



Recycling of residual substrate from *Ganoderma lucidum* mushroom cultivation as biodegradable containers for horticultural seedlings



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ARTICLE INFO

Article history:

Received 5 November 2015

Received in revised form 5 February 2016

Accepted 7 February 2016

Keywords:

Rice agro-residues

Seedling

Solid-state fermentation

Sunflower seed husks

Tomato

Transplantation

ABSTRACT

Cultivation of the medicinal mushroom *G. lucidum* leaves a residual substrate with a matrix that is bound by the mycelium net and presents the necessary mechanical properties to be cut and hollowed. This material was used to make biodegradable containers (*Ganocetas*) which were evaluated for growing horticultural seedlings. Residual substrates from *G. lucidum* cultivation on sunflower seed hull, rice straw and rice husk agro-residues were used for making *Sunflower Seed Hull-based Ganocetas* (SFG) and *Rice agro-based residue Ganocetas* (RG).

Utilization of SFG did not affect the germination in 15 of the 17 plant species tested and it improved seedling growth and/or vigor in 31% of them. A good porosity in these containers produced a cooling effect which reduced the maximum temperature by up to 3 °C on the warmest days.

In the second assay, we evaluated both types of *Ganocetas* (SFG and RG) in tomato seedling transplantation, seedling establishment and tomato production. On transplanting day, seedling growth and vigor in SFG did not differ from the control, whereas both parameters were reduced in RG. Results of seedling establishment under ideal conditions indicated that growth, flowering and early fruit production of tomato using SFG was comparable to the control, whereas plants were reduced in all three parameters when using RG. Tomato production yields were similar between SFG and the control treatments; moreover, physicochemical analysis predicts a promising performance in transplanting and growth of SFG under stress conditions. However, in spite of presenting comparable physical and chemical properties, tomato production using RG was lower. More research is needed to look for possible allelopathic substances coming from the biodegraded lignocellulosic matrix in these RG containers.

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1. Introduction

Nursery culture of seedlings allows uniform seedling growth, control of weeds and diseases and can shorten crop duration (Herrera et al., 2008). However it has a drawback, which is the stress of transplantation, due to the fact that seedling roots are

subjected to an abrupt change in environmental conditions, such as direct exposure to air and new soil, and even to different degrees of mechanical damage. This condition may eventually affect the performance and growth rate (Latimer, 1992).

In order to manage transplantation stress, some physical and chemical approaches are used to increase seedling vigor and stimulate plant resistance to pathogens, e.g., the restriction of water and the application of cupric salts or low doses of paclobutrazol in the case of tomatoes (Argerich and Troilo, 2011).

Nevertheless, when aiming at avoiding mechanical damage to the roots, organic containers are the only adequate options because they can efficiently reduce such stress. Once the containers are buried, the roots can gradually pass through their matrix walls and explore the new soil environment. However, this capacity depends on the material and binding method used in obtaining the seedling container (Schettini et al., 2013). In recent years, interest in studying these containers has increased as they do not generate

Abbreviations: AD, apparent density; AP, air porosity; ATG, average time of germination; DIAM, basal diameter; DWA, aerial dry weight; DWR, roots dry weight; EP, effective pore space; FWA, aerial fresh weight; FWR, root fresh weight; G%, percentage of seed germination; IGR, index of germination speed; ISE, index of seedling establishment; L, number of leaves; LE, soil-apical length; RG, rice agro-based residue *Ganocetas*; RH, relative humidity; SFG, sunflower seed hull-based *Ganocetas*; SQI, seedlings quality index; WP, water porosity.

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any polluting wastes, and also due to the opportunity of recycling the organic material with which they are fabricated. Moreover, some materials reportedly used for organic containers are non-renewable, such as peat and biodegradable plastics (Horinouchi et al., 2008), but on the other hand, organic residues, such as chicken feathers (Evans and Hensley, 2004), paper (Yamauchi et al., 2006), and vegetable fibers (Schettini et al., 2013) have also been used. Generally the bound particles in those containers are achieved through compression or by using biodegradable adhesives. However, some drawbacks are observed, e.g., plant growth may be affected by withdrawal of water from the root system by peat containers, and also the difficulty in degrading the buried matrix, which may remain in the soil even after the cultivation cycle (in the case of chicken feathers) (Evans and Hensley, 2004).

In this study, the growth of seedlings in organic containers was evaluated. The organic containers, hereafter called “*Ganocetas*”, were manufactured using a novel method which includes drying, cutting and hollowing of the “synthetic log” (residual substrate) obtained after solid-state fermentation (SSF) of agro-industrial lignocellulosic wastes by the medicinal mushroom *Ganoderma lucidum*. SSF was performed on sunflower husks and rice by-products used for mushroom production. The substrate biodegradation resulting from this process reduced the lignin and cellulose content, increased mineralization and provided new nutrient combinations (Postemsky et al., 2014; Postemsky and Curvetto, 2015). *G. lucidum* synthetic logs had an adequate mechanical resistance, more convenient than that found under similar conditions with other cultivated mushrooms on the same substrates.

The aim of this study was to evaluate the germination, growth and vigor of 17 vegetable species. And then to study the growth and vigor of tomato seedlings and the effect of transplanting on product yield. Additionally, the physicochemical properties of the organic containers were explored in order to know more about the influence of the matrix wall of the *Ganocetas* on plant growth.

2. Materials and methods

2.1. Seeds and substrate

Seeds for exp.#1 were obtained from INTA (National Institute of Agricultural Technology, Argentina). Tomato seeds for exp.#2 were obtained from Seminis® (Argos hybrid). Plant substrate used for seedlings culture was *Terraferil Growmix MULTIPRO*® (Argentina). Experiments were conducted in Bahía Blanca, Argentina in November–December 2012 (exp.#1) and in September–January, 2013–2014 (exp.#2).

2.2. Experiment#1. Effect of SFG *Ganocetas* on the germination and seedling quality of vegetables

The substrate containers were SFG (Fig. 1C, D, F), which are residual substrates from *G. lucidum* cultivation on sunflower seed hulls, (Bidegain et al., 2015) and were made by drying (60–70 °C, 72 h), cutting and hollowing. Plastic (polypropylene) containers (180 mL) were used as controls. In each experimental unit (1 container) 4–6 seeds were sown in 150 mL substrate under glass greenhouse conditions (average daily temperature 22–25 °C, 13–14 h photoperiod with a maximum of 400–600 μmol/m²s natural light and 50–70% RH). Irrigation was provided manually (15–20 mL/container/day). Evolution thermo-registers (*i-button*, Maxim, EUA) were used to measure the temperature of the substrate.

