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RESEARCH ARTICLE

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Effect of different substrates for organic agriculture in seedling development of traditional species of Solanaceae

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Abstract

Sowing of seedlings is one of the most critical processes on the establishment of a crop, since the future development of the plant depends largely on its health when is planted on the field. Moreover, organic agriculture has to deal with the low application of fertilizers and pesticides, which hinder the growth of seedlings. In this work, we studied the big influence of different mixtures of substrates suitable for organic agriculture based on peat, coconut husk and vermicompost in traditional varieties of tomato, pepper and eggplant. Our results indicate that the use of coconut husk based substrates in organic agriculture can reduce the growth of seedlings between 20 and 30% compared with peat-based substrates. Moreover, the plants growth in this substrate showed lower levels of chlorophyll and lower weight, but the results are strongly dependent on the species tested. Comparison between traditional plants demonstrates that traditional varieties are strongly influenced by the substrate, whereas the growth of a commercial variety of tomato barely differs when different substrates are used. The election of the substrate in organic agriculture is critical to the correct development of the plant, especially when traditional plant varieties are used.

Additional key words: vermicompost; coconut husk; tomato; eggplant; pepper.

Abbreviations used: CH (coconut husk); EC (European Community); ROS (reactive oxygen species); S1, S2, S3 (substrate mix 1, 2, 3); VC (vermicompost).

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Introduction

Organic farming is based in the minimum use of non-renewable natural resources and the absence of chemical fertilizers and pesticides. These agricultural methods have been shown to be more environmentally friendly than intensive agriculture, which is dependent on the routine use of herbicides, pesticides and inorganic nutrient applications in the production of crops and animals. The model of organic agriculture results in less leaching of nutrients and higher carbon storage (Drinkwater *et al.,* 1995), less erosion (Reganold *et al.,* 2001), and lower levels of pesticides in water systems (Kreuger *et al*., 1999).

Organic farming is only a small portion of the global agriculture, representing a minimum percentage of a country's sector. However, organic agriculture currently has promising growth levels worldwide (Florez, 2009). The organic sector in the EU has increased over the past 10 years, mainly due to policy support and market demand for organic products (Sahm *et al*., 2013), turning it in one of the fastest growing sectors of agricultural production.

Seedling production is one of the most important steps in the horticultural production system because it determines the final yield of plants (Carmello & Minami, 1995). The substrate is constituted by a porous material, in which the root system of the plant develops, and takes water and nutrients it needs for development and oxygen necessary for the proper functioning of the root system. The term substrate is applied to any material that allows anchoring the root system and therefore play a supporting role for the plant (Abad *et al.*, 2004, 2005).

The horticultural substrate can be conceptualized as the environment in which the roots of plants grown in nurseries are developed (Carneiro, 1995). This should ensure a solid phase through its maintenance and stability of the root system of the plant; through the liquid phase the supply of water and nutrients; and through the gas phase, the oxygen and carbon dioxide transport between the roots and the outside air (Lemaire, 1995). This medium should also be free of minerals or any other substance in phytotoxic concentrations and plant pathogens, pests and unwanted plants (Minami, 1995). Use of an appropriate substrate is essential to enhance the quality of horticultural products, especially in organic production. It directly influences the growth, development and maintenance of an extensive functional root system. A good substrate should supply secure anchorage, suffice as a source for nutrients and water, permit oxygen dispersion to the roots and permit gaseous exchange between the inside and outside of the root (Bunt, 1988; Urrestarazu *et al.*, 2008).

Therefore, the choice of substrate is one of the most important decisions for producers of crop seedlings. According to Minami (1995), 60% of the success of a culture lay a hotbed of good quality, and the quality of the final fruit can be altered by the substrate mixture used to raise seedlings even when seedlings are transplanted into equally fertilized field soil (Arenas *et al.*, 2002). This is evident in the large number of existing studies on the influence of the substrate on germination and seedling development (Zaller, 2007; Belda *et al.,* 2013; Osman & Rady, 2014). Furthermore, when dealing with a model of organic farming is particularly important to choose a good substrate, as the limitation in the application of non-chemical fertilizers makes the only source of resources available to the plant which the substrate can offer.

