

GIBBERELIC ACID FERMENTED EXTRACT OBTAINED BY SOLID-STATE FERMENTATION USING CITRIC PULP BY *FUSARIUM MONILIFORME*: INFLUENCE ON *LAVANDULA ANGUSTIFOLIA* MILL., CULTIVATED *IN VITRO*

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Abstract

The aim of this study was to evaluate the effects of fermented extract contain GA₃ obtained by Solid State Fermentation and comparing with a commercial source of GA₃ (reagent grade) in *In vitro* culture of common lavender (*Lavandula angustifolia* Mill.). The residual effects of GA₃ also were evaluated in acclimatization. Nodal segments of *L. angustifolia* were cultured on LS culture medium, supplemented with 1320 mg.L⁻¹ CaCl₂, 30 g.L⁻¹ sucrose and solidified with 7 g.L⁻¹ agar. The experimental design was completely randomized in a factorial arrangement (2x4), with the first factor being the type of GA₃ source (pure or fermented extract) and the second factor being the GA₃ level (0, 0.25, 0.5 and 1.0 mg.L⁻¹). Growth parameters were evaluated after 85 days. Acclimatization was performed using Plantmax[®] HT as substrate, plantlets were maintained by 14 days inside greenhouse with intermittent nebulization and after they were transferred to another greenhouse with manual irrigation. Growth parameters were evaluated after 28 days. The fermented extract and GA₃ pure were analyzed by HPLC (High-performance liquid chromatography). In conclusion, the differences between the GA₃ sources (fermented extract and reagent grade) in nodal segments of *Lavandula angustifolia* cultured *In vitro* and the plantlets acclimatized are little. The GA₃ pure increases more the root number than fermented extract. The GA₃ pure promotes highest chlorosis rate than fermented extract. Fermented extract inhibits root formation at higher levels (1.0 mg.L⁻¹). In acclimatization, the fermented extract at 0.25mg.L⁻¹ GA₃ showed a beneficial residual effect promoting plants more vigorous than other treatments.

Introduction

Gibberellic acid (GA₃) is a plant hormone belongs to gibberellins. Plants produce low amount of GA₃, therefore this hormone is produced by microorganisms. Nowadays, it is industrially produced by submerge fermentation, but this process presented low yield with high production costs and hence higher sale value. One alternative process to reduce costs of the GA₃ production is Solid-State Fermentation (SSF) that allows the use of agro-industrial residues. Various processes of GA₃ production using SSF have been studied, with different yields, substrates/supports, strategies and bioreactors (Hollmann *et al.*, 1995; Bandelier *et al.*, 1997; Escamilla *et al.*, 2000; Machado *et al.*, 2002; Shukla *et al.*, 2005; Corona *et al.*, 2005; Rodrigues *et al.*, 2009). Solid-State Fermentation using *Fusarium moniliforme* in citric pulp (CP) extract produces 5.9 g.kg⁻¹ dry CP after 3 days of fermentation (Rodrigues *et al.*, 2009).

Endogenous gibberellins influence various development processes, such as stem elongation, control various aspects of seed germination, including dormancy break and mobilization of endosperm reserves, moreover, gibberellins influence transition from juvenile stage to mature stage, induction of flowering, sex determination and fruit set establishment in the reproductive development (Taiz & Zeiger, 2004). Only a few gibberellins possess activity as hormone, of these the GA₃ is the gibberellin more used. Therefore, GA₃ had diverse applications in agriculture and plant biotechnology.

Several applications of GA₃ in the agriculture and plant biotechnology can be evidenced in these studies: The presence of GA₃ combined with others plant growth regulators promoted best morphogenic events in *Lycopersicon esculentum* (Afroz *et al.*, 2009) and

Lavandula dentata (Machado *et al.*, 2011). Secondary metabolites accumulation can be increased with GA₃ application, as observed in *Lathyrus sativus* (Bano & Sanaullah, 1995). Primary metabolites also can be increased with GA₃ application, one example is the sucrose accumulation in sugarcane due to elongation of internodes during winter (Taiz & Zeiger, 2004). Exogenous GA₃ on culture medium was used to increase height of *Dyckia maritima* shoots to facilitate *In vitro* manipulation (Silva *et al.*, 2004). Grapevine fruits (Thompson seedless) treated with GA₃ had increased its size and production (Abu-Zahra, 2010). Foliar application of GA₃ and nutrients had improved the productivity and quality of lily cut flowers (Sajid *et al.*, 2009). Moreover, we found a great amount of others studies demonstrating various GA₃ applications.