The variables evaluated were: percentage of seed germination (G%), index of germination speed (IGR) and the average time of germination (ATG), according to Cubillos-Hinojosa et al. (2009).

Once germination reached the stationary phase, one seedling per container was allowed to continue.

At the transplanting stage, or when growth of non-transplantable plantlets showed comparable aerial sizes, the following morphological parameters were measured: basal diameter of the seedlings (DIAM), length of principal stem (LE), aerial (FWA) and root (FWR) fresh weight, number of leaves (L) and aerial (DWA) and root (DWR) dry weights (obtained at 60 °C, 72 h followed by 105 °C, 2 h). Seedling quality index (SQI) was obtained using FWA, DIAM and LE (Zhang et al., 2012), in this case, the dry weight of roots was not considered because it was not possible to remove them entirely from the matrix of *Ganocetas*. An improvement in growth was considered when higher values of LE, DWA or L occurred; better vigor was at higher values of DIAM, SQI and loss of vigor when the ratios LE/DIAM, FWA/DWA and FWR/DWR were increased.

2.3. Experiment#2. Effects of *Ganocetas* containers on tomato performance from seedling to crop

The effects of SFG and RG on tomato seedling growth and vigor, as well as on tomato production under plastic greenhouse conditions, were also evaluated (Fig. 1E and F). RG were obtained from residual substrate from *G. lucidum* cultivation on rice-based substrates (Postemsky et al., 2014).

Evaluation of seedling growth and vigor was performed as follows: seeds were pre-germinated (90% RH and 29 °C for 48 h), then introduced into substrates in both organic and plastic (control, n=36) containers. The seedling containers were randomly distributed under similar environmental conditions. Seedling irrigation (15 mL/container/day) was done manually using filtered tap water (during the first three weeks) and then with an aqueous solution of 0.5 g/L (*Zafiro-multiestadios* Argenfert® 19% N: 19% P: 19% K: 2.2% S) at the same rate of irrigation until transplanting time, day 43. The average temperatures in glass greenhouse were 28 °C (day) and 15 °C (night), photoperiod was 12 h with a maximum of 400–500 μmol/m²s natural light irradiation. At transplanting time, seedlings (n=12) were chosen randomly to study seedling growth performance and vigor by the method described in Section 2.2.

Study of the effect of the container on tomato plant establishment and fruit yield production was carried out as follows: seedlings (n=17) were randomly transplanted in rows in a total area of c.a. 20 m². Soil (sandy loam) was raked over, and an irrigation hose and polyethylene mulch (bicolor black and white, 100 μm) were used. The seedlings were irrigated daily and fertigation was applied once a week using the mineral supplement mentioned above. Fertilization dosage was 0.6 g/L until flowering and then 1.2 g/L, which represented a dosage of 70 and 140 mg/plant and 1 and 2 kg/ha/week, respectively). Plant management included pruning the lateral branches until day 60 after transplanting and tutoring of the main branch and pruning of old leaves at the plant base (in the period of 60–90 days after transplanting). The insecticides chlorpyrifos (0.9 g L⁻¹) cypermethrin (0.3 g L⁻¹) and imidacloprid (0.2 g L⁻¹) were applied with utmost discretion.

Analysis of plant establishment after transplanting was calculated using non destructive measures of plant length, stem basal diameter, and number of leaves at 1 and 15 days after transplanting using an *index of seedling establishment* (ISE):

$$ISE = \frac{\text{basaldiameteratday15} - \text{basaldiameteratday1}}{\text{lengthatday15} - \text{lengthatday1}} \times \left(\frac{1}{3}La + \frac{1}{2}Lb + Lc \right)$$



Fig. 1. Organic pots made of residual substrate from *Ganoderma lucidum* cultivation. Synthetic logs were dried, cut (A) and hollowed (B). *Solanum melongena* grown in SFG (C), split container (D) showing a rough root morphology. Seedlings of *Solanum lycopersicum* grown in a plastic container (E) and in SFG (F).

where L_a , L_b and L_c are the number of leaves of less than 5 cm, between 5–10 cm and >10 cm in longitudinal leaf length, respectively.

Fruit yield was determined as the number of mature fruits (full orange–red, no green parts) per plant recorded on the day of collection. Fruit quality was studied by recording the equatorial diameter of the tomatoes, together with the fresh and dry weight (48 h at 70 °C). These data were recorded from day 60 to 120, following transplantation.

2.4. Determination of containers physicochemical properties

Container pieces c.a. 2 g dry weight (85 °C, 24 h) were obtained from samples of exp.#2 ($n=12$) collected at initial time (day 1), transplant time (day 43) and after transplanting, when the production cycle was considered finished (day 120).

Physical properties at “initial” and “transplanting” times were studied as follows: samples with known dry weight (air dried and kept at 25 °C and 30% RH, mean water content 4.5 and 5.0 in SFG and RG, respectively) were placed in tared Falcon tubes (50 mL), and a viscose agar solution (0.04% p/v, density 1 g/mL) at 4 °C was rapidly added to reach 40 mL. Sample volume was obtained gravimetrically by difference. Apparent density (AD) was: $AD \text{ (g/mL)} = \text{Dry weight/sample volume}$. Then, the tube was sealed and conserved at 4 °C for 48 h, and subsequent volume reduction was used to calculate the effective pore space (EP): $EP \text{ (\%)} = 100 \times \text{volume reduction/sample volume}$. After that, the liquid

was drained (3 h) and sample fresh weight was obtained. Water porosity (WP) was calculated as follows: $WP \text{ (\%)} = 100 \times (\text{fresh weight} - \text{dry weight})/\text{sample volume} \times 1 \text{ g/mL}$ and air porosity (AP) was obtained by difference: $AP = EP - WP$.

Physical properties of recovered vestiges of *Ganocetas* after the tomato crop (“final”) were analyzed as follows: samples with known dry weight (air dried and kept at 25 °C and 30% RH) were placed in tared Falcon tubes (50 mL), the volume was recorded and apparent density was calculated with the equation: $AD \text{ (g/mL)} = \text{dry weight}/\text{volume}$. After that, pure water (4 °C) was added over a period of 24 h until saturation point was reached and the saturated weight of the sample was obtained. The effective pore space was calculated as follows: $EP \text{ (\%)} = 100 \times (\text{saturated weight} - \text{dry weight})/\text{volume} \times \text{water density}$. Then, the substrate was drained (3 h) and the fresh weight of the sample was recorded; water porosity was calculated with the formula: $WP \text{ (\%)} = 100 \times (\text{fresh weight} - \text{dry weight})/\text{volume} \times \text{water density}$ and air porosity was obtained by difference: $AP \text{ (\%)} = EP \text{ (\%)} - WP \text{ (\%)}$.

