Organic fertilizer on the *in vitro* cultivation of the *Cattleya labiata* orchid

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ABSTRACT

Propagation of *in vitro* plants through other culture media rather than the traditional ones has been widely researched. However, to increase the effectiveness of these media, the addition of organic products has been presented satisfactory results. Therefore, the objective of this work was to evaluate the influence of the FishFértil® organic fertilizer on the *in vitro* cultivation of the Brazilian orchid *Cattleya labiata*. Treatments included FishFértil® fertilizer concentrations of 0; 1; 2; 3; 4; 5 and 6 mL L^{-1} in a simplified culture medium. At 180 days, shoot height, number of leaves, leaf area, number of roots, root average length, shoot and root dry mass and the shoot: root ratio were evaluated. The experimental design was entirely randomized, with 10 replications, each one containing 10 plantules. Data were submitted to an analysis of variance and regression analysis, at 5% of significance. The FishFértil® organic fertilizer at the concentration of 6 mL L^{-1} promoted better plantule growth of *Cattleya labiata*, subcultivated *in vitro*, in a simplified culture medium.

Key words: Orchidaceae, culture medium, *in vitro* propagation.

INTRODUCTION

The *Cattleya labiata* Lindl., a native Brazilian orchid, flourishes in the summer and produces symmetrical flowers of 15 x 15 cm, which last approximately 10 days (Watanabe et al., 2002). Such species has significant participation in the global orchid culture, not only for being an ornamental plant but for its participation in several genetic crosses, bringing to the sector an infinite number of high quality hybrids extensively cultivated worldwide (Reis 2011).

Multiplication of this species and hybrids occurs, basically, through in vitro propagation. Thus, the preparation of a culture medium adequate to the species is important for the success of this technique. Among the most used culture media for the *in vitro* propagation of orchids are the MS (Murashige and Skoog 1962), Knudson C (Knudson 1946) and Vacin and Went (Vacin and Went 1949).

Many studies suggest the substitution of these culture media by simplified media, using commercial fertilizers and organic products in their formulations. According to Stancato et al. (2008) and Ferreira et al. (2010), the addition of organic composites such as domestic sugar and fruit pulp to chemical formulas, mostly NPK, is necessary to increase the effectiveness of simplified media.

The use of simplified media cultivation has brought satisfactory results to the *Catasetum fimbriatum* and *Cyrtopodium paranaensis* (Rego-Oliveira and Faria 2005), Oncidium nanum and *Cattleya forbesii* (Unemoto et al., 2007), *Cattleya luddigesii* (Moraes et al., 2009) *Laeliocattleya schilleriana* (Cunha et al., 2011), and *Brassavola tuberculata* (Herrmann et al., 2011) orchids. Satisfactory results were also found for hybrids of *Phalaenopsis* (Colombo et al., 2012), *Dendrobium nobile* (Su et al., 2012) and *Vanda tricolor* (Favetta et al., 2014).

FishFértil® is an organic fertilizer (MAPA/SP-80116 10004-2) originated from a seafood natural enzymatic fermentation process. It acts as a natural plant nutritional balance regulator and is a source of nitrogen, phosphorous, C organic and amino acids recommended for food and ornamental crops fertilization (Agrobac 2013). However, its effect on plantules cultivated *in vitro* is still unknown.

Thus, the objective of this work was to evaluate the effect of the organic fertilizer FishFértil® on the i*n vitro* cultivation of the Brazilian orchid *Cattleya labiata*.

MATERIAL AND METHODS

This work was developed at the Londrina State University (UEL) Tissue Culture Laboratory in 2014, with *Cattleya labiata* Lindl orchid plantules originated from seeds germinated *in vitro*, in a MS culture medium, with half of the macronutrients concentration (½ MS). Treatments consisted of supplementing the simplified culture medium with concentrations of 0.0; 1.0; 2.0; 3.0; 4.0; 5.0 and 6.0 mL L^{-1} of the fertilizer FishFértil®.

The basis of the simplified culture medium was formulated with the fertilizer Peters®, NPK (20-20-20) (3 g L^{-1}); banana pulp (*Musa* sp. AAA Cavendish), at the four maturation stadia (60 g L^{-1}); activated vegetable charcoal (1 g L^{-1}); saccharose (30 g L^{-1}) and agar (8 g L^{-1}). Mean pH was adjusted to 6.0 ± 0.2. Glass flasks of, 350 mL received 50 mL of the culture medium and were sterilized in autoclave at 121 °C and 1 pressure atmosphere, for 25 min.