Due to fact wherein *In vitro* growth of explants facilitates the research of physiological effects of nutrients, elicitors and plant growth regulators (Bisognin *et al.*, 2008; Carvalho *et al.*, 2011; Scheidt *et al.*, 2011). The aim of this study was to evaluate the effects of fermented extract contain GA₃ obtained by Solid State Fermentation and comparing with a commercial source of GA₃ (reagent grade) in *In vitro* culture of common lavender (*Lavandula angustifolia*) Mill. The residual effects of GA₃ also were evaluated in acclimatization.

Materials and Methods

Gibberellic acid sources: The pure GA₃ was a commercial powder (reagent degree) dissolved with 1N NaOH and solubilized with distilled water. The GA₃ extract was a fermented extract obtained by Solid-State Fermentation using *Fusarium moniliforme* LPB 03 in citric pulp extract supplemented with sucrose, it was used two columns with

forced aeration (30mL.min⁻¹) under 70% humidity during 5 days, the GA₃ was extracted with sodium phosphate buffer (pH 7.4) and filtered, this material was precipitated with 20% ethanol and cleaned by an adsorption column of activated charcoal (Rodrigues *et al.*, 2009). Quantitative determination of GA₃ in fermented extract was performed by spectrophotometry with acidification of fermented extract with 30% HCl during 60 to 80min at 20°C and 254nm absorbance (Lu *et al.*, 1995). The fermented extract and GA₃ pure also were analyzed by HPLC (High-performance liquid chromatography) using the methodology proposed by Machado *et al.*, (2002). All GA₃ solutions were sterilized by microfiltration (0.22µm) and added to cooled autoclaved media at about 60°C inside the flux chamber.

Culture establishment: Shoot tips (5mm height) of *Lavandula angustifolia* Mill. were used as explants. These explants were collected from donor plants cultivated in vases with soil inside greenhouse. Disinfection was performed by washing with tap water during one hour, followed by immersion in 2% Cercobin[®] for 40 minutes, immersion in 70% ethanol for 20 seconds, immersion in 1% NaOCl with 0.2% Tween-20 for 20 minutes under shaking and four washings in distilled sterilized water. Shoot tips disinfected were cultured on MS medium (Murashige & Skoog, 1962), 30 g.L⁻¹ sucrose, 0.5 µM 6-benzylaminopurine (BAP), 6 g.L⁻¹ agar and the pH was adjusted to 5.8. Shoots were subcultured each 40 days on same medium described above, but supplemented with 1.0 µM BAP and 1320 mg.L⁻¹ CaCl₂ (Machado *et al.*, 2010).

Culture in different source of gibberellic acid: Nodal segments (1cm height) were used as explants. Basal medium was LS (Linsmeyer & Skoog, 1965), 1320 mg.L⁻¹ CaCl₂, 30 g.L⁻¹ sucrose and solidified with 7 g.L⁻¹ agar. The experimental design was completely randomized in a factorial arrangement (2x4) with five replicates of five explants, with the first factor being the type of GA₃ source (pure or fermented extract) and the second factor being the GA₃ level (0, 0.25, 0.5 and 1.0 mg.L⁻¹). The amounts of the fermented extract used in media were: 7.8mL.L⁻¹ to 0.25mg.L⁻¹ GA₃, 15.6mL.L⁻¹ to 0.5mg.L⁻¹ GA₃ and 31.2mL.L⁻¹ to 1.0mg.L⁻¹ GA₃. The rooting percentage, root number, height of the aerial part (cm), leaf number, fresh mass (g), survival percentage, chlorosis percentage, oxidation percentage, lateral shoot percentage and number of lateral shoots were evaluated after 85 days of *In vitro* culture.

