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ORIGINAL ARTICLE

Growing of Salt Tolerant Vegetables in an Experimental Compost in Greenhouse

José Venegas González, Martha Alicia Velázquez Machuca and José Luis Montañez Soto
Instituto Politécnico Nacional (CIIDIR MICHOACÁN). Justo Sierra 28, CP 59510, Col Centro, Jiquilpan, Michoacán, México. E-mail: jvenegasg@ipn.mx

ABSTRACT
The objective of this research was to know the salinity tolerant vegetables response to a compostusing the broccoli (Brassica oleracea) as a response crop. The compost was prepared using, sugar cane by-product,rice husk, cow manure, coconut fiber dust phosphate rock and yeast mixture. The compost (S_1) was mixed with other ingredients to generate 7 treatments.A greenhouse experiment was established under a completely randomized statistical design, broccoli Claudia variety was used as a compost quality indicator crop. The composthad high nutrients content, except Cu. pH and salt content were high and theP content was extremely high. The yield data was applied to analysis of variance and a $Tukey_{0.05}$ test. The yields were affected by treatments: T_6 (S_1 + chemical fertilizer) and T_2 (S_1 + fish meal) treatments had higher yields (52.8 and 48.4 g plant^{-1} respectively), while T_1 (S_1) and T_5 (S_1 + mycorrhiza+ Azospirillum sp.) treatments yielded 38.7 and 40.7 g plant^{-1} respectively. Plants with mycorrhiza absorbed the largest amounts of phosphorus (0.081%), indicating that somehow the mycorrhiza interacts with broccoli roots. The plants grown in T_5 (S_1 + mycorrhiza + Azospirillum sp.) treatment showed the highest foliar nitrogen contents (2.55%) but inhibited phosphorus and others nutrients absorption.
***Key words**: compost, mycorrhiza, Azospirillum, coconut fiber, broccoli crop, phosphorus uptake*

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INTRODUCTION

A substrate is a solid material which put it in a container provides a suitable rhizosphere for the plant roots anchorage and is a source of nutrients enough to meeting up the crop demands, in addition, it slows the control of the nutrients in the plant rhizosphere and water management [21]. From here the importance of its physical and chemical characterization,especially when it is a system component with gradual degradation and plant nutrients continua source [1].

The application of substrates based on organic solid wastes such as coconut fiber, animal wastes, rice husk or sugar cane rum as a soil amendment, improves the physical and chemical soil properties such as soil structure, organic matter content, cation exchange capacity; in addition it increase the crop yields and reduce the use of synthetic chemical fertilizers to crop production; it constitute an alternative strategy in the exploitation of agricultural production systems to mitigate the climatic change [12, 13].

Coconut fiber is the palm fruit fibrous skin, it is a by-product arising from processing of the palm fruit; it has physical properties suitable for preparing alternative substrates with high stability and great water holding capacity, low bulk density, high content of available water and nutrients for plant development [21] increases the ion transport mechanisms between the soil and the plant and therefore the cation exchange capacity and soil damping properties, promotes the nutrients conversion to available forms [3].

The coconut fiber dust has favorable physical properties for preparing substrates for growing different horticultural species, because it has high water holding capacity (50-81%), total porosity greater than 92%, low bulk density (0.08 to 0.12 gcm^{-3}) and low true density (1.48-1.49 gcm^{-3}) [21]. The substrates derived from this material have good conditions for growing of various horticultural species [15] in addition they can be used for the species propagation of both agricultural and medical interest [11].

The *Azotobacter, Azospirillum* and *Rhizobium* bacteria have had positive effects on the seed germination, plant development as well as the N and P assimilation on some crop tissues such as pepper (*Capsicum annum*) and corn (*Zea mays*). In addition, a bacteria strain *Azospirillum* increased the contents of N and P in corn tissues (17). The co-inoculation bacteria-mycorrhizal and fungi such as *Azospirillum brasilense - Glomus intraradix* has synergistic effects on fixation and uptake of N and P in plants [22,9,4].

The different Mycorrhizal strains (*Glomus intraradix*) have an influence on nutrimental absorption in different ways on plant organs under different salinity levels; it has been found that the K concentration in the carnation root was increased with a mycorrhiza strain compared to another one which increased the K concentration in flowers a low salinity level (1dS m^{-1}); with the first strain increased nitrates in leaves , while roots inoculated with the same strain the highest salinity level (6 dSm^{-1}) the highest P concentrations [14].
The aims of this study were: 1) To determine the substrate nutritional quality for the broccoli (*Brassica oleracea*) production in greenhouse and 2) To know the mycorrhizal fungi and *Azospirillum* bacteria effects on broccoli plants growth.

