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RESPONSE OF *LACTUCA SATIVA* L. 'BABÁ DE VERÃO' EXPOSED TO DIFFERENT SUBSTRATES FOR LABORATORY BIOASSAYS

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Abstract

Plant bioassays are used to evaluate the biological effect of biotic and abiotic agents and its substrate must be carefully chosen. The objective of this study was to elucidate the effects of four different substrates (filter paper, agar, vermiculite and organic substrate) on the germination and initial growth of *Lactuca sativa* L. 'Babá de verão'; 30 seeds were used for each treatment, with 3 replicates. After 7 days, the following parameters were evaluated: germination (%G) and emergence (%E) percentages, Germination (GSI) and Emergence (ESI) Speed Index, number of seedlings (NS), fresh biomass (FB), root length (RL) and shoot length (SL). Filter paper and agar were equally satisfactory for %G, GSI and NS; the same was observed for vermiculite and the organic substrate concerning %E, ESI and NS. The parameters FB, RL and SL were higher in the seedlings submitted to vermiculite and lower in the seedlings submitted to filter paper. It is concluded that vermiculite yielded the greatest seedling growth among the tested substrates; however, it does not allow the evaluation of germinative parameters, only emergence. In cases where it is essential to analyze germination parameters and evaluate seedling growth, agar is the most suitable substrate.

Key words: Filter paper; Agar; Vermiculite; Organic substrate; Initial growth.

Introduction

Plant bioassays have been widely used in order to evaluate the biological effect of environmental samples, bioactive principles of plants and/or synthetic substances (Leme & Marin-Morales, 2009; Iqbal, 2016; Priac *et al.*, 2017; Santos *et al.*, 2017; Silveira *et al.*, 2017). Several species can be used as target plants of biological activity, and Lettuce and Onion are the most usual since they have particularities that facilitate their use (Simões *et al.*, 2013; Palsikowski *et al.*, 2017). Lettuce (*Lactuca sativa* L.) has some advantages in relation to other bioassays, such as high sensitivity, low cost, germination in approximately 24 hours, growth in a wide pH range and low sensitivity to osmotic potentials (Smiderle *et al.*, 2001; Simões *et al.*, 2013; Moraes *et al.*, 2015).

In addition to the target species selected for use, the substrate for the bioassay must be carefully chosen. Among several types of substrates used for evaluation of biological effect and/or plant development, it is possible to mention filter paper, agar, vermiculite and organic substrate.

Filter paper is the most used substrate in bioassays for evaluation of biological effect (Buss & Masek, 2014; Naeem *et al.*, 2015; Zhang *et al.*, 2015; Grichi *et al.*, 2016; Haq *et al.*, 2016; Souza *et al.*, 2018). Its main advantages are the low cost and speed in the evaluation of the bioassay, considering that the seeds are visible and facilitate the analysis of germinative parameters. However, according to Wang (1993), the use of filter paper has some disadvantages, such as inconveniences to evaluate the biometry of individuals, once the roots can be strongly adhered to the substrate making it difficult to collect whole roots. Besides, promotes horizontal or nonlinear root growth and interferes in the bioavailability of the tested sample, due to adsorption to the paper.

The use of agar is less common than filter paper for bioassays (Cândido *et al.*, 2010; Appiah *et al.*, 2015). Although little used for this purpose, agar has considerable advantages, such as the formation of a gelatinous consistency that allows root fixation and seedling support, moisture conservation in the container used – since it is properly sealed – and the possibility of keeping substances in suspension that are insoluble in water, leaving them bioavailable for seedling absorption. A negative point to its use would be the high risk of contamination (Abreu *et al.*, 2005).

Vermiculite is a clay-mineral consisting of hydrated silicates of aluminum, magnesium and iron that is widely used as a substrate in many research areas and has high cation exchange capacity (Malandrino *et al.*, 2006; Abollino *et al.*, 2008). It is a readily available substrate, with low density, uniformity in its chemical and granulometric composition, high porosity and considerable water retention capacity (Martins *et al.*, 2009), besides being microbiologically inert, which eliminates possible interferences in responses of target plants. A considerable disadvantage of vermiculite in bioassays lies precisely in its high cation exchange capacity, which can lead to the adsorption of compounds in the substrate particles making them unavailable for plant absorption (Quartarone *et al.*, 2012; Tito *et al.*, 2012).