Peat is the most widely studied and utilized substrate in the world, however, peatlands are under the safeguard of the Directive 92/43/EEC (EC, 1992) and peat is considered a non-renewable resource and thus, its use in the organic agriculture must be progressively reduced. Vermicompost (VC) and coconut husk (CH) are environmentally friendly materials that are often part of bedding substrates. Vermicompost has many characteristics that make it suitable for mixture in substrates including high porosity and good aeration (Edwards & Neuhauser, 1988). It also contains essential available nutrients such as nitrates, phosphates, exchangeable calcium and soluble potassium (Orozco *et al.*, 1996). Coconut husk is a promising substrate being cheap, mixable, and with high cation exchange. It is mainly used in soilless culture techniques. CH is a lightweight material and its total porosity is above 94% by volume. It also exhibits high air content, from 24% to 89% by volume (Abad *et al.*, 2005). CH has also been shown to be suitable for mixture in substrates in numerous production trials (Evans *et al.*, 1996; Inden & Torres, 2004). The use of traditional plant varieties is particularly relevant because modern agriculture has resulted in a loss of diversity in the agricultural landscape (Stoate *et al.*, 2001), and it has been suggested that large-scale conversion to organic farming could partly ameliorate this loss. It is estimated that more than 95% of organic agriculture is based on crop varieties that are bred for the conventional high-input sector with selection in conventional breeding programs. Recent studies have shown that such varieties lack important traits required under organic and low-input production conditions (Murphy *et al.*, 2007). Moreover, the use of traditional varieties is often more difficult because these varieties use to be less productive and more disease susceptible than industrial varieties (Cebolla-Cornejo *et al.,* 2012).

The establishment of seedlings is a key step that may affect subsequent plant growth. This part has a huge importance in the organic farming where the application of fertilizers is limited. For all these reasons, the aim of this work was to evaluate the influence of organic substrates in the emergence and growth of traditional varieties of Solanaceae.

Material and methods

Substrate mixtures

Three substrate were used: (i) peat (Gramoflor GmbH & Co. Vechta, Germany; average nutrient concentrations pH: 5.8; organic matter: 92-98%; N: 100 mg/L; P_2O_5: 140 mg/L K_2O: 140 mg/L; electrical conductivity: <1.2 mS/cm); (ii) vermicompost (KKCuc. Sueca, Spain; pH: 5.5; N: 3000 mg/L; P_2O_5: 2600 mg/L; K_2O:3000 mg/L; Mg: 710 mg/L; EC: 1.5 mS/cm); (iii) coconut husk (Cocogreen. Manchester, UK; pH: 6.3; N: 30 mg/L; P_2O_5>3 mg/L; K_2O: 4 mg/L; Mg>3 mg/L; EC: <0.6mS/cm); and (iv) perlite (P.V.P industries Inc., OH, USA).

Mixtures were designed according to Arenas *et al.* (2002) using peat, coconut husk as substitute of peat and addition of 30% of vermicompost (Edwards *et al.,* 2010) Substrate 1 (S1) was composed by 100% peat because is one of the most used substrates and serves as control; Substrate 2 (S2) was composed by 60% coconut husk + 30% vermicompost + 10% perlite; and Substrate 3 (S3) by 60% peat + 30% vermicompost + 10% perlite.

Plant material

Plant material consisted of three traditional varieties from east of Spain: tomato (*Solanum lycopersicum*) 'Valencia', pepper (*Capsicum annum*) 'Cuatro cantos' and eggplant (*Solanum melongena*) 'Rallada'. In addition, a commercial variety of tomato "Rio grande" was used. All varieties were obtained from a local farmer in Castellón (Spain).

Seeds were sowed in plug trays with 128 cells of 34.6 cm^3 (3.5 × 3.5 × 6.2 cm). The plug trays were placed in a greenhouse with controlled temperature (18-24ºC). Each tray was filled with a particular substrate mixture and 32 seeds of each variety were sown into cell plugs arranged in rows. Plants in plug cells were watered three times per week using a drip irrigation system. The duration of the experiment was adjusted to the recommended time that the plants stay in the greenhouse, before transplanted to the field (Leskovar *et al.,* 1991). No additional fertilizer was applied to seedlings in plug trays. The experiment was conducted in spring between February and May of 2014.