Both, pH and electric conductivity were measured in a saturated paste and at 1:6 (1 mL fresh weight basis in 6 mL of distilled water) suspension solutions. Ash content was obtained by calcinations at 550 °C for 4 h. Chemical analysis of both samples collected on day 1 (initial time) and control materials used for synthetic log production without inoculation, was undertaken with a pool of milled container pieces (20 g dry weight, 85 °C, 24 h), according to Postemsky et al. (2014).

Table 1
Effect of the Sunflower seed Hull-based Ganocetas (SFG) on the percentage of seed germination (G%), index of germination rate (IGR) and average time of germination (ATG) of seeds. Plastic containers (Ctroll) were used for comparison. Data are mean values \pm SD. The number of experimental units (n) in each container and the results for pair comparison using t-test are shown (NS: no significant differences, *: $p < 0.05$, **: $p < 0.01$).

Taxonomic family	Common name, species	Container	n	G% ^a	IGR ^b	ATG ^c
Amaranthaceae	Kiwicha, <i>Amaranthus caudatus</i>	Ctroll	5	76 \pm 9 ^{NS2}	.21 \pm .07 ^{NS}	3.9 \pm 1.0 ^{NS}
		SFG	5	76 \pm 17	.17 \pm .02	4.8 \pm 1.0
	Spinach, <i>Spinacia oleracea</i>	Ctroll	4	20 \pm 16 ^{NS}	.02 \pm .01 ^{NS}	13.5 \pm 2.8 ^{NS}
		SFG	5	60 \pm 31	.06 \pm .03	11.3 \pm 1.8
Amaryllidaceae	Leek, <i>Allium ampelo-prasum</i> var. <i>porrum</i>	Ctroll	5	64 \pm 9 ^{NS}	.06 \pm .01 ^{NS}	12.5 \pm 1.6 ^{NS}
		SFG	5	68 \pm 27 ^{NS}	.06 \pm .03	13.4 \pm 2.0
Apiaceae	Fennel, <i>Foeniculum vulgare</i>	Ctroll	5	85 \pm 26 ^{NS}	.10 \pm .03 ^{NS}	10.0 \pm 1.2 ^{NS}
		SFG	5	96 \pm 9	.11 \pm .01	8.8 \pm 0.5
Asteraceae	Lettuce, <i>Lactuca sativa</i>	Ctroll	5	56 \pm 22 ^{NS}	.08 \pm .03 ^{NS}	7.9 \pm 1.6 ^{NS}
		SFG	5	64 \pm 26	.08 \pm .02	8.6 \pm 1.7
Brassicaceae	Cabbage, <i>Brassica oleracea</i> var. <i>viridis</i>	Ctroll	6	43 \pm 8 ^{**}	.08 \pm .03 ^{NS}	11.8 \pm 1.8 ^{NS}
		SFG	6	73 \pm 16	.09 \pm .03	10.7 \pm 1.6
Cucurbitaceae	Watermelon, <i>Citrullus lanatus</i>	Ctroll	6	79 \pm 29 ^{NS}	.09 \pm .03 ^{NS}	10.7 \pm 1.6 ^{NS}
		SFG	6	88 \pm 21	.08 \pm .03	11.8 \pm 1.8
	Cucumber, <i>Cucumis sativus</i>	Ctroll	5	95 \pm 11 ^{NS}	.15 \pm .02 ^{NS}	6.3 \pm 0.3 ^{NS}
		SFG	5	10 \pm 0	.15 \pm .01	6.6 \pm 0.3
	Pumpkin, <i>Cucurbita maxima</i>	Ctroll	6	83 \pm 20 ^{NS}	.11 \pm .03 ^{NS}	8.4 \pm 2.0 ^{NS}
		SFG	6	91 \pm 13	.09 \pm .02	11.0 \pm 3.0
	Zucchini, <i>Cucurbita pepo</i>	Ctroll	6	17 \pm 28 ^{NS}	.03 \pm .01 ^{NS}	18.0 \pm 0.0 ^{NS}
		SFG	6	17 \pm 28	.03 \pm .01	17.0 \pm 1.4
	Calabash, <i>Lagenaria siceraria</i>	Ctroll	5	80 \pm 27 ^{NS}	.06 \pm .02 ^{NS}	14.2 \pm 1.1 ^{NS}
		SFG	5	60 \pm 22	.04 \pm .01	16.0 \pm 1.8
	Loofah, <i>Luffa</i> sp.	Ctroll	5	80 \pm 45 ^{NS}	.05 \pm .03 ^{NS}	11.8 \pm 6.7 ^{NS}
		SFG	5	10 \pm 0	.06 \pm .02	18.4 \pm 2.2
Fabaceae	Pea, <i>Pisum sativum</i>	Ctroll	5	60 \pm 28 ^{NS}	.11 \pm .05 ^{NS}	5.5 \pm 0.4 ^{**}
		SFG	5	53 \pm 30	.08 \pm .05	6.9 \pm 0.8
Lamiaceae	Basil, <i>Ocimum basilicum</i>	Ctroll	5	84 \pm 21 ^{NS}	.74 \pm .22 ^{NS}	6.4 \pm 0.7 ^{NS}
		SFG	6	83 \pm 8	.70 \pm .13	6.7 \pm 0.6
Solanaceae	Peppers, <i>Capsicum annuum</i>	Ctroll	6	73 \pm 24 ^{NS}	.04 \pm .02 ^{NS}	17.2 \pm 1.2 ^{NS}
		SFG	6	80 \pm 31	.05 \pm .02	17.5 \pm 1.9
	Tomato, <i>Solanum lycopersicum</i>	Ctroll	6	100 \pm 0 ^{NS}	.16 \pm .02 ^{NS}	6.2 \pm 0.6 ^{NS}
		SFG	6	97 \pm 8	.17 \pm .01	6.3 \pm 0.5
Eggplant, <i>Solanum melongena</i>	Ctroll	6	60 \pm 33 ^{NS}	.04 \pm .02 ^{NS}	17.3 \pm 2.0 ^{NS}	
	SFG	6	53 \pm 16	.03 \pm .01	19.4 \pm 2.6	

^a G% = 100 \times n° germinated seeds/n° sown seeds.

^b IGR = $\sum [G\%(\text{at day } n)/(\text{day } n^\circ)]$.

^c ATG = $[\sum (n^\circ \text{ germinated seeds (at day } n^\circ) \times \text{day } n^\circ)]/n^\circ \text{ total days}$.

2.5. Data analysis

Pair comparison of data from exp.#1 was done using t-test with Satterwait protection. Data of assay exp.#2 were subjected to a one way ANOVA, means were then further analyzed by Tukey test ($\alpha = 0.05$), or by the Kruskal–Wallis test, using the Infostat software (Di Rienzo et al., 2010).