Organic substrates can be understood as the medium in which plants grow that simulates the soil, but they differ from it, since they have been removed from their places of origin and are artificially produced or enriched (Menezes-Júnior *et al.*, 2000). The main advantage of using organic substrates in bioassays is their approximation with soil characteristics, which allows elucidating the possible interactions that occur in natural environments. On the other hand, the same characteristic can be considered negative if it is necessary to eliminate

possible interferences of the soil microbiota on the biological effect of samples. It is important to note that vermiculite and organic substrate do not allow the analysis of germination parameters, only emergence, since it is only possible to verify the germinated seeds that emerge on the surface of the substrate.

Substrates have a direct influence on bioassays, and their physical and chemical characteristics are directly related to the initial growth of target plants, such as aeration, water retention capacity, degree of pathogen infestation, among others (Varela *et al.*, 2005; Silva *et al.*, 2012). Therefore, the objective of this study was to elucidate the effects of different substrates on the germination and initial growth of *Lactuca sativa* L. 'Babá de verão'.

Material and Methods

Lactuca sativa L. 'Babá de verão' (ISLA, lot 40112-S2) seeds were used, since they were the most suitable genotype for this type of experiment, according to Santos *et al.*, (2017). For the bioassay, 30 seeds were used in: Petri dish (diameter: 7 cm) with two sheets of filter paper (Whatman nº 2) and 3 mL distilled water; Petri dish (diameter: 7 cm) with 10 mL agar (6 $g.L^{-1}$); Gerbox containing half its volume (200 mL) of vermiculite with distilled water; and Gerbox containing half of its volume (200 mL) of Mococa® (organic substrate containing peat, pine dust and charcoal, enriched with macro-and micronutrients) with distilled water. For each treatment, 3 replicates were used.

The treatments were maintained in a B.O.D. chamber for 7 days, at 24°C and 12-hour photoperiod. The following pre- and post-emergence parameters were analyzed: Germination percentage (%G) and Emergence percentage (%E), Germination Speed Index (GSI) and Emergence Speed Index (ESI). Germination parameters were used for Petri dishes and emergence parameters were used for Gerbox. %G and %E were obtained 24 hours after the beginning of the experiment and on the 7th day. Seeds that had radicle protrusion were considered germinated, and those considered emerged were visibly exposed to the substrate surface. Number of seedlings (NS), fresh biomass (FB), root length (RL) and shoot length (SL) were also evaluated.

GSI and ESI were determined according to the formulas (Maguire, 1962):

$$\text{GSI or ESI} = \frac{N_1}{1} + \frac{N_2}{2} + \frac{N_3}{3} + \cdots + \frac{N_n}{n}$$

where, N_1, N_2, N_3, N_n, correspond to the number of germinated seeds or seedlings emerged in the first, second, third, to the seventh evaluation, respectively; n is the evaluation number.

FB, NS, RL and SL data were collected on the 7th day after the beginning of the experiment; all the plant material in each bioassay was weighed in an analytical balance, and the germinated plant material that developed root and shoot was considered as seedlings. For the evaluation of RL and SL, 10 visibly larger seedlings of each treatment were selected, as described by Santos *et al.*, (2017). Measurements were performed using a digital caliper (DIGIMESS® 150 mm).

The experimental design was completely randomized (CRD) and data were submitted to analysis of variance (ANOVA); the means were compared using the Scott-Knott test at 5% significance.

Results

Germination (%G) and Emergence (%E) percentages had the same behavior at 24 hours and on the 7th day of experiment, in which the substrates filter paper and agar did not differ (Table 1). The same can be observed for vermiculite and the organic substrate.

For Germination (GSI) and Emergency (ESI) Speed Index, seedlings exposed to the filter paper and agar did not differ among themselves, as well as vermiculite and the organic substrate (Fig. 1a).

Filter paper and agar had a higher number of seedlings (NS) than vermiculite and the organic substrate, but there was no significant statistical difference between filter paper and agar and between vermiculite and the organic substrate (Fig. 1b).

For fresh biomass (FB), there was a significant statistical difference among all the treatments. The highest FB was observed in the treatment with vermiculite. In decreasing order, agar, organic substrate and filter paper (Fig. 1c).