Water availability of the different substrates

Plug trays with 128 alveoli of 34.6 cm^3 were filled with 4.5 L of freshly mixed substrates, then were watered until saturation and after draining the excess of water were weighted to obtain the wet mass of wet substrate. The trays were placed in the greenhouse with the same conditions that the plants and weighted every day for one week. To obtain the dry weight of the substrates, the same amount of each mixture was dried in oven at 105ºC during 2 days. The water content was determined each day by the difference between the mass of wet substrate and the mass of the dried substrate, expressed in grams of water.

Growth and biomass measurements

Seeds were considered emerged when the cotyledons came through the surface of the potting substrate. Seedling elongation was measured in all the plants every 3 days from soil surface until the apical shoot to calculate the differences in the germination. Total growth was measured at the last day of the experiment following the same criteria. Five seedlings of each species were sampled from each substrate mix for determination of fresh and dry weights of shoots and roots at the end of the experiment. Harvested plant biomass was dried at 70ºC for at least 48 h and weighed.

Chlorophyll content

The chlorophyll level of the leaves was measured using a chlorophyll meter (SPAD; Minolta, Tokyo, Japan). Three measurements were taken per leaf on each side of the central vein with 10 plants per treatment. The three SPAD readings taken on one leaf for each of the 10 plants per treatment were averaged to represent one observation. The results were obtained as SPAD values (S, dimensionless).

Total protein content and peroxidase activity

Fresh leaves (100 mg) were homogenized in an ice-cooled mortar with 1 mL of 100 mM potassium phosphate buffer (pH 6.0). Homogenized was centrifuged (10000 g, 5 min at 4ºC). Supernatant was used for determinations. Protein content was determined using bovine seroalbumine as a standard, according to the method of Bradford (1976). The peroxidase activity was determined following the oxidation of guaiacol by the method of Hemeda & Klein (1990). In brief, the assay mixture contained 1 mL of 50 mM phosphate buffer (pH 6.0), 10 µL of 33% H_2O_2 and 5 µL of the enzyme extract. After addition of 25 µL guaiacol, the rate of increase in absorbance as guaiacol oxidized, measured at 470 nm for 3 min. The enzyme activity was expressed as activity per gram of protein.

Statistical analyses

Statistical analyzes were performed using the software Statgraphics Centurion XVI (Statpoint Technologies, Warrenton, VA, USA). Data were submitted to an ANOVA analysis for population groups that follow a normal distribution and the means were separated using Fisher's least significant difference (LSD) at 95%. Seed germination was analyzed with "Germinator curve fitting". It was considered that there are significant differences when p values are <0.05.

Results

Seedling emergence and growth

Seedling emergence was used as the determining factor regarding seed germination with different substrates in plug trays. Between the substrates tested, no statistical differences were found in the promotion of seed germination in any substrate for any variety

(Table S1 [online resource]). However, seedling elongation of the four varieties was significantly affected by the substrate used (Fig. 1A). For three varieties (tomato "Valencia", tomato "Rio grande" and pepper) commercial peat substrate amended with vermicompost showed highest elongation, only the eggplant showed highest elongation at 100% peat (Fig. 1A). The three traditional varieties showed lowest elongation with coconut husk substrate. Tomato "Valencia" and pepper growth in substrate S2 showed a reduction of 31 and 37% compared with results obtained in substrate S3. Eggplant growth in substrate S2 showed a reduction of 22% compared with results obtained in substrate S1. However, the substrate S1 and S2 did not show differences for tomato "Rio grande" elongation resulting in a reduction of 10% compared with results in substrate S3. Root length only showed statistical differences in both tomato varieties, where the substrate based in coconut husk showed roots 30 and 15% longer for tomato "Valencia" and tomato "Rio grande" compared with the other two substrates (Table 1).