3. Results

3.1. Effect of SFG on germination and seedling quality of vegetables

The percentage of seed germination (G%), the index of germination rate (IGR), or the average time for germination (ATG) showed no differences in 15 of 17 species when sown in substrate in either plastic or SFG type containers (Table 1).

The effect of SFG organic containers on seedling performance was inferred considering the seedling morphology and calculated growth indexes (Table 2). Growth improvements using SFG were seen in *Brassica oleracea* var. *viridis*, *Citrullus lanatus*, *Lactuca sativa*, *Capsicum annuum*, *Solanum lycopersicum* and *Solanum melongena*. It is worthwhile mentioning that the LE/DIAM ratio was a better predictor of vigor than FWA/DWA, due to the distinct shape nature of *L. sativa*. With regards to *C. annuum*, a lower LE/DIAM ratio in the control container does not necessarily mean higher vigor since SFG seedlings in this species also presented higher DIAM, LE and L and therefore vigor was considered equal for both treatments.

When the root systems of the cultivated species in SFG were observed in detail, it was possible to get new insight on the performance of root growth *vis-à-vis* its controls. Indeed, it was found that the root systems were more branched and rustic, i.e., thicker, hard and dark as compared to the controls (Fig. 1 C–F).

Moreover, an interesting growth response was observed in the seedlings: their root tips protruded outwards from the SFG, an effect not observed in younger seedlings; hence those seedlings exhibited a more advantageous transplanting size.

A higher water evaporation rate was deduced in SFG, as a result of the thermal differences in the substrate of each type of container (Fig. 2). When using SFG there are reductions from 0.5 to -3°C in the substrate temperature over considerable periods during the warmest days.

3.2. Effects of SFG and RG containers on tomato performance from seedling to harvest

Various growth parameters from seedlings were obtained at the time of transplanting (Table 3). Data revealed that growth of seedlings in SFG containers was similar to that obtained in plastic containers, but vigor parameters were better in SFG. In the case of RG, data showed that seedlings had a lower growth rate and also exhibited a lower vigor status. After 15 days transplanting, seedling establishment was evaluated by using nondestructive measures (Table 4). ISE and flowering records showed that seedlings from SFG were comparable to the controls after two weeks. At day 40, the number of green fruits was recorded (Table 4), and data revealed

Table 2
Effect of SFG organic containers on seedling performance. Seedlings grown in plastic containers (Ctrl) or Sunflower seed Hull-based Ganocetas (SFG), were measured: base width (DIAM), length to the apical bud (LE) and their ratio (LE/DIAM), aerial dry weight (DWA), fresh weight to dry weight of the aerial parts ratio (FWA/DWA) and roots ratio (FWR/DWR), number of leaves (L) and the seedling quality index (SQI). Values are means ± SD at the transplanting time (days). The number of experimental units (n) in each container and results for pair comparison using t-test are included.