Root length (RL) showed higher values in seedlings developed in vermiculite. Intermediate values can be observed in seedlings grown on agar and organic substrate, which did not differ from each other. The lowest RL value observed belongs to the seedlings developed in filter paper (Fig. 1d). Regarding shoot length (SL), all treatments differed statistically from each other. Seedlings grown on vermiculite, organic substrate, agar and filter paper (Fig. 1d) are in descending order.

Table 1. Germination percentage (%G) and Emergence percentage (%E) means ± standard error obtained for bioassays in Petri dishes and Gerbox, respectively, at 24 hours and on the 7th day of experiment.

Bioassay	%G 24h	%E 24h	%G 7th day	%E 7th day
Filter paper (Petri dish)	75.55 ± 6.18 a		97.77 ± 1.11 a	
Agar (Petri dish)	74.44 ± 4.84 a		95.55 ± 2.93 a	
Vermiculite (Gerbox)		15.55 ± 10.59 a		77.77 ± 8.67 a
Organic substrate (Gerbox)		17.77 ± 5.87 a		73.33 ± 5.09 a

Values followed by the same letter in the column do not differ by the Scott-Knott test at 5% significance

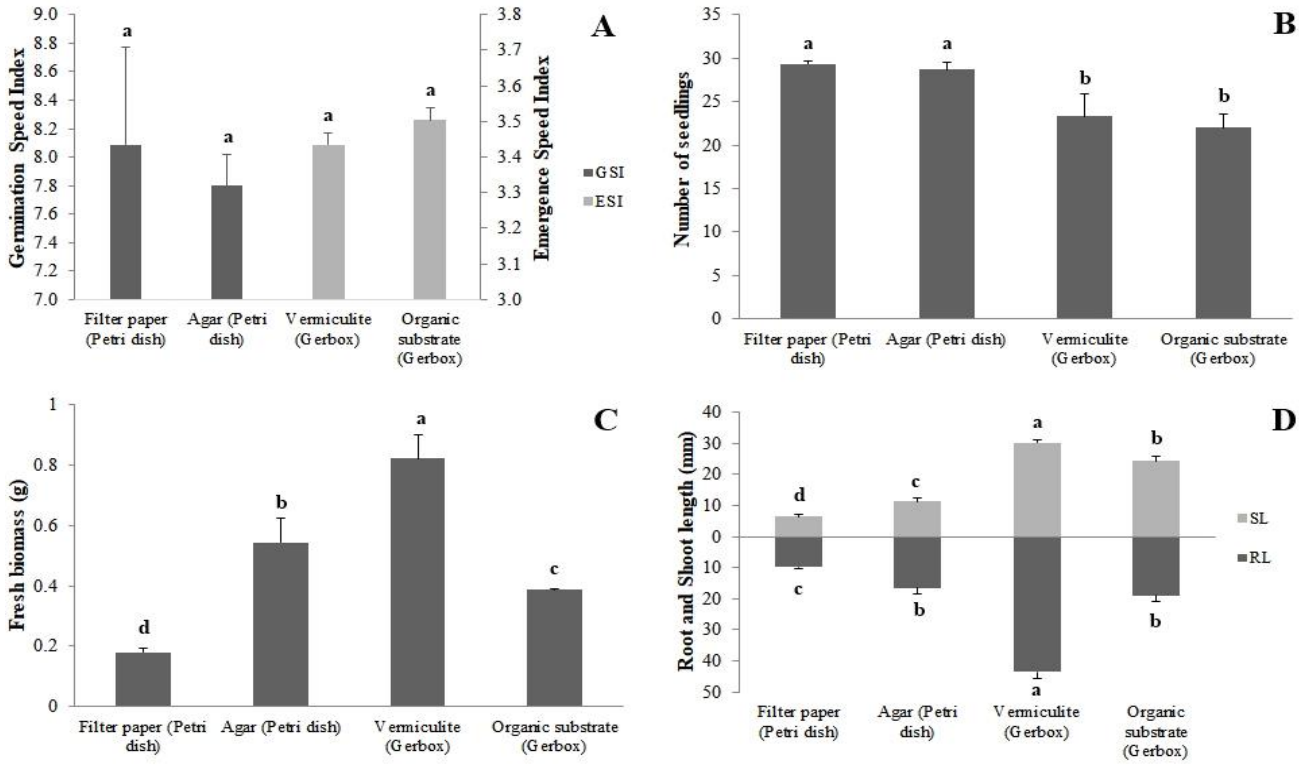

Fig. 1. **A.** Germination Speed Index (GSI) and Emergence Speed Index (ESI) means, obtained for bioassays in Petri dishes and Gerbox, respectively **B.** Number of seedlings (NS) means of *Lactuca sativa* L. grown in different bioassays. **C.** Fresh biomass (FB) means of *Lactuca sativa* L. seedlings grown in different bioassays. **D.** Root length (RL) and shoot length (SL) means of *Lactuca sativa* L. seedlings grown in different bioassays. Columns with the same color followed by the same letter do not differ by the Scott-Knott test at 5% significance. Bar: Standard error.