Each flask received 10 plantules with an average height of 2.5 ± 0.3 cm; average number roots: 1.5 ± 0.6; average

number of leaves: 2.6 ± 0.8. Flasks were kept in a growth room at 25 ± 2 °C, 25 micro-mol.m^{-2} s^{-1} of luminosity and photoperiod of 16 h. The experimental design was totally randomized with 10 replications, including 10 plantules. After 180 days, shoot height (SH), number of leaves (NL), leaf area (LA), number of roots (NR), root average length (RAL), shoot dry mass (SDM) and root dry mass (RDM) and the shoot/root dry mass ratio (S/R) were calculated. For the leaf area parameter, leaves were scanned and the area was determined with the help of the SisCob program.

For the SH, NL, NR, RAL parameters, replication was considered as the mean of 10 plantules of each flask and for LA, SDM, RDM, S/R the sum of 10 plantules of each flask. Data were submitted to ANOVA and polynomial regression analysis, testing the linear, quadratic and square root models. Based on the most efficient concentration, the relative increase was calculated for all characteristics by the IR = 100 (x-y)/y formula where x is the mean for the most efficient concentration and y the mean for the treatment at concentration 0.0 mL L^{-1}, being these treatments means submitted to the Scheffé contrast and the Bonferroni test, at 0.01 de significance.

RESULTS AND DISCUSSION

The increase in concentrations of FishFértil® resulted in shoot development linear increases since the shoot height, leaf area and shoot dry mass variables had increasing linear adjustments, (Figures 1A, C, and D), resulting in relative increases of 76.0, 71.1 and 66.6% respectively, in the concentration of 6.0 mL L^{-1}, compared to plantules subcultivated in the absence of FishFértil® (Table 1). The increase in shoot dry mass is related mainly to leaves expansion, resulting in an increase in leaf area, since the increase in leaf number was not significant (Table 1).

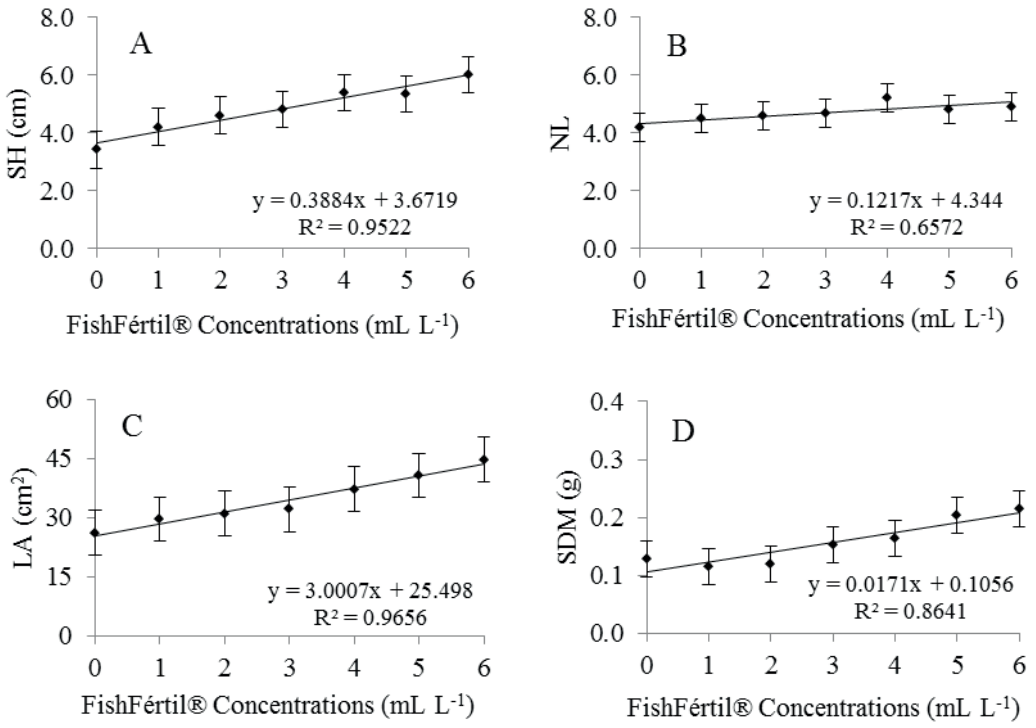

Figure 1. Effect of FishFértil® concentrations on (A) shoot height (SH), (B) number of leaves (NL), (C) leaf area (LA) and (D) shoot dry mass (SDM) in the *in vitro* cultivation of *Cattleya labiata*, after 180 days.