Species	C	n	Time (d)	DIAM (mm)	LE (cm)	LE/DIAM	DWA (g)	FWA/DWA	FWR/ DWR	L (n#)	SQI ^a	Effect of SFG ^b
<i>Amaranthus caudatus</i>	Ctrl	5	49	1.7±0.4 ^{NS}	9.7±2.1 ^{NS}	5.7±0.4 ^{**}	0.07±0.05 ^{NS}	5.6±0.4 [*]	5.0±1.5 ^{NS}	3.3±1.5 ^{NS}	.012±.009 ^{NS}	>Growth
	SFG	5		1.5±0.6	12.4±5.3	8.3±0.6	0.08±0.08	7.1±0.9	4.5±2.2	4.6±2.4	.010±.009	<Vigor
<i>Spinacia oleracea</i>	Ctrl	4	42	1.1±0.1 ^{NS}	5.3±2.6 ^{NS}	4.7±2.7 ^{NS}	0.06±0.03 ^{NS}	8.2±3.3 ^{NS}	0.1±0.1 ^{NS}	5.0±1.0 ^{NS}	.011±.001 ^{**}	=Growth
	SFG	5		1.1±0.1	11.4±5.6	9.7±4.7	0.02±0.01	17.7±10.4	0.2±0.3	6.2±2.3	.003±.001	<Vigor
<i>Allium ampeloprasum</i>	Ctrl	5	84	1.5±0.3 ^{NS}	2.6±0.9 ^{NS}	1.7±0.3 ^{**}	0.03±0.02 ^{NS}	4.4±0.6 ^{NS}	8.4±1.9 ^{NS}	3.4±1.5 ^{NS}	.037±.016 ^{NS}	=Growth
	SFG	5		1.5±0.7	3.8±1.9	2.7±0.3	0.05±0.07	5.2±1.6	9.1±1.8	2.2±0.8	.015±.017	<Vigor
<i>Foeniculum vulgare</i>	Ctrl	5	56	2.4±0.5 ^{NS}	4.0±0.5 ^{NS}	1.7±0.4 ^{NS}	0.14±0.07 ^{NS}	6.3±0.5 ^{NS}	8.9±0.8 ^{NS}	3.6±0.9 ^{NS}	.090±.064 ^{NS}	=Growth
	SFG	5		2.4±1.1	4.2±1.6	1.8±0.3	0.19±0.20	6.9±2.7	12.1±7.4	4.0±2.1	.116±.132	=Vigor
<i>Lactuca sativa</i>	Ctrl	5	49	1.9±0.2 ^{NS}	13.8±5.0 ^{NS}	7.4±2.8 [*]	0.18±0.13 ^{NS}	7.5±1.1 ^{**}	8.3±0.8 ^{NS}	4.8±1.3 ^{NS}	.023±.009 ^{NS}	=Growth
	SFG	5		2.3±0.5	9.2±4.3	3.9±1.4	0.18±0.11	14.1±3.1	8.6±1.0	5.8±1.6	.046±.025	>Vigor
<i>Brassica oleracea</i>	Ctrl	6	112	0.9±0.2 ^{**}	3.2±1.6 [*]	3.5±1.0 ^{NS}	0.06±0.04 ^{NS}	6.0±0.8 ^{NS}	4.4±1.4 [*]	3.6±0.9 [*]	.017±.008 [*]	>Growth
	SFG	6		2.2±0.6	6.0±2.0	2.7±0.3	0.77±0.72	6.7±1.2	6.4±1.2	6.2±1.7	.275±.229	>Vigor
<i>Citrullus lanatus</i>	Ctrl	6	49	3.1±0.3 ^{NS}	9.5±1.9 ^{NS}	3.1±0.6 ^{NS}	0.20±0.07 ^{NS}	6.5±0.9 ^{**}	11.4±1.6 ^{**}	2.0±0.9 ^{**}	.072±.026 ^{NS}	>Growth
	SFG	6		3.0±0.2	14.3±7.1	4.7±2.0	0.31±0.17	9.4±1.1	8.1±1.3	4.5±1.5	.062±.016	>Vigor
<i>Cucumis sativus</i>	Ctrl	5	49	4.0±0.4 ^{NS}	18.4±3.2 ^{NS}	4.6±0.8 ^{NS}	0.50±0.07 ^{NS}	7.3±0.9 ^{NS}	12.1±1.3 ^{NS}	3.8±0.5 ^{NS}	.109±.015 [*]	=Growth
	SFG	5		3.5±0.3	15.4±3.5	4.3±0.8	0.36±0.13	8.2±1.8	9.9±1.9	3.2±0.8	.081±.019	<Vigor
<i>Cucurbita maxima</i>	Ctrl	6	42	3.6±0.3 ^{NS}	8.8±5.5 ^{NS}	2.5±0.8 ^{NS}	0.45±0.12 ^{NS}	6.5±0.6 [*]	10.3±0.6 ^{**}	3.7±0.5 ^{NS}	.221±.105 ^{NS}	=Growth
	SFG	6		3.5±0.5	8.3±3.4	2.5±1.5	0.44±0.27	9.4±2.5	8.3±0.8	3.5±0.6	.196±.138	=Vigor
<i>Cucurbita pepo</i>	Ctrl	6	49	3.4±0.1 ^{NS}	11.5±0.7 ^{NS}	3.3±0.1 ^{NS}	0.34±0.00 ^{NS}	14.5±1.8 ^{NS}	11.5±0.4 ^{NS}	6.0±1.4 ^{NS}	.103±.004 ^{NS}	=Growth
	SFG	6		3.6±0.5	10.0±2.8	2.8±1.1	0.40±0.13	12.9±1.7	9.8±2.1	5.5±0.7	.165±.113	=Vigor
<i>Lagenaria siceraria</i>	Ctrl	5	49	3.8±0.3 ^{NS}	15.0±1.6 ^{NS}	4.0±0.6 ^{NS}	0.42±0.12 ^{NS}	9.2±1.0 ^{NS}	14.5±0.9 ^{**}	4.0±0.7 ^{NS}	.106±.027 [*]	=Growth
	SFG	5		3.5±0.4	14.2±3.0	4.1±0.6	0.30±0.10	9.4±1.0	10.2±0.6	3.2±1.3	.074±.019	<Vigor
<i>Luffa sp.</i>	Ctrl	5	49	3.6±0.2 [*]	38.3±10.5 ^{NS}	12.7±3.9 ^{NS}	0.60±0.22 ^{NS}	7.5±1.1 ^{NS}	14.0±1.8 ^{**}	6.8±0.5 [*]	.049±.022 ^{NS}	<Growth
	SFG	5		2.7±0.1	23.2±12.6	8.5±4.6	0.40±0.24	7.5±0.5	9.4±1.6	4.6±1.5	.046±.008	<Vigor
<i>Pisum sativum</i>	Ctrl	5	42	2.7±0.4 ^{NS}	23.5±7.1 ^{NS}	8.7±1.5 ^{NS}	0.55±0.35 ^{NS}	8.9±0.3 ^{NS}	14.1±1.3 ^{**}	9.0±1.6 ^{NS}	.060±.027 ^{NS}	=Growth
	SFG	5		2.6±0.2	22.6±4.7	8.7±1.8	0.46±0.20	9.0±0.6	11.0±1.1	8.6±1.5	.051±.016	<Vigor
<i>Ocimum basilicum</i>	Ctrl	5	56	1.9±0.3 ^{NS}	11.8±3.8 ^{NS}	6.3±0.7 ^{NS}	0.18±0.07 ^{NS}	6.3±0.7 ^{NS}	11.0±0.7 ^{NS}	6.0±1.4 ^{NS}	.028±.008 ^{NS}	=Growth
	SFG	6		2.4±0.8	21.7±18.0	8.3±4.3	0.68±0.76	6.3±1.9	23.9±32.8	12.0±9.2	.068±.053	=Vigor
<i>Capsicum annum</i>	Ctrl	6	112	1.6±0.2 [*]	4.2±0.8 [*]	2.6±0.2 [*]	0.02±0.01 ^{NS}	7.5±1.1 ^{NS}	7.6±1.7 ^{NS}	2.0±0.1 ^{**}	.007±.002 ^{NS}	>Growth
	SFG	6		2.4±0.7	7.3±2.9	3.0±0.3	0.21±0.23	6.6±1.9	7.2±2.4	5.7±0.8	.065±.063	=Vigor
<i>Solanum lycopersicum</i>	Ctrl	6	56	2.2±0.2 ^{NS}	8.0±0.6 ^{NS}	3.6±0.4 ^{NS}	0.04±0.01 [*]	8.6±0.7 ^{NS}	10.8±2.3 ^{**}	2.0±0.1 ^{**}	.011±.002 [*]	>Growth
	SFG	6		3.0±0.8	13.3±6.8	4.2±1.4	0.38±0.32	8.0±1.1	7.6±1.0	5.7±2.0	.078±.058	>Vigor
<i>Solanum melongena</i>	Ctrl	6	112	1.5±0.0 ^{**}	4.6±0.9 ^{**}	3.0±0.6 ^{NS}	0.02±0.00 ^{**}	6.2±0.3 ^{**}	10.8±2.0 [*]	3.1±0.5 ^{**}	.008±.001 ^{**}	>Growth
	SFG	6		2.9±0.2	9.3±1.0	3.2±0.3	0.57±0.18	5.4±0.2	8.5±1.5	4.5±0.8	.179±.069	>Vigor

^a Seedling Quality Index: $SQI = DIAM/LE \times DWA$.

^b Better growth is a significant increase in LE, DWA or L, while better vigor is a significant increase of DIAM, SQI, or decreases in LE/DIAM, FWA/DWA and FWR/DWR ratios.