Biomass allocation

Shoot mass showed significant differences between varieties depending on the substrate. For all the varieties tested, seedlings showed significant lower fresh weight in S2. This reduction is more pronounced in the traditional varieties, where the reduction achieved between 65 and 75% lower fresh weight, whereas the commercial variety of tomato only achieve a reduction of 50% (Table 1). Moreover, when we compared the dry weight, all the traditional varieties showed similar results, achieving the higher biomass with S3 and a reduction of near 60% when S2 was used. Fresh and dry root weight showed similar results than obtained in the shoot weight, no significant differences were found between S1 and S3, but significant lower results were obtained with S2 (Table 1). The ratio weight/length reveals that all the varieties tested showed roots with less weight per centimeter when S2 was used, achieving values between 60 and 70% lower than other substrates.

Plants sowed in S3 had a protein content that was significantly higher than in the rest of the substrates, with the exception of Tomato "Valencia" that did not show differences in protein content between the different substrates (Fig. 1B).

Availability of water in the substrate

Capacity of three substrates to hold water was significant different. In Fig. 2 we can observe that S1 and S3 are able to maintain 491.85 and 471.52 g of water/L of substrate. On the other hand, S2 only was able to retain 425.33 g of water/L of substrate. After three days in the greenhouse the three substrates had lost between 81% (S1) and 85% (S2) of its initial content of water. At this point, S2 only contained 65.4 g of water/L of substrate, whereas S1 and S3 contained 91.8 and 70.8 g of water/L of substrate, respectively.

Table 1. Effect of different potting substrates on growth of tomato 'Valencia' (1), tomato 'Rio grande' (2), pepper and eggplant. Values represent the average of three experiments.

	Shoot fresh weight (g)	Root fresh weight (g)	Fresh shoot/ root ratio	Shoot dry weight (mg)	Root dry weight (mg)	Dry shoot/ root ratio	Root lenght (cm)	Root dry weight/length
Tomato 1								
S1	1.15b	0.85b	1.35a	178.11b	84.78b	2.10a	11.44b	7.41b
S2	0.53c	0.44c	1.20b	62.89c	40.00c	1.57b	15.94a	2.51c
S3	1.49a	1.05a	1.41a	243.33a	102.78a	2.37a	11.89b	8.64a
Tomato 2								
S1	1.81b	1.40a	1.29b	338.78b	151.33a	2.24b	11.21b	13.50a
S2	1.10c	0.71b	1.55a	162.67c	68.44b	2.38b	12.94a	5.29b
S3	2.31a	1.29a	1.79a	419.00a	139.67a	3.00a	11.01b	12.68a
Pepper								
S1	1.14b	0.63a	1.79a	105.11b	58.67b	1.79b	11.14a	5.26b
S2	0.37c	0.24b	1.54b	37.11c	28.33c	1.31c	10.39a	2.73c
S3	1.39a	0.71a	1.95a	152.00a	74.11a	2.05a	10.56a	7.02a
Eggplant								
S1	1.50a	0.64a	2.37a	227.22a	65.78a	3.45a	9.24a	7.12b
S2	0.52b	0.37b	1.41b	76.33b	37.11b	2.06c	9.74a	3.81c
S3	1.40a	0.66a	2.12a	209.00a	78.67a	2.66b	8.54b	9.21a

S1: 100% peat, S2: 60% coconut husk + 30% vermicompost + 10% perlite, S3: 60% peat + 30% vermicompost + 10% perlite. Numbers followed by the same letter are not significantly different at $p \leq 0.05$ according to the LSD test.