MATERIALS AND METHODS

Materials

Coconut fiber dust (obtained from Artifibras, Uruapan, Michoacán,México). Rice husk (obtained from Gabriel Zamora rice mill, México). Cattle manure was obtained from a cattle ranch on the outskirts of the Jiquilpan de Juarez city, México. Sugar cane by-product was obtained in a sugar cane mill (Tamazula, México). *Saccharomyces cereviciae* yeast was acquired in a bakery supply store (Jiquilpan, México). Whole tilapia fish dehydrated and grinding (Cuitzeo, Mexico). Dolomite,urea, phosphate rock, calcium triple superphosphate and potassium chloride (Agrochemical from Zamora, México).Arbuscular mycorrhizal fungi(*Glomusclarum*) and nitrogen-fixing bacteria (*Azospirillum* spp) were acquired from CIIDIR microbiolobyloboratory.

Substrate Formulation

The substrate (S_1) was prepared with the following materials at the percentage indicated in a volume/volume (v/v) ratio: a) coconut coir fiber dust (58%),b) rice husk (12%),c) cattle manure (17%),e)yeast (1%) and sugar cane rum (12%). The coconut fiber dust was selected because of its content in potassium, phosphorus and its high water holding capacity [21],the rice husk was selected because of its low apparent density [15], the cattle manure was chosen because of its N compounds, micronutrient contents and its microorganism populations;the sugar cane by-product was chosen for its high content of N, P and Ca and *S. cereviciae* yeast was used to accelerate the composting process.

Substrate composting

All of substrate ingredients were mixed homogeneously and then, the mixture were watered homogeneously at ca. 60% of water holding capacity; when the temperature was higher than 60ºC the mixture was aerated and watered homogeneously, this cyclic process was replicated until the temperature was stabilized in 27ºC. Later the mixture was aerated and watered homogeneously weekly for a six months period, until the compost (S_1) was obtained and it was analyzed for its physical and chemical characteristics (18, y16).

Treatment design

The substrate (S_1) was mixed with each of the following ingredients (Table 1) at percentage indicated in a volume/volume (v/v) ratio: fish flour (10%), was chosen because of its high N content, dolomite (2%) was selected as a Ca and Mg source, phosphate rock (2%) was included as a slow release P source, arbuscular mycorrhizal fungi (*Glomusclarum*) ($1x10^6$ufc/g), *Azospirillum* bacteria ($1x10^8$ufc/g), and chemical fertilizers (150-60-200): urea (46% N) as N source, calcium triple super phosphate (46%P_2O_5) as a P source and, potassium chloride (60% K_2O) as a K source. Broccoli (*Brassica oleracea* L. Italica group CV Claudia) was used as an indicator crop of the substrate quality.

Experiment setup

The experiment was placed during the 2010-11 winter season at the greenhouse of the CIIDIR-IPN-MICH (Centro Interdisciplinario de Investigaciónpara el Desarrollo Integral Regional del InstitutoPolitécnicoNacional), located in northwestern Michoacán, México. There were testing 7 treatments with a completely randomized design with 4 replications each one and the containers were filled with 10 L of substrate with each treatment. A broccoli seedlings transplant was made in November 8, 2010. The seedlings were irrigated every third day during the first growth weeks to substrate water holding capacity, later were watered daily. On February 9, 2010 agronomic data were taken such as plant height, leaves length and width, stem diameter and dry matter yield; three youngest fully matured leaves were sampled from each replication and weighed fresh, oven dry at 65^{0}C for 48 h, and then reweighed to permit calculation of moisture content. The dried sample was ground and sieved through 40 mesh and the resulting powder was used for determination of total N and P, K, Ca, Mg, Fe, Cu, Mn, Zn and B were determined by Atomic Absorption Spectrophotometry, after a wet digestion of the sample [10]. The analyses were performed in CIIDIR-IPN Soil Chemistry Laboratory. Dry matter yield data were subjected to analysis of variance and means comparison test ($Tukey_{0.05}$) [7].

RESULTS AND DISCUSSION

According to the criteria established by [21], the results obtained over the physical characteristics of the substrate (S_1) indicated a high porosity and water holding capacity, and consequently a low apparent density (Table 2), properties that make it a suitable substrate for growing vegetables.

The chemical analysis of the substrate (S_1) indicated a high content of macronutrients (N, P, K, Ca and Mg) and micronutrient (Fe, Zn and Mn), except Cu whose contents were in low levels (Table 3).

The pH and electrical conductivity of the substrate were too high for the development of many crops, which have limitations related primarily to the micronutrients availability such as Fe, Zn and Mn. On the other hand, the P content was extremely high and it can interacts with Ca, Mg and Fe however, in this substrate it is possible to grow some species of the *Solanaceae* and *Brassicaceae* families, such as sweet pepper, cabbage, broccoli, including salt-tolerant species.