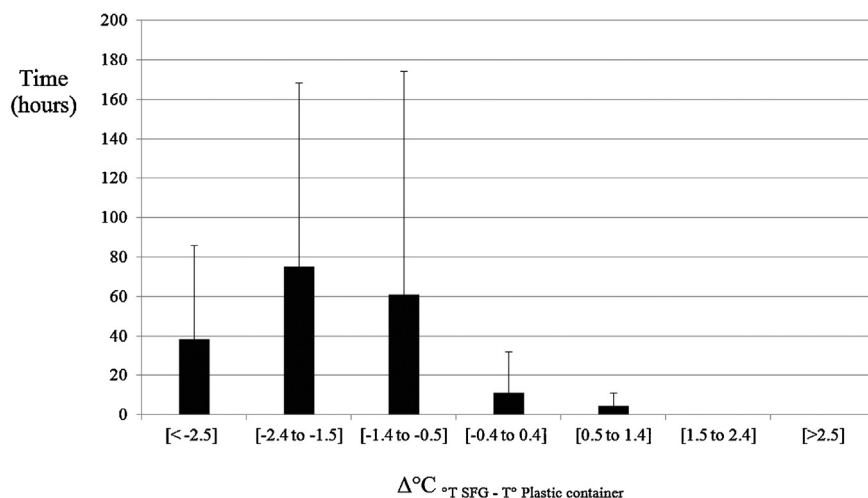


Fig. 2. Cooling effect when temperature was over 28 °C. Cumulated time (h) for differences recorded between temperatures of growing media in SFG and plastic containers. Six pairs of SFG and plastic containers were used and records when the containers temperature was over 28 °C in a study period of 50 days were selected. Values are the means ($n=6$) and the maximum value for each range of temperature.

Table 3
Effect of container type on the performance of tomato seedlings. Seedlings ($n=12$) grown in SFG and RG and plastic (Control) containers were studied: base width (**DIAM**), length to the apical bud (**LE**) and their ratio (**LE/DIAM**), aerial dry weight (**DWA**), fresh weight to dry weight of the aerial parts ratio (**FWA/DWA**) and roots ratio (**FWR/DWR**), number of leaves (**L**) and the *seedling quality index* (**SQI**). Values are means \pm SD at the transplanting time (43 days), which were differentiated using Anova and Tukey test at $\alpha=0.05$.

Container	DIAM (mm)	LE (cm)	LE/DIAM (cm/mm)	DWA (g)	FWA/DWA	FWR/DWR	L (n#)	SQI ^a	Global effect ^b
Control	3.2 \pm 3 ^b	18 \pm 5 ^a	5.4 \pm 1.2 ^a	.34 \pm .08 ^a	9.5 \pm 1.3 ^b	11.2 \pm 0.7 ^a	3.5 \pm 7 ^b	.063 \pm .014 ^b	
SFG	3.8 \pm 3 ^a	14 \pm 3 ^b	3.7 \pm 0.8 ^b	.40 \pm .15 ^a	11.8 \pm 1.2 ^a	8.6 \pm 2.3 ^b	4.9 \pm 7 ^a	.107 \pm .029 ^a	=growth, > vigor
RG	2.8 \pm 5 ^c	8 \pm 2 ^c	2.9 \pm 0.4 ^b	.10 \pm .05 ^b	12.8 \pm 1.0 ^a	9.5 \pm 1.4 ^b	3.5 \pm 9 ^b	.032 \pm .014 ^b	< growth, < vigor

^a Seedling Quality Index: $\text{SQI} = \text{DIAM}/\text{LE} \times \text{DWA}$.

^b Better growth is a significant increase in **LE**, **DWA** or **L**, while better vigor is a significant increase of **DIAM**, **SQI**, or decreases in **LE/DIAM**, **FWA/DWA** and **FWR/DWR** ratios.

Table 4
Evaluation of tomato seedling establishment with non-destructive measurements. At day 15 after transplanting, increment in basal diameter (Δ DIAM) and in shoot length (Δ LE) and also *index of seedling establishment* (**ISE**) and number of flowers were studied. At day 40 after transplanting, the number of fruits was compared. Data are the means \pm SD, means differentiated using Anova and Tukey test at $\alpha=0.05$.

Container	Δ DIAM (mm)	Δ LE (cm)	ISE ^a	Flowers (n#)	Fruits (n#)
Control	2.2 \pm 0.9 ^a	6.6 \pm 1.7 ^a	1.5 \pm .3 ^a	0.9 \pm 1.4 ^a	3.9 \pm 1.5 ^a
SFG	1.8 \pm 1.2 ^{ab}	5.6 \pm 2.1 ^a	1.5 \pm .4 ^a	0.8 \pm 1.3 ^a	3.4 \pm 1.6 ^a
RG	1.4 \pm 0.9 ^b	3.5 \pm 1.7 ^b	0.9 \pm .5 ^b	–	0.5 \pm 1.3 ^b

^a Index of Seedling Establishment $\text{ISE} = \frac{\text{basaldiameteratday15} - \text{basaldiameteratday1}}{\text{lengthatday15} - \text{lengthatday1}} \times (\frac{1}{3}L_a + \frac{1}{2}L_b + L_c)$.

that SFG containers did not affect fruit production; but a lower number of fruits per plant were found in the case of the RG containers.

The tomato plant yields and quality parameters of tomato fruits are given in Table 5 and production yields in terms of the growing area are given in Fig. 3. During this time period, SFG containers did not affect production either in precocity and quantity (Fig. 3), whereas RG containers affected both variables. In fact, fruits per plant in the latter were 56–67% lower than in SFG and control, respectively (Table 5). Nevertheless, when considering fruit weight

Table 5
Tomato fruiting performance at the end of cropping. Accumulative production values shown as mean \pm SD in number of fruits per plant, average fresh and dry weight, water content and equatorial diameter, at 60–120 days after transplanting are given. ANOVA and Tukey test were performed in normal data and Kruskal–Wallis in the rest ones (both at $\alpha=0.05$).

Treatment (seedling container)	Fruits per plant (n#)	Fresh weight (g)	Dry weight (g)	Water content (%)	Diameter (mm)
Control	12.9 \pm 5.7 ^a	116 \pm 66 ^a	7.8 \pm 5.0 ^a	92 \pm 2 ^a	61 \pm 13 ^a
SFG	14.2 \pm 4.7 ^a	126 \pm 122 ^a	7.6 \pm 4.7 ^a	92 \pm 2 ^a	62 \pm 11 ^a
RG	8.0 \pm 5.6 ^b	96 \pm 48 ^a	7.1 \pm 3.6 ^a	91 \pm 1 ^b	58 \pm 12 ^a
Statistical analysis and <i>P</i> -values	Anova–Tukey $p=0.0036$	Kruskall–Wallis $p=0.2913$	Kruskall–Wallis $p=0.9503$	Kruskall–Wallis $p=0.0009$	Anova–Tukey $p=0.1637$

(fresh or dry) or diameter, no differences were found between treatments. In general, plants derived from seedlings grown in RG containers showed symptoms of drought stress, such as reduced plant size and leaf area, and also lower water content in fruits (Table 5).