These results may indicate that the fertilizer FishFértil® acts as a conditioner in the culture medium, complementing its chemical composition, once most of the commercialized chemical formulations used in simplified culture media show lack or low availability of some macro and micronutrientes, the main source of NPK (Rego-Oliveira and Faria 2005). In addition, the product supplies amino acids and other components originated from fermentation (Agrobac 2013), which act as source of vitamins and micronutrients.

Colombo et al. (2012) and Favetta et al. (2014) cultivated *in vitro* a hybrid of *Phalaenopsis* and *Vanda tricolor*, respectively, and found results superior to that obtained by the ½ MS medium, using a medium based on the commercial product Biofert® and banana pulp. The authors attribute this effect to the supplementation of media with banana pulp to provide organic composites to plantules, considering that the chemical composition of these culture media (inorganic salts) was practically the same. Corroborating with these results, Galdiano Junior et al.

Table 1. Mean values and relative increase (RI) for the following variables: shoot height (SH), number of leaves (NL), leaf area (LA), shoot dry mass (SDM), number of roots (NR), roots dry mass (RDM), root average length (RAL) and shoot/root ratio (S/R). Means and relative increase calculated in function of the supplementation of the organic fertilizer FishFértil® in the culture medium during the *in vitro* cultivation of *Cattleya labiata*, after 180 days.

Treatments	SH	NL	LA	SDM	NR	RDM	RAL	S/R
mL L^{-1}	cm		cm^2	g		g	cm	
0.0	3.42	4.21	26.13	0.129	6.16	0.467	4.06	0.276
1.0	4.22	4.49	29.60	0.115	3.97	0.223	3.95	0.536
2.0	4.61	4.61	31.06	0.120	3.37	0.163	4.08	0.754
3.0	4.82	4.70	32.13	0.153	3.69	0.247	4.14	0.628
4.0	5.40	5.22	37.18	0.164	4.46	0.232	3.99	0.736
5.0	5.36	4.80	40.69	0.204	4.39	0.363	4.71	0.578
6.0	6.02	4.93	44.70	0.214	4.75	0.345	4.92	0.625
RI (%)	76.0**	17.2^{ns}	71.1**	66.6**	-22.8**	-26.2**	21.4^{ns}	126.0**

**significance at 0.01 of probability; ^{ns} non - significant, determined by Bonferroni t-test. RI = 100 (x-y) /y, where x is the treatment mean for the treatment at the concentration of 6.0 mL L^{-1} and y is the mean for the treatment at the concentration of 0.0 mL L^{-1}.

(2012) verified that the *in vitro* development of the *Cattleya trianaei* was favored in cultivation based on simplified medium, NPK (10-30-20) + micronutrients, supplemented with banana pulp in detriment of the ½ MS medium.

The number (Figure 2A) and root dry mass (Figure 2B) variables adjusted themselves to the square root model when, in the absence of FishFértil® they presented the highest means while, with the addition of concentrations of 1.0 and 2.0 mL L^{-1}, they were reduced. However, from concentration 3.0 mL L^{-1} and up, there was a tendency to increase due to the FishFértil® concentrations. Thus, these two variables behaved similarly considering that the roots dry mass is given in function of number of roots.

The highest number of roots mean was observed for the treatment without the addition of FishFértil®. The inflexion observed in root number and dry mass occurs as a response to shoot growth, since the root system is favored in more elongated plants. (Souza and Pereira 2007).