Acclimatization: Plantlets originated from culture with different treatments of GA₃ as above described were removed from culture *In vitro*. Their roots were washed in distilled water to remove culture medium residues. Plantlets from treatment with 1.0mg.L⁻¹ GA₃ of fermented extract did not use, because they did not have roots. These plantlets were cultivated in Plantmax[®] HT inside greenhouse with intermittent nebulization during 14 days and after they were transferred to another greenhouse with manual irrigation. The experimental design was completely randomized with five replicates of five explants. Height of the aerial part (cm), root fresh mass (mg), fresh mass of the aerial part (mg), total fresh mass

(mg), root number, number of leaves, root dry mass (mg), dry mass of the aerial part (mg), total dry mass (mg), percentage of lateral shoots, number of lateral shoots and percentage of survival were evaluated after 28 days of *ex vitro* culture.

Culture conditions and statistical analysis: All media had the pH adjusted to 5.8 and were autoclaved at 1 atm and 121°C for 20 min. The cultures were kept at 25 ± 2°C under white fluorescent light (28µM m⁻² s⁻¹) with a 16 h photoperiod. The data was submitted in a homogeneity analysis for the Bartlett's method and, followed by analysis of variance (ANOVA) followed by Duncan's test (qualitative treatments) or regression analysis (quantitative treatments), both at the levels of p<0.01 and 0.05. Variables from counting were transformed to $\sqrt{x+0.5}$ and variables from percentage were transformed to $\arcsin\sqrt{x/100}$. All of statistical analyses were performed using the software GENES (Cruz, 2001).

Results and discussion

Gibberellic acid sources: The fermented extract after precipitation and cleaning by an adsorption column of activated charcoal presented 62 mg.L⁻¹ GA₃. The HPLC chromatograms of the fermented extract and GA₃ pure had demonstrated some impurities (Fig. 1). The retention time of GA₃ was identified at 3 minutes; it was observed the presence of other compounds in lower quantities (a small pick at right and other pick at left of GA₃), these compounds can be due to 1% impurities specified by manufacturer of GA₃ (reagent grade) (Fig. 1A). Similar result was presented by HPLC chromatogram of fermented extract, the retention time was very close to showed by chromatogram of GA₃ (reagent grade), nevertheless we observed two picks, one at left and other at right of GA₃ (Fig. 1B). Others substances that also can be present in fermented extract are: residues of ethanol (used in the precipitation), sodium phosphate buffer (used in the extraction) and probably some carbohydrates from fermentation process.

Culture in different sources of gibberellic acid: There are no statistical differences between the two sources of GA₃ (Pure and Extract) for rooting percentage, fresh mass, height of the aerial part, leaf number, survival percentage, oxidation percentage, number and percentage of lateral shoots. Nevertheless, there are statistical differences between the two sources of GA₃ for chlorosis percentage and root number (Table 1). The GA₃ pure demonstrated best results for root number than GA₃ from fermented extract, the general mean for root number was 2.9 root per explants in treatments with GA₃ (reagent grade) and 1.8 root per explants in treatments with GA₃ (fermented extract). The chlorosis percentage was highest in GA₃ pure (24.6%) than in the fermented extract (9%). The GA₃ level presented significant differences for fresh mass, height of the aerial part, leaf number, chlorosis percentage, oxidation percentage, rooting percentage, number and percentage of lateral shoots. Survival Percentage and percentage and number of roots did not show significant differences for GA₃ level. The only interaction significant was the root percentage (Table 1).