Broccoli plants were affected by different treatments applied to the base substrate (S_1), for example, T_6 and T_2 treatments had higher dry matter yields with 52.82 and 48.45 g plant^{-1} respectively,on the contrary T_1 and T_4 treatments had the lower dry matter yields 38.70 and 38.15 g plant^{-1} respectively (Table 4).

Different treatments influenced on the earliness of the broccoli plants, which with the dolomite treatments (T_7) were the earliest (99 days to flowering); this phenomenon may result from the dolomite influence on the dynamics of various essential elements because it was observed that plants with this treatment had lower levels of N, P, K, Fe, Mn and Cu; on the contrary, plants with T_6 and T_4 treatments, (with chemical fertilizers and *Azospirillum* bacteria respectively), were the latest (110 and 109 days to flowering respectively); however [20] found the opposite in maize co-inoculated with *Azospirillum* and *mycorrhizal* fungi, as were earlier compared with those which were not inoculated with microorganisms. Presumably this phenomenon was influenced by the contents of foliar N, P, Mg and Mn which were the highest) (Table 5).

Table 1. Formulation of the different treatments

Treatments	Ingredients
T_1	S_1
T_2	S_1 + Fish flour
T_3	S_1 + arbuscular mycorrhizal fungi
T_4	S_1 + nitrogen-fixing bacteria
T_5	S_1 + arbuscular mycorrhizal fungi + nitrogen-fixing bacteria.
T_6	S_1 + chemical fertilization
T_7	S_1 + dolomite ($CaCO_3$ + $MgCO_3$)

Table 2. Physical characteristics of the substrate (S_1)

Parameter	Value
Total Porosity (%)	125.8 ± 2.5
AerationPorosity (%)	45.6 ± 1.8
Water Holding Capacity (%)	80.5 ± 2.3
ApparentDensity (g /cm^3)	0.38 ± 0.02
ParticlesDensity (g/cm^3)	1.47 ± 0.03

Table 3. Chemical and physicochemical analysis of the substrate (S_1)

pH	CE	Macronutrients (%)					Micronutrients (ppm)			
	(µScm^{-1})	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
8.2	6000	1.64	0.54	1.30	0.42	0.75	9000	30	100	365

Table 4. Agronomic performance of broccoli plants (*Brassica oleracea* L. Italica group CV Claudia) grown on seven different treatments on a substrate sustainable naturally

Treatment	Plantheight(cm)	Foliagewidth (cm)	Stemdiameter(cm)	Leaflength(cm)	Leafwidth (cm)	Flowering days	Dry matterper plant (g)
T_1	44.50	61.50	1.83	32.00	14.25	105	38.70 c
T_2	48.75	74.75	2.17	20.25	16.00	106	48.45 a
T_3	46.50	63.75	2.05	15.75	14.25	102	42.02 b
T_4	44.50	61.50	1.71	14.00	14.50	109	38.15 c
T_5	43.25	59.67	1.51	15.34	13.34	103	40.67 b
T_6	43.50	76.25	2.44	20.75	16.50	110	52.82 a
T_7	43.50	64.00	2.13	13.25	14.50	99	43.97 b

Different letters indicate significant statistical differences according to Tukey test ($p<0.05$)

Table 5. Foliar analysis of broccoli plants (*Brassica oleracea* L. Italica group CV Claudia) grown on seven different treatments on a substrate sustainable naturally

	Concentration (%)			Concentration (ppm)				
Treatments	N	P	K	Ca	Mg	Fe	Mn	Cu
T_1	1.16	0.066	0.67	25	47.50	1.00	6.40	0.15
T_2	1.73	0.058	0.98	50	55.00	15.5	10.30	1.40
T_3	1.53	0.081	1.55	425	52.50	1.00	7.30	0.10
T_4	2.12	0.023	0.77	25	50.00	3.10	6.75	0.45
T_5	2.55	0.047	1.05	25	57.50	0.75	8.00	0.35
T_6	1.81	0.019	0.92	150	82.50	1.85	11.30	0.30
T_7	1.78	0.022	0.47	42.5	50.00	0.90	7.30	0.15

This nutrimental phenomenon associated with the presence of arbuscular mycorrhizal fungi in the substrate-broccoli system rhizosphere, differs from that observed for other authors [19] in the sense that the arbuscular mycorrhizal fungi do not develop in this system due to the substances excreted by plants belonging to the *Brassicaceae* family, which turn out to be antifungal. It is possible that the rhizosphere physicochemical conditions of the substrate-broccoli system modify the plant exudates action, then the arbuscular mycorrhizal fungi uptake P, K and Ca and then transported to the plant.

Respect to Ca, again it was hypothesized that fungal infection caused wounds in the crop root walls and membranes, and the plant used more Ca compared with uninfected ones; the arbuscular mycorrhizal fungi uptake the nutrients from the substrate and provided it to the plant.