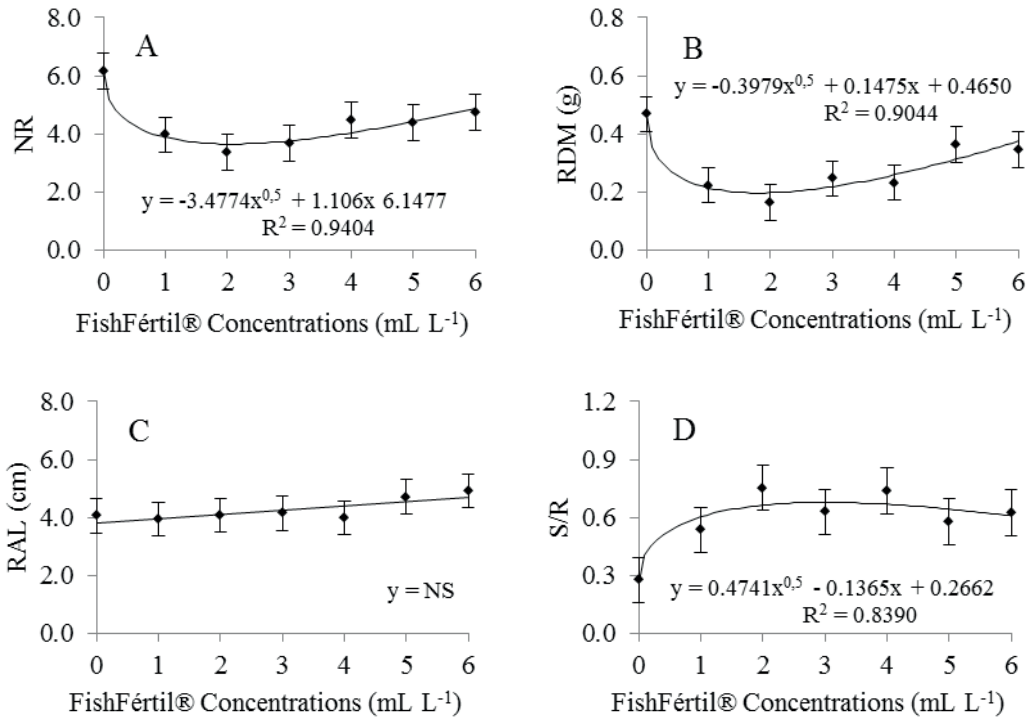

Figure 2. The effect of FishFértil® concentrations on (A) number of roots (NR), (B) root dry mass (RDM), (C) root average length (RAL) and (D) shoot/root ratio (S/R) in in vitro cultivation of *Cattleya labiata*, after 180 days.

On the other hand, the use of the product in concentrations between 1.0 and 4.0 mL L^{-1} reduced plantules total dry mass significantly (Figure 3), which can be attributed mainly to the reduction in the roots dry mass (Figure 2B). The other treatments do not differ in regards to total dry mass; however, the shoot/root dry mass

ratio (S/R) was altered (Figure 2 D). For this variable, an adjustment to the square root model is observed, being the lower mean observed in the absence of FishFértil® (Figure 2D). These results show that in the absence of FishFértil® the plantules had excessive roots, presenting a relationship of approximately 0.276, which increases to 0.536 by the addition of 1.0 mL L^{-1} of the product; reaching 0.625 at the concentration of 6.0 mL L^{-1}.

Based on these results, it is possible to observe that FishFértil® does not act directly on biomass accumulation but rather on its distribution between the shoot and the roots. Stancato et al. (2008), observed that the shoot/root ratio in plantules grown in the Knudson and MS media are close to 1, however, this relationship is different among different species when cultivated in NPK formula-based media. Yet, figure 3 shows that plantules subcultivated in the presence of FishFértil® tend to present a shoot/root ratio close to 1.