3.3. Physicochemical properties of containers

Physical properties of the *Ganocetas* were studied in exp.#2 (Table 6). AD was not modified in SFG during the experiment, while in RG it was reduced at the end of the cropping cycle.

Toward the end of the assay both *Ganocetas* showed higher EP values. In both organic containers, WP increased while AP remained constant from the beginning of assay until transplanting time. However, at the end of the experiment, containers showed differences in the source of porosity, AP being higher in SFG and WP higher in RG. Roots of tomato grew into the *Ganocetas* matrix until transplanting time, without any disintegration of the container structure. However, at the end of the cultivation cycle, about 50% of the SFG and RG containers were completely broken down in the soil.

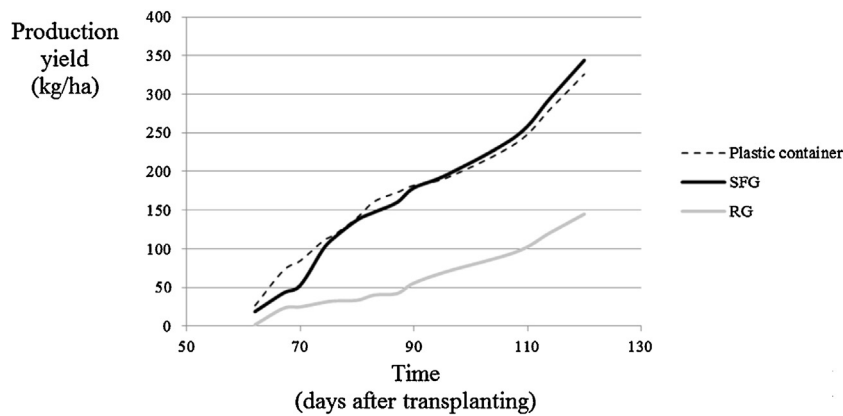


Fig. 3. Tomato production as accumulated yield per surface. Fresh weight of tomatoes per hectare was calculated during 60 and 120 days after transplanting.

Table 6

Evolution of physicochemical properties of *Ganocetas* during the cultivation stages. Mean values \pm SD ($n=3$) at sowing, transplanting and at the end of the cropping cycle (samples were obtained from the buried containers). Different letters means Tukey differences within SFG or RG values ($\alpha=0.05$).

Physicochemical properties ^a	SFG			RG		
	Initial	Transplanting	Final	Initial	Transplanting	Final
AD (g/cm ³)	.20 \pm .03 ^a	.2 \pm .02 ^a	.18 \pm .04 ^a	.14 \pm .02 ^b	.15 \pm .01 ^b	.20 \pm .05 ^a
EP (%)	34 \pm 8 ^b	58 \pm 19 ^a	66 \pm 17 ^a	30 \pm 4 ^c	45 \pm 13 ^b	76 \pm 11 ^a
WP (%)	29 \pm 6 ^b	45 \pm 14 ^a	29 \pm 20 ^b	21 \pm 3 ^c	31 \pm 8 ^b	42 \pm 11 ^a
AP (%)	6 \pm 4 ^b	13 \pm 10 ^b	36 \pm 9 ^a	10 \pm 5 ^b	14 \pm 8 ^b	34 \pm 9 ^a
pH saturated paste	4.1 \pm .1 ^c	6.2 \pm .6 ^b	7.2 \pm .3 ^a	4.7 \pm .2 ^c	6.3 \pm .4 ^b	7.1 \pm .7 ^a
pH 1:6 (v/v)	4.3 \pm .1 ^c	6.4 \pm .7 ^b	7.6 \pm .4 ^a	5.0 \pm .2 ^c	6.5 \pm .6 ^b	7.4 \pm .7 ^a
EC saturated paste	10.3 \pm 2.9 ^a	8.4 \pm 2.2 ^a	4.4 \pm 1.4 ^b	10.1 \pm 1.5 ^a	4.9 \pm 0.9 ^b	1.6 \pm 0.6 ^c
EC 1:6 (v/v)	1.6 \pm .2 ^a	1.4 \pm .2 ^{ab}	1.2 \pm .5 ^b	1.2 \pm .2 ^a	0.8 \pm .2 ^b	0.3 \pm .1 ^c
Ashes	30.1 \pm 2.9 ^b	28.7 \pm 2.6 ^b	40.8 \pm 6.9 ^a	30.8 \pm 3.9 ^b	28.7 \pm 2.6 ^b	62.9 \pm 10.8 ^a
C (%)	34.5 \pm 1.4	–	–	34.1 \pm 1.4	–	–
Ca (%)	3.03 \pm .10	–	–	1.80 \pm .07	–	–
K (%)	0.09 \pm .01	–	–	1.20 \pm .06	–	–
Mg (%)	.28 \pm .01	–	–	.18 \pm .01	–	–
N (%)	1.34 \pm .06	–	–	0.91 \pm .03	–	–
Na (%)	.07 \pm .01	–	–	.04 \pm .01	–	–
P (%)	.08 \pm .01	–	–	.16 \pm .01	–	–
S (%)	.88 \pm .02	–	–	.22 \pm .01	–	–
As (mg/Kg)	1.1 \pm .2	–	–	1.1 \pm .1	–	–
Cu (mg/Kg)	6.1 \pm .3	–	–	0.7 \pm .3	–	–
Fe (mg/Kg)	2300 \pm 170	–	–	2200 \pm 165	–	–
Zn (mg/Kg)	19 \pm .1	–	–	26 \pm .1	–	–
C/N	26	–	–	37	–	–
C/P	431	–	–	213	–	–

^a AD: apparent density, EP: effective pore space, WP: water porosity, AP: air porosity.

Electrical conductivity, pH and chemical content of the main mineral components of *Ganocetas* are shown in Table 6. In both SFG and RG, the initial pH was low and it increased until transplanting time was reached. With respect to EC, it was observed that both saturated paste and dilution methods were consistent. At initial time SFG and RG presented similar EC values, but when the seedlings continued to grow, the EC values were higher in SFG.

Ash content revealed a high initial mineral content which continued to increase as the organic matter was further biodegraded in the soil. Mineral analyses revealed lower C/N ratios in SFG (26) and RG (37) when compared with the values of raw constituents prior to biodegradation by *G. lucidum*: sunflower seed hulls (69), rice straw (57) and rice husks (47) (data not shown).

With regards to micronutrients, SFG were rich in copper content and both SFG and RG in ferric and zinc contents. Arsenic content was analyzed for the presence of toxic concentrations, but it was found to be under the no-toxic levels of 1.1 mg/Kg in both substrates.

Some interesting observations were that shrinkage of the substrate contained in *Ganocetas* was lower than that observed in the

plastic containers (5 vs. 30%, results not shown) and that airborne fungus *Trichoderma* spp. grew on the walls of *Ganocetas*.