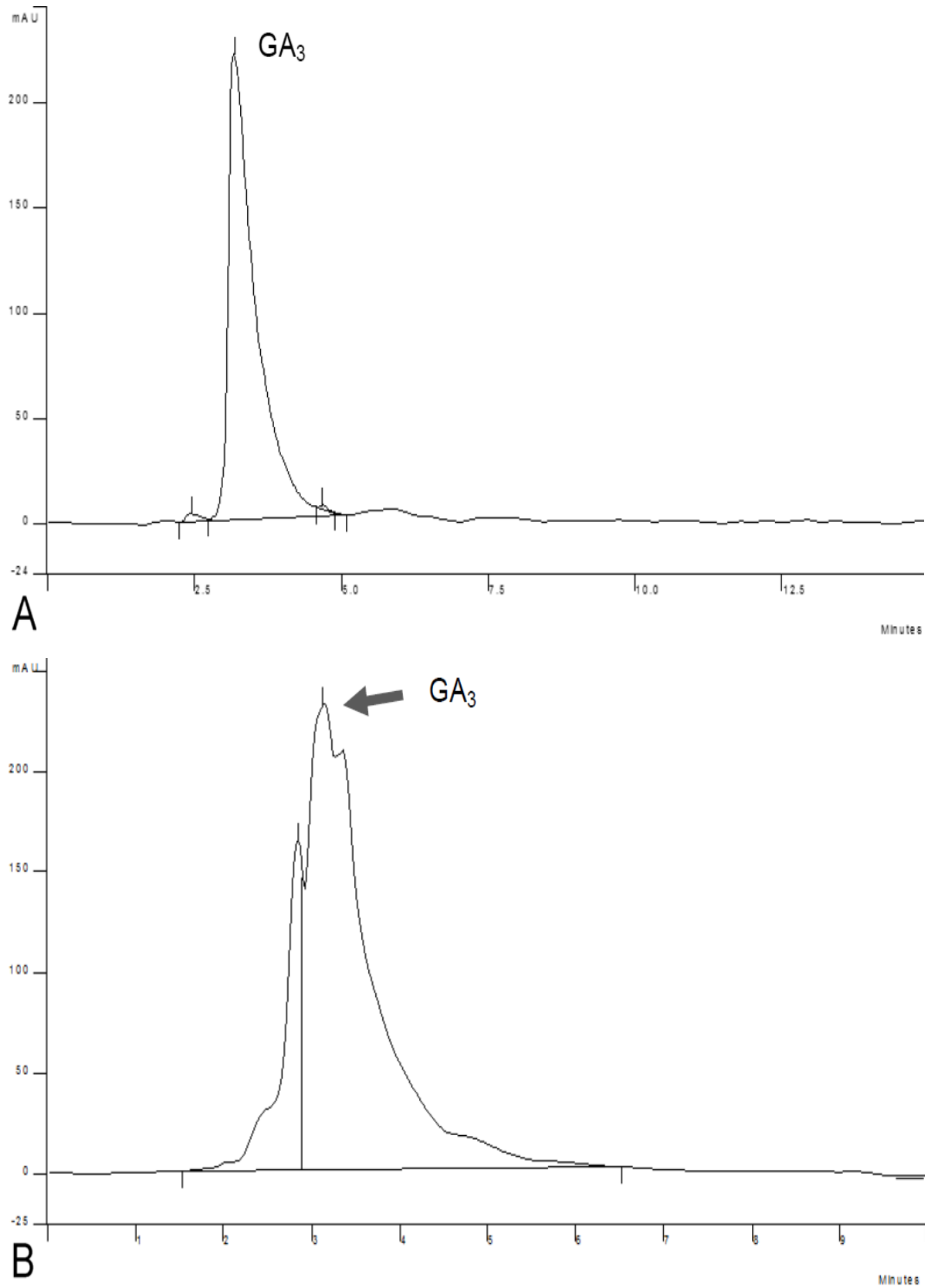


Fig. 1. HPLC chromatograms of the fermented extract and GA₃ pure (reagent grade). (A) Analysis of GA₃ solution at 0.125 g.L⁻¹ (reagent grade, 99%) AKROS Trademark, (B) Fermented extract contents GA₃ according the conditions used in the present study.

Table 1. Summary of two-way ANOVA (analysis of variance) on growth of *Lavandula angustifolia*. Rooting (R%), root number (NR), height of the aerial part (A cm), leaf number (LF), fresh mass (FM g), survival percentage (S%), chlorosis percentage (C%), oxidation percentage (O%), lateral shoot percentage (LS%) and number of lateral shoots (SN) of two sources of gibberellic acid and four levels to the 85 days of *In vitro* culture.

Mean square						
Source of variation	d.f.	R %	NR	A cm	LF	FM g
A ¹	1	607.5 ^{ns}	1.143 ^{**}	0.633 ^{ns}	0.589 ^{ns}	0.00001 ^{ns}
B ²	3	2608.4 [*]	1.252 ^{ns}	2.626 [*]	3.451 [*]	0.03105 [*]
A x B	3	1067.7 ^{**}	0.436 ^{ns}	0.144 ^{ns}	0.553 ^{ns}	0.00026 ^{ns}
Residual	32	207.0	0.234	0.186	0.199	0.00449
CV(%)		41.3	30.3	20.2	11.7	76.0
		S %	C %	O %	LS %	SN
A	1	47.22 ^{ns}	1598.2 ^{**}	213.79 ^{ns}	88.511 ^{ns}	0.00201 ^{ns}
B	3	70.92 ^{ns}	2905.9 [*]	2512.4 [*]	3520.7 [*]	0.12563 [*]
A x B	3	130.3 ^{ns}	628.04 ^{ns}	71.367 ^{ns}	83.912 ^{ns}	0.00573 ^{ns}
Residual	32	438.4	241.11	163.90	588.48	0.01883
CV(%)		38.5	92.5	82.2	44.1	13.0