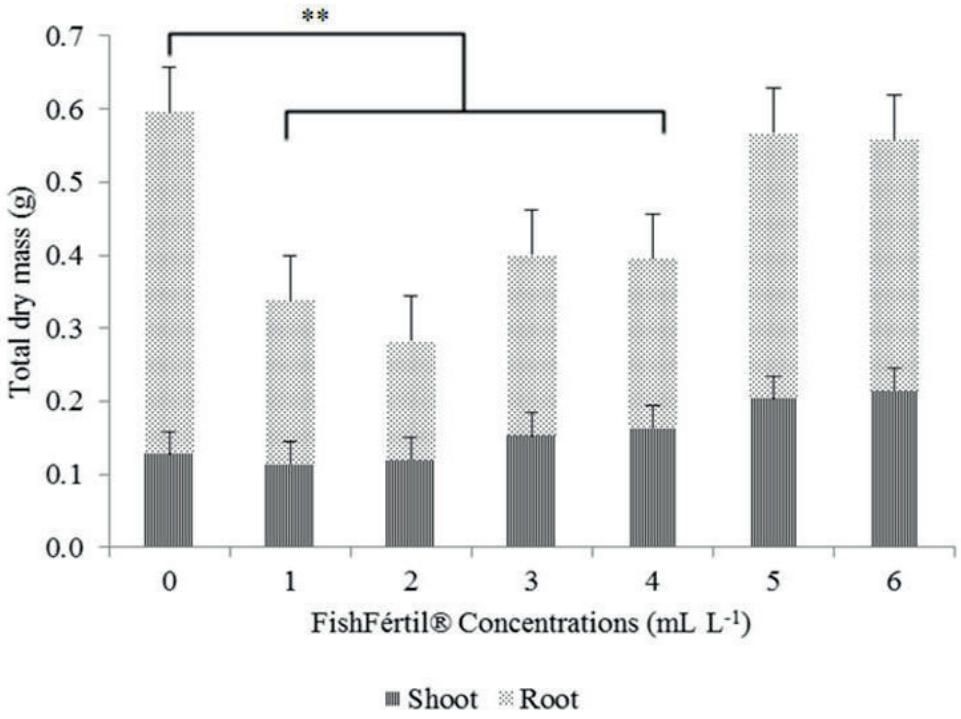

Figure 3. *Cattleya labiata* plantules total dry mass and its distribution between shoot and root. Error bar = standard deviation. **Means statistically different by the Scheffé contrast at 0.01 of significance.

Greater root system development in detriment of shoot development is not desirable in *in vitro* cultivation, since the artificial conditions to which they are submitted make them less functional due to changes caused in the anatomy and morphology of these organs (Pierik 1990; Mayer et al., 2008). On the other hand, those grown *ex vitro*, during acclimatization, will give support to later growth stages. So, the use of the organic fertilizer FishFértil® as conditioner in a simplified culture medium brought more balance to seedlings development (Figure 4), being its use at the 6 mL L^{-1} concentration viable for the *in vitro* growth of *Cattleya labiata*.

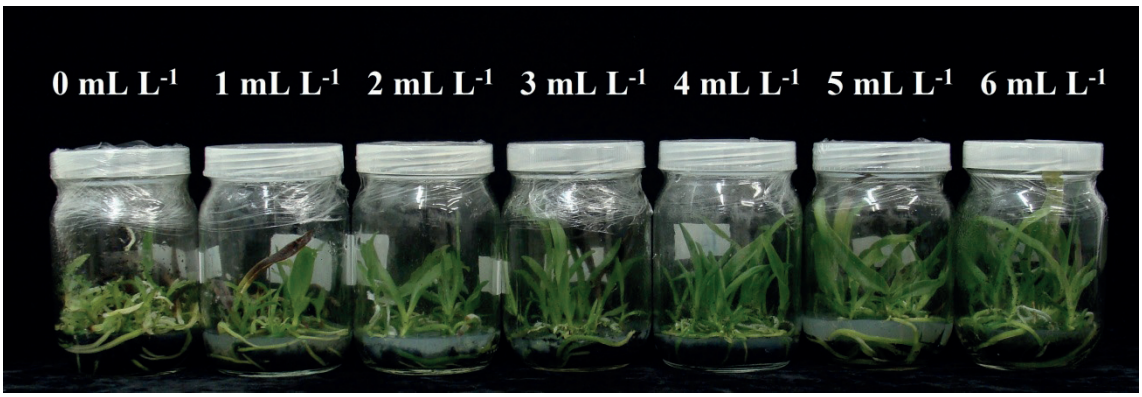

Figure 4. *In vitro* growth of *Cattleya labiata*, after 180 days of cultivation in a simplified culture medium, supplemented with the FishFértil® fertilizer in concentrations of 0.0; 1.0; 2.0; 3.0; 4.0; 5.0 and 6.0 mL L^{-1}.

CONCLUSION

Simplified culture medium supplementation with 6 mL L^{-1} of the organic fertilizer FishFértil® promotes improvements in the quality of *Cattleya labiata* orchid seedlings grown *in vitro*.

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