenced the increasing of plant height when compared to water control, in area 2. However, no significant differences were observed for stem diameter

TABLE 3. Plant height, root length and stem diameter in seedlings of Piracicaba precoce hybrid of broccoli treated with high dilution preparations.

Preparations	Plant height (cm)			Root length (cm)			Stem diameter (cm)		
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
Arnica montana 6CH	12.25±0.44 a	12.90±0.28 a	12.98±0.24 a	9.05±0.39 ab	8.01±0.34 abc	9.36±0.36 a	1.18±0.09 a	1.25±0.08 a	1.18±0.02 ab
Arnica montana 30CH	11.75±0.47 a	12.78±0.21 a	11.85±0.31 a	9.59±0.48 ab	6.19±0.42 bc	8.61±0.46 a	0.92±0.05 bc	1.21±0.04 a	1.11±0.06 abc
Carbo vegetabilis 6CH	10.59±0.24 a	11.22±0.24 a	11.10±0.33 a	7.10±0.31 b	5.76±0.31 c	8.63±0.47 a	1.02±0.06 abc	1.15±0.09 a	0.91±0.04 c
Carbo vegetabilis 30CH	11.48±0.20 a	11.08±0.37 a	10.89±0.30 a	9.28±0.43 ab	7.29±0.40 abc	8.84±0.31 a	1.00±0.04 abc	1.21±0.07 a	1.04±0.04 bc
Silicea terra 6CH	11.82±0.28 a	11.77±0.48 a	11.43±0.31 a	9.16±0.49 ab	7.78±0.49 abc	8.97±0.46 a	1.00±0.03 abc	1.16±0.05 a	1.01±0.03 bc
Silicea terra 30CH	12.34±0.37 a	12.61±0.35 a	11.72±0.30 a	10.22±0.44 a	8.57±0.49 ab	9.63±0.47 a	1.04±0.08 ab	1.35±0.09 a	1.28±0.05 a
Sulphur 6CH	11.41±0.24 a	11.47±0.31 a	11.22±0.21 a	9.20±0.49 ab	9.85±0.37 a	9.06±0.37 a	0.86±0.03 bc	1.14±0.06 a	1.27±0.07 a
Sulphur 30CH	11.71±0.46 a	11.48±0.42 a	11.73±0.33 a	9.77±0.42 ab	8.56±0.42 ab	9.22±0.47 a	0.79±0.05 bc	1.10±0.08 a	1.02±0.03 bc
Water	10.63±0.33 a	11.10±0.26 a	11.12±0.31 a	8.88±0.47 ab	6.95±0.40 bc	7.57±0.49 a	0.76±0.04 c	1.08±0.04 a	0.92±0.03 c
CV (%)	12.4	12.1	12.8	13.3	15.3	14.4	11.9	12.4	8.1

Means ± S.E. followed by the same letter in the column did not differ significantly from each other by Tukey test ($P \leq 0.05$). Exp1 = Sep-Oct (2011); Exp2 = Feb- Mar (2012); Exp3 = Aug-Oct (2012).

TABLE 4. Fresh and dry mass of inflorescences, plant height and stem diameter of Piracicaba precoce hybrid of broccoli treated with high dilution preparations.

Preparations	Fresh mass inflorescences (g)		Dry mass inflorescences (g)		Plant height (cm)		Stem diameter (mm)	
	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2
Arnica montana 6CH	178.32±1.68 a	207.84±0.93 ab	55.09±1.55 a	64.62±1.35 ab	46.13±0.74 a	41.92±0.91 ab	16.94±0.49 a	16.33±0.28 a
Arnica montana 30CH	151.56±1.01 a	205.20±1.00 bc	47.50±1.20 a	64.07±1.20 ab	42.31±0.91 a	44.22±0.65 ab	16.09±0.19 a	15.63±0.50 a
Carbo vegetabilis 6CH	136.80±1.43 a	154.20±1.57 c	42.13±0.99 a	47.29±1.11 c	42.65±0.66 a	39.77±0.82 ab	15.16±0.45 a	15.99±0.41 a
Carbo vegetabilis 30CH	178.92±1.13 a	195.36±1.32 bc	55.49±1.05 a	60.50±1.45 bc	49.86±0.86 a	44.54±0.90 a	17.38±0.34 a	16.41±0.37 a
Silicea terra 6CH	164.28±1.43 a	208.68±1.68 ab	50.68±1.82 a	64.08±1.63 ab	44.28±0.86 a	45.45±0.84 a	14.55±0.34 a	17.29±0.11 a
Silicea terra 30CH	158.69±0.77 a	157.44±1.31 bc	49.28±1.51 a	48.87±1.72 bc	41.08±0.78 a	42.48±0.73 ab	14.92±0.36 a	15.88±0.18 a
Sulphur 6CH	177.24±1.49 a	253.44±1.00 a	54.59±1.60 a	78.01±0.89 a	38.07±0.99 a	43.93±0.86 ab	15.45±0.42 a	18.13±0.40 a
Sulphur 30CH	155.88±1.34 a	168.78±1.44 bc	48.62±1.64 a	52.64±0.73 bc	46.66±0.85 a	44.61±0.65 a	16.21±0.39 a	15.59±0.34 a
Water	171.12±1.37 a	174.54±1.44 bc	52.63±1.37 a	53.82±1.43 bc	45.50±0.66 a	37.53±0.74 b	15.73±0.27 a	15.11±0.39 a
CV (%)	12.6	11.2	14.2	11.5	12.4	6.5	13.8	7.4

Means ± S.E. followed by the same letter in the column did not differ significantly from each other by Tukey's test ($P \leq 0.05$). Area 1 = Experimental Station of Lages - Epagri, Dec-Apr (2011/2012); Area 2 = Farm in Pedras Brancas, Lages, May-Sep (2012).

in broccoli among treatments in both experimental areas (Tab. 4).

Fresh and dry mass of the inflorescences, plant height and stem diameter in broccoli did not differ among treatments applied in area 1 (Tab. 4). Andrade *et al.* (2012) also found no significant differences between the treatments in high dilutions in the characteristics of fresh and dry mass in plants of *Justicia pectoralis.* However, preparation of *Arnica montana* at 3CH, 30CH, 60CH, 100CH and 200CH dynamizations directly influenced the secondary metabolism of plants, causing changes in the coumarin content.

Conclusions

High dilution preparations influence the growth of broccoli plants under organic system, since first phenological stages V_0 (seedlings stage) until R_2 (phase of flower formation). Preparation of *Silicea terra* at 30CH dynamization stands out by promoting the increase in the evaluated features: stem diameter, root length, and dry mass of shoot and root in broccoli seedlings of Piracicaba precoce hybrid. Broccoli plants treated with the homeopathic preparation of *Sulphur* 6CH showed increases of fresh and dry mass of the inflorescences, thus the preparations of *Silicea terra* 6CH, *Carbo vegetabilis,* and *Sulphur* at 30CH increment the height of broccoli plants.

Finally, high dilution preparations can influence the seedling growth and production of broccoli crops under an organic system.

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