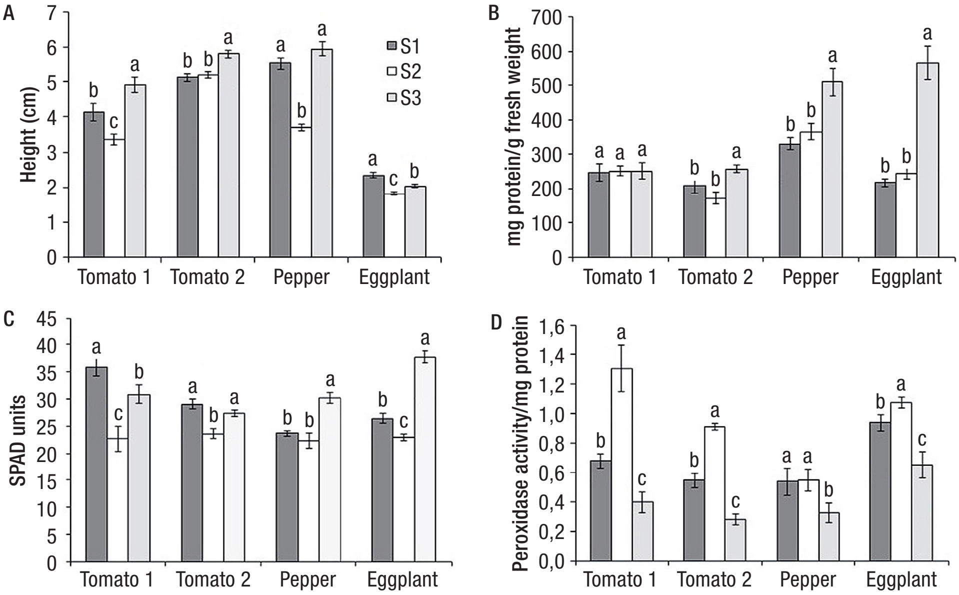

Figure 1. Physiological parameters of different plant varieties (Tomato 'Valencia' (1), Tomato 'Rio grande' (2), pepper and eggplant) grown in plug cells with different substrate mixtures. S1: 100% peat, S2: 60% coconut husk + 30% vermicompost + 10% perlite, and S3: 60% peat + 30% vermicompost + 10% perlite. A: Seedling elongation; B: Leaf protein content; C: Chlorophyll content and D: Peroxidase activity. Bars represent standard error of the mean values, and different letters represent significant differences ($p \leq 0.05$, LSD test) between substrates for the same variety.

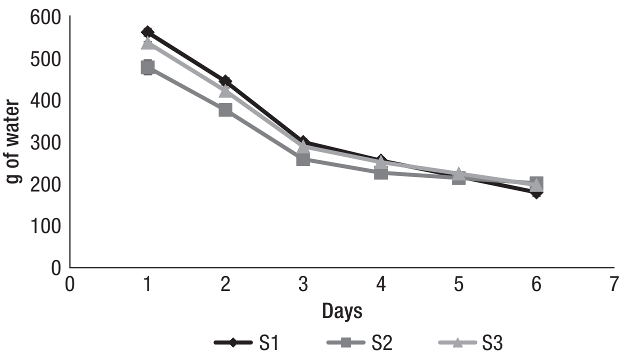

Figure 2. Water availability in the substrates. S1: 100% peat, S2: 60% coconut husk + 30% vermicompost + 10% perlite, and S3: 60% peat + 30% vermicompost + 10% perlite. Bars represent standard error of the mean values.

Chlorophyll and peroxidase

Levels of chlorophylls observed in both varieties of tomato were higher in S1. Again, the tomato 'Valencia' showed more sensitivity to the substrate, showing a chlorophyll content 36% lower when S2 was used, whereas the tomato 'Rio grande' showed a reduction of 18%. Both pepper and eggplant showed higher chlorophyll content with the substrate composed by peat + vermicompost (S3). Chlorophyll content in pepper did not show significant differences when S1 and S2 was used, resulting in values 20% lower than observed in S3. Eggplant showed the highest differences with results 40% lower when S2 was used, compared with the results obtained with S3 (Fig. 1C).

The peroxidase activity was consistent in both tomato varieties. The use of substrate based in coconut husk (S2) showed the highest results, whereas the use of S1 and S3 reduced the peroxidase activity in tomato a 30 and 70%, respectively.

Moreover, in pepper and eggplant S3 showed a peroxidase activity 40% lower than observed in S2. However, the pepper did not show statistical differences between S1 and S2, and eggplant sowed in S1 showed levels only 12% lower than in S2 (Fig. 1D).

