Short note [Nota corta]

USE OF ALTERNATIVE SUBSTRATES FOR BROCCOLI SEEDLING PRODUCTION UNDER GREENHOUSE CONDITIONS[1]

[USO DE SUSTRATOS ALTERNATIVOS PARA LA PRODUCCIÓN DE PLÁNTULAS DE BRÓCOLI EN CONDICIONES DE INVERNADERO]

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SUMMARY

In this study, the effect of two alternative growing substrates (corncob and *Azolla anabaena*) on some vegetative parameters (days to emergence, stem diameter, plant height and root volume) in seedlings of broccoli hybrid Coronado were evaluated. Both materials were ground and used according to the following treatments: 100% corncob (T1), 100% *Azolla* (T2), 50% corncob + *Azolla* 50% (T3), corncob 75% + *Azolla* 25% (T4), 25% corncob + *Azolla* 75% (T5) and then compared to a commercial substrate (BM2) (T6). Substrates were uniformly mixed, deposited in germination trays and watered at field capacity. Additionally, physic-chemical characteristics were determined in the different substrates used for broccoli seedling production. Stem diameter, height plant and root volume showed to be statistically higher in seedlings grown in commercial substrate (0.18 cm, 5.27 cm and 0.61 cm^3, respectively) followed by those seedlings grown in 100% *Azolla* (0.17 cm, 4.92 cm and 0.49 cm^3, respectively) ($p< 0.001$). Seedlings growing in substrates with higher corncob proportion showed lower values in these vegetative parameters. Based on our results, *Azolla* showed potential to be used as a seed substrate for production of broccoli seedlings, thus decreasing the use of peat and consequently the production costs in nurseries.

Keywords: *Brassica*, substrates, *Azolla*, maize residues, organic agriculture.

RESUMEN

En este estudio, se evaluó el efecto de dos sustratos alternativos de siembra (tusa de maíz y *Azolla anabaena*) sobre los parámetros vegetativos (días a la emergencia, diámetro del tallo, altura de planta y volumen de raíces) en plántulas de brócoli híbrido Coronado. Ambos materiales fueron pulverizados y usados de acuerdo a los siguientes tratamientos: tusa de maíz al 100% (T1), *Azolla* al 100% (T2), tusa de maíz 50% + *Azolla* 50% (T3), tusa de maíz 75% + *Azolla* 25% (T4), tusa de maíz 25% + *Azolla* 75% (T5), los cuales fueron comparados con un sustrato comercial (BM2) (T6). Los sustratos fueron mezclados uniformemente, colocados en bandejas de germinación y humedecidos a capacidad de campo. Adicionalmente, fueron determinadas las características físico-químicas en los diferentes sustratos usados en la producción de plántulas de brócoli. Los valores de grosor de tallo, altura de planta y volumen de raíz fueron estadísticamente superiores en las plántulas sembradas en el sustrato comercial (0,18 cm; 5,27 cm y 0,61 cm^3, respectivamente), seguidos de aquellas plántulas sembradas en el sustrato con 100% de *Azolla* (0,17 cm; 4,92 cm y 0,49 cm^3, respectivamente) ($p< 0,001$). Las plántulas que crecieron en aquellos sustratos con mayor proporción de tusa mostraron menores valores en estos parámetros vegetativos. Basados en los resultados, *Azolla* mostró potencialidad para ser usado como sustrato de siembra en la producción de plántulas de brócoli, lo cual podría disminuir el uso de la turba y consecuentemente los costos de producción durante la fase de vivero.

Palabras clave: *Brassica*, sustratos, *Azolla*, residuos de maíz, agricultura orgánica

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INTRODUCTION

Broccoli (*Brassica oleracea* var. *italica*) is an annual vegetable of importance for human nutrition and vegetable oil production (Lopes *et al.*, 2012), however, its yield and quality can be affected by several factors including fertilization regime (Feller and Fink, 2005), as well as, for the date and method of sowing (transplanting or direct sowing), among others (Reta *et al.*, 2004). In relation to the sowing method, transplant is preferred because it ensures to obtain more vigorous seedling from a few number of seeds. In addition, it allows to get an early harvest (Laviola *et al.*, 2006) and to increase productivity (Andreoli *et al.*, 2002).