It was hypothesized it is possible that microorganisms which lives in mutualistic symbiosis with plant roots, uptake all the essential elements in a very close relationship so they did not cause nutrient imbalances in the plant, and these did not behave as if the mycorrhizal fungi was an invasive species, however, in fact it was. In this work it could see that broccoli plants inoculated with mycorrhizal fungi (T_3) absorbed 32.71% more P than that those without mycorrhizal fungi; it was able also to verify the influence of these on K and Ca nutrition, as they uptake 1.7 times more K and 2.84 times more Ca than that which was uptake by the chemically fertilized plants (T_6) (Table 5).

However, this increased foliar nutrient contenthadnot been shown neither on dry matter yields nor on agronomic characteristics considered (Table 4). The absorption of K and Ca was stimulated by mycorrhizal fungi; these nutrients are involved in the transport of photosynthate from plant to mycorrhizal fungi structures; in addition, it was seen that the copper uptake was inhibited by the mycorrhizal fungi.This phenomenon indicates that somehow there was an interaction between mycorrhizal fungi and broccoli plant roots in this production system [4] indicated that mycorrhizal fungi and bacteria can interact synergistically to stimulating plant growth through several mechanisms including improvement in nutrient acquisition.In addition, the K promotes the translocation of sugars to storage sites in the broccoli plant. According to results herein it was hypothesized that mycorrhizal fungi provided K to the broccoli plants in greater amounts, because this is necessary for the carbohydrates translocation (Table 5).

On the other hand, the broccoli plants grown in T_4 treatment (S_1 + *Azospirillum* sp.), accounted 25.82% more N than those developed in T_3 treatment (S_1 + mycorrhizal fungi), even that the fertilized treatment (T_6), although this has not been shown by the plant agronomic characteristics data (Table 4).This plant N nutrition pattern influenced by rhizobacteria, partly agrees with the findings of other researchers (8 y6) who found that these bacteria stimulates plant growth, because N is an essential element for plant growth; but when broccoli plants were co-inoculated with mycorrhizal fungi and *Azospirillum* bacteria (T_5), a synergistic effect was observed on N uptake; *Azospirillum* sp. bacteria was favored by the mycorrhizal fungi, but this one was not favored by the rhizobacteria in the P absorption.

These results differs from that obtained by some authors [22,9,4], because they found that the co-inoculation of *Azospirillum* bacteria-mycorrhizal fungi had synergistic effects on plants N fixation and P uptake through several mechanisms including improvement in nutrient acquisition.However, in this work it was observed that the interaction between mycorrhizal fungi-*Azospirillum* bacteria encouraged the absorption of some nutrients but inhibited others. In addition this co-inoculation mycorrhizal fungi and *Azospirillum* bacteria had a positive effect on N uptake but a negative one on Fe absorption by broccoli plant roots which absorbed 1.2 times more N but 4.1 times less Fe than plants inoculated only with rhizobacteria. Ca and Fe uptake was inhibited by this association, as well it could be observed in leaf nutrient analysis, on the other hand, they also inhibited the P absorption by the mycorrhizal fungi, as plants absorbed only 56.21% of P compared with that absorbed by the plants inoculated only with mycorrhizal fungi (T_3). The enhanced in the absorption of N caused by the association of mycorrhizal fungi-*Azospirillum* bacteria observed in this study, it is agree with those reported by [5] who concluded that the N-fixing *Rhizobium* bacteria improved the N bioavailability for plants, but this attribute is intensified when co-inoculation with mycorrhizal fungi.

According to [2] from essential elements for plant growth contained in a compost, between 70 and 80% of P and between 80 and 90% of K are available the first year after application, however, all N is in organic compounds, which makes it a limiting factor for crops growth, therefore these must be mineralized (oxidized) first and then absorbed and assimilated by plants; in addition, during the first year only 11% of N is mineralized, generating a N deficiency in crops if does not supplied it with external sources;but integral components of this substrate and the composting system which underwent, transform this into an open thermodynamic system that exchanges energy and matter with its environment, and under these circumstances, it becomes on a degradation material with nutrient gradual release, so hypothetically, it is expected that the substrate functions as a nutritionally self-enough system for several crop seasons, thus contributing to reducing environmental pollution, damp climate change and global warming.

CONCLUSIONS

Although broccoli plants were developed apparently without nutrition problems, it is necessary to make some changes in the substrate in order to adjust the salt and P content thus as some micronutrients.

Mycorrhizal fungi had a positive effect on P uptake by broccoli plants developed in the substrate.

The *Azospirillum* bacteria co-inoculated with mycorrhizal fungi had a synergistic effect in the atmospheric N fixation in this substrate-broccoli system.

The co-inoculation mycorrhiza-*Azospirillum* bacteria inhibited the P, Ca and Fe absorption by broccoli plant roots.

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