Discussion

The results obtained in this work showed that the use of different substrates makes a big difference in the growth and development of plants both between species and between varieties of the same species, evidencing that the same substrate gives different results depending on the variety that is planted on it. Moreover, the use of traditional varieties of plants, that usually are less vigorous than commercial varieties, increases the need for a substrate that provides all essential nutrients for seedling development.

In terms of growth, the substrate composed of coconut husk and vermicompost showed lower growth and less weight (fresh and dry) in all tested species, showing plants that can be 30% shorter and with 50% less weight. Moreover, the tomato 'Valencia' and tomato 'Rio grande' showed longer roots when grown in this substrate. The size of the root is an indicator of development and the state of the plant. In this way, our results agree with studies by Nahar & Gretzmacher (2011) showing that moderate water stress promotes excessive growth of the roots of several tomato species. Comparing data with those obtained in fresh and dry root weight observed that tomato roots planted in S2, are larger but had less weight, giving less vigorous roots. This may suggest that this substrate causes a slight stress forcing the plant root growth.

The test of water availability of the substrates showed that, after saturation, S2 retains between 50 and 65 g of water/L of substrate less than the other two substrates tested. This result indicates that the amount of water available for the plant is between 10 and 15% lower than in the other substrates during all the experiment, which can be directly correlated with the lower growth and lower biomass achieved by the plants in this substrate. On the other hand, despite S1 retains more water after each irrigation, plants grown in S3 achieved higher biomass and height. This result is probably related with the higher amount of nutrients provided by the addition of vermicompost to the mixture. However, the same amount of vermicompost added to S2 (based in coconut husk) seems not to be enough to promote a correct development of the plant. In this way, previous studies suggest that the coconut husk may reduce plant growth when concentrations higher than 50% are used because of a microbial N immobilization (Arenas *et al.,* 2002) that, coupled with the lower water availability, can dramatically affect the plant growth.

Chlorophyll pigments are essential for the conversion of light energy into chemical energy. Therefore, the chlorophyll content can directly determine the production potential and photosynthetic primary (Filella *et al.,* 1995). We found that both substrates based in peat showed higher amount of chlorophyll per leaf, while the substrate containing coconut fiber shows the lower chlorophyll levels. The amount of these pigments gives an indirect estimate of the nutrient status, since most of the nitrogen is incorporated to the leaf chlorophyll (Filella *et al.,* 1995; Moran *et al.,* 2000). In addition, the chlorophyll content of leaves is closely related to plant stress and senescence (Peñuelas & Filella, 1998; Merzlyak *et al.,* 1999).

The stress in plants may produce a rapid accumulation of reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$) and singlet oxygen ($^1O_2$), in chloroplasts and mitochondria. The accumulation of these radicals can have very serious effects on plants such as degradation of photosynthetic pigments, lipid peroxidation, alteration in the selective permeability of cell membranes, protein denaturation and DNA mutations. To repair and mitigate the damage caused by ROS, plants use protection mechanisms such as the synthesis of peroxidases that degrade these compounds, which can serve as an indicator of the level of stress to which plants are subjected. Our data show a significant increase of peroxidase activity in tomato 'Valencia' and tomato 'Rio grande' planted in coconut husk substrate. However, in pepper plants no significant differences between planted in peat substrate and coconut fiber were observed. In all species studied the lowest level of peroxidase activity was observed in the substrate based in peat and vermicompost, which could indicate that this substrate causes less stress to plants. These results agree with that obtained in the root size. In both cases, the results suggest that tomato varieties 'Valencia' and 'Rio grande' may suffer stress when cultivated on substrate containing coconut. All the variables studied in this work suggest that substrates based in coconut husk as a main component may affect the development of the plant.

In conclusion, in this report we highlight that the substrate is a key component in the seedling establishment, especially in organic agriculture, where the addition of fertilizers can be limited. The use of traditional varieties, that usually are less vigorous than commercial varieties or new hybrids, adds a new challenge to the choice of a suitable substrate. Our results demonstrate that the election of an inadequate substrate can reduce the growth and weight of the seedlings. For this reason, is important to choose a substrate that covers the needs of our crop in order to improve the vigorousness of our plants.

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