Additionally, quality of seedling depends on the quality of seeds and the type of substrate (Lopes *et al.*, 2012). Substrates must show good water retention capacity, porosity to promote oxygen diffusion, to be free of pathogens and provide nutrients for the plant (Silva *et al.*, 2001, Smiderle and Minami, 2001). The overexploitation of some types of substrates to obtain commercial seedlings can cause an ecological impact in places where they are extracted from, for example: in peat exploitation sites in northern Europe (Maroto, 2000). Therefore, there is a growing concern to use alternative substrates, which include by-products from either wood industry (pine bark compost) or agricultural production (coconut fiber, some plant straw fibers) (Maroto, 2000).

In recent years, the use of *Azolla* has gained popularity as a sowing substrate because it has shown to confer positive effects on agriculture productivity since its symbiosis with a nitrogen fixer seaweed, *Anabaena azollae* (Petruccelli *et al.*, 2015). Although the *Azolla-Anabaena* complex has been mainly used as fertilizer in rice plantings, it has a wide potential to be used as bio fertilizer in other crops (Pabby *et al.*, 2003). Previous works that included the use of *Azolla* mixed with other types of substrates have proved to be easy handling and to have an acceptable water content to be used either as fertilizer or as a substrate in nurseries in different crops (Ríos, 2014). Thus, the use of *Azolla* in combination to kekilla allowed to obtain good quality broccoli plants (Gavilanez, 2015).

On the other hand, corncob is also a viable alternative for use as sowing substrate since of the total hemicellulose (34%), approximately 94% corresponds to xylan, making it attractive for the development of nitrogen fertilizers with prolonged or slow action (Córdoba *et al.*, 2013). In consideration of the above, in this study the effect of corncob and *Azolla anabaena* as substrate for sowing on the vegetative parameters of the *B. oleracea* seedlings were evaluated

MATERIALS AND METHODS

Location and substrate collection

Study was conducted in Parroquia Montalvo, Canton Ambato, Province of Tungurahua (01º24'00 "S, 78º23'00" W, 2600 masl). Corncob was obtained from the waste of the grain harvest while *A. anabaena* was collected from the water reservoir of the Experimental Farm at the Faculty of Agricultural Sciences (FCAGP), Technical University of Ambato (UTA) in Querochaca, Province of Tungurahua. Previuos to be used, *Azolla* was shade dried at room temperature for five days, as described by Petruccelli *et al.* (2015).

Preparation of substrates and treatments

Each substrate was ground in a one-HP electric mill (Fritsch™; model Pulverisette 15; Germany) to obtain 1 mm particles. Firstly, all substrates were disinfected using Vitavax Flow™ (2 mL/20 L of water) and uniformly mixed, according to the following treatments: T1; corncob 100%, T2; *Azolla anabaena* 100%, T3; corncob 50% + *Azolla anabaena* 50%, T4; corncob 75% + *Azolla anabaena* 25%, T5; corncob 25% + *Azolla anabaena* 75%, T6; BM2 commercial substrate. After that, substrates were placed on germination trays and watered to field capacity. Previously, a chemical analysis of each substrate was made in order to determine the nutrient supply in each case (Table 1). This research was conducted in a plastic-covered greenhouse providing 35% shade.

Variable considered

The number of days to emergence was determined taking into account the days from sowing until 50% of the seedlings had emerged in each treatment. On the other hand, stem diameter, plant height and root volume were measured 28 days after sowing (before being transplanted).

Physical and chemical analysis

Electrical conductivity (EC) and pH were determined according to Violante and Adamo (2000). Total nitrogen and carbon content were determined by the Dumas method, using the CHN LECO 628 elemental analyzer (LECO Corporation). The total phosphorus content was determined by colorimetric method of the vanado-molybdate complex (Genesys 20 Spectrophotometer) (Anderson and Ingram, 1993). Potassium, calcium, magnesium, cooper, manganese and zinc contents were determined by wet digestion (AA Perkin Elmer 100 spectrophotometer) (Shirin et al., 2008), while K2O, CaO and MgO were calculated by transformation from pure elements to their respective oxide forms. All analyzes were replicated three times.