4. Discussion

4.1. Effect of SFG on germination and seedling quality of vegetables

Germination can be affected by ligninolytic products arising from microorganism degradation of organic substrates (Muratalla-Lúa et al., 2006). Bio-products from SFG matrix did not interfere with seed germination. Lower values were observed in the mean germination time of *Pisum sativum*, which could be attributed to the higher demand for water that these seeds require for imbibition as they are larger in size.

When the root systems of cultivated species in SFG containers were observed in detail certain characteristics were found to be related with a more advanced stage of maturation and thus they had greater capacity to explore the surrounding soil (López-Bucio

et al., 2003). In addition, the root system in the control plants grew on the circumference in contact with the wall of the plastic container with an undesirable morphology, a phenomenon frequently observed in this type of container, which impacts on both growth and implantation after transplantation (Evans and Hensley, 2004).

Thus it was predicted that SFG containers would be degraded after transplantation. Ideal organic containers should keep their shape until transplanting and then lose them readily to let the roots grow into new soil (Schettini et al., 2013). Peat is the most common material for organic containers but it may persist in the ground after one production cycle (Evans and Hensley, 2004). In contrast, *Ganocetas* containers have an advantage: the mycelium cell walls (composed of polysaccharides, such as chitin and chitosan) of their matrix are initially hydrophobic but then they gradually become hygroscopic without losing the container structure. In fact, they endured irrigation for up to 4 months, with *S. melongena*.

The substrates present in plastic containers showed less capacity to drain the daily-irrigation solution on account of the irrigation protocol for both types of containers. Indeed, a cooling effect reduced the temperature on the warmest day in the organic-type container. However, a higher evaporation rate from the SFG would require greater control of irrigation.

4.2. Effects of SFG and RG containers on tomato performance from seedling to harvest

Seedlings from SFG were comparable to the controls as shown by the consistency of results found in the seedling evaluation at transplantation and the establishment period.

Data revealed that in terms of productivity, SFG were comparable to the control in the plastic containers under optimum environmental conditions. However, the better morphology of the plants cultivated in SFG showed that they would be able to tolerate stress factors, e.g., drought or wind, better during the establishment phase.

Plants derived from seedlings grown in RG showed symptoms of drought stress, such as reduced plant size and leaf area, and also lower water content in the fruits. These symptoms could be explained by the higher salt content in the RG container or by a possible allelopathic-related phenomenon which is discussed below.

4.3. Physicochemical properties of containers

Modification in the **AD** of RG containers towards the end of the cropping cycle was attributed to lower cohesive-strength between the particles, as a result of mycelium growth and the matrix of vegetative fibers of rice straw and rice husks as compared to this property in sunflower seed hulls. It could also be possible that this increment in the **AD** values was a result of a better particle biodegradation in RG, which occurred with an increase in **EP** during the experiment, when the particle size diminishes. These **EP** values were also found near to decomposed peat (Ansorena Miner, 1994). In addition, the use of this kind of organic container would be helpful for the introduction of organic matter into horticultural soils.

Shrinkage is a physical phenomenon which, in this particular case, means a substrate-volume reduction, caused by multiple irrigation events. In this regard, the values obtained with organic containers were close to that recommended for ideal substrates (Ansorena Miner, 1994). This positive effect was attributed to the roughness inside the walls of the *Ganocetas* which favored the adherence of substrate mass onto the inner walls, therefore reducing substrate movements during irrigation.

It is worth mentioning that roots of several species grew into the *Ganocetas* matrix in both experiments, without any disintegration of the container structure until the time of transplanting.

Related studies from labs using organic containers also reported that roots grew through them and radiated widely throughout the soil (Yamauchi et al., 2006). On the other hand, although the RG containers presented similar physical properties, they showed inhibition of seedling growth and lower vigor which could be ascribed to chemical inhibition. In addition, this undesirable effect was also observed when analyzing plant growth and tomato production yields. Such inhibition with RG cannot be ascribed to either the physical or chemical properties studied here and could eventually be explained by other unexplored causes, e.g., the presence of rice allelopathic substances (Le Thi et al., 2014).

Moreover, higher EC values in SFG with cultivation of tomato were not as harmful as revealed by a similar performance of the seedlings grown in plastic containers.

A lower C/N ratio in SFG and RG, with regards to the initial values for the main components (sunflower seed hulls, rice straw and rice husks), indicates an advanced degree of *Ganocetas* biodegradation produced during solid-state fermentation by *G. lucidum*. However, if the highest value of C/N < 30 is considered as indicative of an acceptable stability for the mineralization process in organic substrates (Burés, 1997), then only SFG fulfills this condition.

In the case of the mineral content of *Ganocetas*, it was found that these structures were adequate as good amendment for holding the tomato plants during the first stages of vegetative growth (Argerich and Troilo, 2011). Actually, it is expected that these nutrients would be released at a low rate during the final biodegradation of the material. Indeed, the use of residual substrates from mushroom cultivation is a known practice for improving horticultural-soil fertility (Medina et al., 2012). The estimated rate of amendment was of 30 and 50 t/ha for RG and SFG, respectively, a conservative value when compared with the higher ones of 77–85 t/ha used by those authors.

With regards to some biological issues, the fortuitous growth of *Trichoderma* species were found to benefit horticultural practices, as several *Trichoderma* species show some positive growth-regulating activities and also act as natural biological controllers (Harman, 2006). In addition, it is known that the residual substrate from mushroom cultivation greatly favors the growth of such ambient microorganisms (Trillas et al., 2006; Colavolpe et al., 2014). On the other hand, it is also mentioned that several secondary metabolites of *G. lucidum* possess antibacterial, nematocidal, antiviral and antifungal activity (Patterson, 2006), hence the use of *Ganocetas* would provide a additional source of beneficial metabolites for controlling phytopathogenic microorganisms.

5. Conclusion

Sunflower seed hull-based *Ganocetas* did not affect either the germination of 15 vegetable species or tomato production whereas it improved the growth and vigor of six species including two varieties of tomato. Rice agro-based residue *Ganocetas* evaluated with tomato did not improve growth and vigor and they also affected growth and productivity.

The biomatrix obtained resulted in good properties for sustaining both seedling and plant growth following transplantation.

These conclusions lead to the proposal of additional studies in order to evaluate the use of container-shaped templates made by solid-state fermentation of sunflower seed hulls using *G. lucidum* mycelium as the bioadherent agent.

Acknowledgments

This research was supported by grants from the CONICET and Universidad Nacional del Sur, Argentina. We thank Ramón López Castro for his critical review of the manuscript.

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