Discussion

The results obtained for filter paper show that, although it is advantageous for evaluation of germinative aspects – Germination percentage (%G) and Germination Speed Index (GSI) – and number of seedlings (NS), it does not allow great development, since it presented the lowest values for the parameters fresh biomass (FB), root length (RL) and shoot length (SL). The seeds germinate and develop root and shoot, but the seedlings have a reduced size in relation to the other substrates tested. This may be justified by the fact that filter paper does not provide depth for root development nor nutrients. As the root is responsible for the uptake of water and nutrients to plants, the smaller it is, the less contact surface there will be for this organ to perform its function, which negatively influences seedling growth and development (Gusman *et al.*, 2008; Andrade *et al.*, 2009; Moraes *et al.*, 2015; Novaes *et al.*, 2016).

Agar was also satisfactory for the evaluation of %G, GSI and NS, compared to filter paper. However, in contrast to the low development observed in the seedlings that grew on filter paper, agar provided better conditions for seedling growth, allowing a high value for FB, which was only lower than vermiculite. FB is a parameter commonly used in the evaluation of biological effect (Javaid *et al.*, 2006; Nunes *et al.*, 2014; Moraes *et al.*, 2015), since it provides efficiency in water uptake by plants. In addition, the values of RL and SL were higher in seedlings exposed to agar, compared to those exposed to filter paper, once agar provides depth for root growth and consequent seedling development. The increase in these parameters is related to the water availability provided by agar to the plants, which corroborates Coutinho *et al.*, (2001), who correlate the increase in FB to the greater development of RL and SL.

Vermiculite and the organic substrate are also satisfactory for the evaluation of emergence parameters – Emergence percentage (%E) and Emergence Speed Index (ESI) – and NS. However, vermiculite has advantages over all other substrates in terms of initial growth, with the highest values for FB, RL and SL. This is related to its water retention capacity and the consequent water availability to the plants, besides providing support for root development, since it is uniform in the granulometric composition and allows greater seedling growth, due to the homogeneous chemical composition (Ugarte *et al.*, 2005; Martins *et al.*, 2009). As well as the seedlings exposed to agar, the highest values of RL and SL directly influence the highest value observed for FB.

In turn, the organic substrate had a low value regarding to the FB which was only higher than filter paper. In contrast, it presented RL similar to agar and SL intermediate between agar and vermiculite. Silveira *et al.*, (2002) and Costa *et al.*, (2007) correlate the growth of tomato seedlings with the physical and chemical characteristics of the substrates used, and those with higher availability of water and nutrients are responsible for the highest values of the tested parameters. Based on this fact, the results obtained for the organic substrate

show that, although it provides good support to root development and has nutrients that support seedling growth – which justifies the values obtained for RL and SL –, it is not a substrate that has water retention capacity as satisfactory as agar and vermiculite, which explains the lower value of FB, compared to them.

It should be taken into account that the statistical difference observed in NS could have occurred due to the fact that not all the seedlings emerged visually on the surface of vermiculite and the organic substrate during the 7 days of experiment, demonstrating that it is more advantageous to evaluate this parameter on filter paper or on agar for short experiments, which is congruent to Nunes *et al.*, (2014). For experiments that require longer time intervals, NS can be applied using vermiculite or organic substrate, since the seedlings will have more time to emerge, as occurred with the experiment developed by John *et al.*, (2010).

Conclusion

It is concluded that vermiculite yielded the highest seedling growth, which is verified by the higher values of fresh biomass, root length and shoot length. However, it is a substrate that does not allow the evaluation of germinative parameters, only emergence. In cases where the analysis of germination parameters is indispensable, agar is the most suitable substrate, since it allows considerable seedling growth.

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