Experimental design and statistical analysis: The study was carried out in a completely randomized design with six treatments and six repetitions. All the variables were subjected to analysis of variance and mean comparison was made by using a Tukey test (p <0.05) (INFOSTAT version 2016).

Table 1. Chemical analysis to the substrates used for broccolis seedling production

Sustrate	pH	CE µS\cm	N	P_2O_5	K_2O	CaO (%)	MgO	C	C:N	Cu	Mn ppm	Zn
T1	6,05	1275	1,14	0,87	0,34	2,96	0,67	45,58	39,38	19	38	58
T2	6,35	1948	1,51	1,38	0,16	2,86	0,54	35,18	23,30	19	353	37
T3	6,33	1888	1,39	0,90	0,10	2,50	1,28	42,98	30,92	20	159	20
T4	6,33	1493	1,25	0,76	0,10	1,37	0,28	43,65	34,92	39	96	39
T5	6,29	2004	1,50	0,85	0,26	2,70	0,47	39,12	26,08	20	223	20
T6	4,96	720	0,71	1,35	0,09	6,12	3,28	35,24	49,63	20	79	40

T1: corncob 100%; T2: *Azolla* 100%; T3: corncob 50% + *Azolla* 50%; T4: corncob 75% + *Azolla* 25%; T5: corncob 25% + *Azolla* 75%; T6: BM2 (commercial)

RESULTS AND DISCUSSION

Agronomic parameters

Days to emergence: the type of substrate used affected the number of days to the emergence of broccoli seedlings (p <0.001) (Table 2). Lower number of days (from sowing up to 50% of the seedlings emerged) was observed in the T6 treatment (commercial substrate) with an average of 5.0 days, while the longest time was observed in T3, in the which seeds were delayed up to 5.5 days as compared to T6. The rest of the treatments showed intermediate values. In general, broccoli seeds have shown to require between 8 and 15 days for total emergence, depending on the depth of sowing and covering of seed (Corpocauca, 2007), soil aeration conditions and the speed of plant growth (Quesada and Méndez, 2005). Additionally, seed germination also depends on the substrate used (Oliveira *et al*., 2015). Thus, higher germination rate in *Tabebuia heptaphylla* and *Terminalia argentea* was observed in substrates with predominance of clay particles compared to sandy soils or vermiculite (Bocchese *et al*., 2008, Oliveira and Farias, 2009)

Stem diameter: significant differences by effect of the substrate type were detected in stem diameter of broccoli seedlings (p <0.001). Maximum values were observed in seedlings grown on substrate containing 100% *Azolla* (T2) and on the commercial substrate (T6) (Table 2). Contrarily, lower values were shown in seedlings planted in substrates with higher content of ground corncob (T1, T3, T4 and T5). Petruccelli *et al*. (2015) found that olive plants caused showed better growth and accumulation of biomass when grown in substrates with 50% *Azolla*. These authors suggested that *Azolla* could be an excellent component of the substrates used in nursery conditions for plants production.

Plant height: the type of substrate used also caused significant differences in plant height (p <0.001) (Table 2). Higher height was observed in plants growing or in the substrate containing 100% *Azolla* (T2) or on the commercial substrate (T6). Similar to that observed with stem diameter, plants that grew on those substrates containing higher proportion of corncob showed lower height, being 2.38 to 2.55 times smaller when 100% was used of corncob (T1) in comparison to T2 and T6, respectively, whereas when the proportion of corncob used in the substrate decreased to 25% (T5) this difference was smaller, being 1.32 and 1.41 times lower in comparison with the same treatments (T2 and T6, respectively).

Probably higher values obtained in plants grown on the substrate with 100% *Azolla* (T2) could be because nitrogen, phosphorus and potassium contents in *Azolla* are similar to those in the commercial substrate BM2 (T6). On the other hand, lower values in plant height in T1 (100% corncob) could be explained by low phosphorus as well as high zinc content since phosphorous can cause general stunting (Bertsch, 2009) whereas zinc provokes competition for P_2O_5 and Mg absorption, which are important for stem growth (Table 1).

Root volume: In general, plants grown on the commercial substrate (T6) showed the maximum root volumes at 28 days after sowing, followed by those grown on 100% *Azolla* (T2) substrate in which root volume was only 20% lower (Table 2). Conversely, plants cultivated with substrates containing higher corncob percentage showed root volumes 68.9 and 57.4% lower when sown in 75% corncob + 25%

Azolla (T4) and 100% corncob (T1), respectively. Although corncob plays a role in the conservation of soil, water and soil carbon, it is still unknown what its contribution of nutrients (Wienhold *et al*., 2011), so that more detailed studies are still required to measure its impact on other crops. Similar to that observed by Petruccelli *et al*. (2015), both the pH and EC values tended to increase as *Azolla* content increased (Table 1), however, this does not seem to have negatively affected growth of broccoli plants since they show acceptable development in conditions close to neutrality and these particular salinity conditions (Sánchez-Monedero *et al*., 1997).

Table 2. Variation in the agronomic parameters (days to emergence, stem diameter, plant height and root volume) in Coronado hybrid broccoli seedlings grown on different substrates

Substrate	Days to emergence	Stem diameter	Plant height	Root volume
T1	7,17±0,983ab	0,12±0,028 c	2,06±0,396 c	0,26±0,057 c
T2	8,67±1,366ab	0,17±0,010 a	4,92±0,470 a	0,49±0,036 b
T3	9,50±2,074ab	0,12±0,011 c	2,66±0,349 c	0,26±0,048 c
T4	10,50±1,975b	0,12±0,008 c	2,53±0,249 c	0,19±0,0256 c
T5	9,33±2,251ab	0,14±0,013bc	3,73±0,294 b	0,40±0,034 b
T6	5,00±2,041a	0,18±0,014 a	5,27±0,488 a	0,61±0,070 a
Valor P	0,001	0,001	0,001	0,001

Values in a column followed by the same letter did not show significant differences according to Tukey's test at $p< 0,001$. T1: corncob 100%; T2: *Azolla* 100%; T3: corncob 50% + *Azolla* 50%; T4: corncob 75% + *Azolla* 25%; T5: corncob 25% + *Azolla* 75%; T6: BM2 (commercial)

On the other hand, those substrates containing *Azolla* 100 or 75% showed 2.12 and 2.11 times more N in relation to the commercial substrate, which could be explained by the high concentration of this element in its dry biomass (Bhuvaneshwari and Singh, 2015). Most of the studies have indicated that broccoli is highly demanding in nitrogen, therefore high N content in T2 could explain better plant growth; however, requirements may vary as cultivation conditions and cultivars (Rincón-Sánchez *et al*., 2001). Additionally, the presence of calcium in 100% *Azolla* (T2) could favor the activation of several plant enzyme systems, which stimulate root and leaf development (INPOFOS, 1997). Likewise, there is a positive effect between the amount of Mg that the crop absorbs and the root and aerial growth (Cakmak and Yazici, 2010), so that together with Ca and N, they could have influenced positively the growth of the seedlings.

Additionally, C/N ratio could also have influenced the best development of broccoli seedlings. On the one hand, C/N ratio influences the availability of mineral nitrogen absorbed by plants and, along with the soil humidity, temperature and aeration favors the microbial activity. According to previous studies, it is estimated that microorganisms generally use 30 parts of C for each part of N (Jhorar *et al*., 1991). Based on this, the observed C/N ratio in the substrates with Azolla could ensure a source of mineral nitrogen available for seedling growth.

According to Bilderback *et al*. (2005), substrates used for plant production in nurseries should show physical-chemical characteristics within acceptable ranges to ensure quality of seedlings. However, these values should not be generalized, but should be adapted to certain groups of plants with similar requirements (Petruccelli *et al*., 2015).

CONCLUSIONS

Azolla proved to be the best substrate since it allowed the seeds of broccoli to germinate in the shortest time, in addition to the seedlings showing better characteristics of stem diameter, plant height and root volume, comparable with the seedlings obtained with the commercial substrate. In this regard, the use of *Azolla* as substrate for broccoli seedlings production could be a sustainable alternative because it is a source of nutrients necessary for plant growth and development in nursery.

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