¹ Gibberellic acid source (pure and extract) ² Levels of gibberellic acid (0, 0.25, 0.5 and 1.0 mg.L⁻¹) * Significant at the level of 1% of error probability for the test F, ** Significant at the level of 5% of error probability for the test F, ^{ns} Not significant

The variables height of the aerial part, number of leaves and fresh mass followed a negative linear regression (Fig. 2A, B and D). It seems that GA₃ in explants of the *Lavandula* species must be associated with others plant growth regulators to produce best results, as demonstrated in *Lavandula dentata*, which shoot tips cultured on LS medium supplemented with 0.5µM BAP, 2.5µM IBA and 0.3µM GA₃ allows explants with 2.7cm height of the aerial part comparing with 0.9cm height obtained in explants cultured on control treatment (Machado *et al.*, 2011). The synergistic effect of GA₃ with others plant growth regulators already was observed in others studies, such as in cell suspension of *L. angustifolia*, wherein the addition of 1 mg.L⁻¹ BAP and 0.5 mg.L⁻¹ GA₃ promoted highest multiplication rate of the cells (Bona *et al.*, 2012) and in *Solanum tuberosum* cultivated *In vitro* it was demonstrated that the addition of GA₃ (0.5 mg.L⁻¹) combined with low cytokinin concentration was effective in the shoot growth (Farhatullah *et al.*, 2007). Nevertheless, there is some species that do not need their association between GA₃ and others plant growth regulators, as demonstrated in *Mentha piperita* (Lamiaceae) wherein the addition of 1 mg.L⁻¹ GA₃ alone promoted the shoot elongation (Ghanti *et al.*, 2004). Nevertheless, GA₃ is not the only plant growth regulators used to promote shoot elongation, BAP alone also can be used, as demonstrated in watermelon cultivated in 0.05 mg.L⁻¹ BAP (Wang *et al.*, 2013).

The leaf number is associated with the shoot height, as smaller the height of the aerial part, minor is the number of leaves, whereas are smaller internodes number. None equation was adjusted to root number and survival percentage due to the fact that these variables are not significant for GA₃ level (Fig. 2C and E). When the nodal segments were isolated during manipulation to experiment installation, it is possible that some explants has been dehydrated during the peeling and transferring to another culture medium, what explain the survival rate (Fig. 2E). Different species demonstrated varied answers when cultured on GA₃ for root number. In potato, the 0.248 mg.L⁻¹ GA₃ level had doubled the root number per

plantlet (5.6) as compared to control (2.3) (Farhatullah *et al.*, 2007). In *Nidularium innocentii* and *Nidularium procerum*, the supplementation of GA₃ (4.1 and 8.2 µM) decreased the root number (Silva *et al.*, 2012).

The fresh mass accumulation influenced by GA₃ can have different effects depending on the species, in *Sinningia speciosa*, which increases of GA₃ level decreased fresh mass (Araújo *et al.*, 2004). On the other hand, in *Nidularium innocentii* and *Nidularium procerum*, the supplementation of GA₃ did not influence the fresh mass accumulation (Silva *et al.*, 2012). The chlorosis percentage follows a positive quadratic regression (Fig. 2F). The chlorosis observed in shoots has been associated to the larger shoot elongation promoted by the GA₃, as in *Ficus carica* that shoots excessively elongated due to the GA₃ effect; they became chlorotic (Fráguas *et al.*, 2004). However, in the present study the chlorosis is not associated to the shoot elongation. Nevertheless, it is possible that GA₃ could be associated to the synthesis of secondary metabolism or even, these concentrations are elevated too much, promoting phytotoxic effects. Other observation that supports this idea is the oxidation percentage, which follows a negative quadratic regression; wherein the higher oxidation rate was 40% at 1.0 mg.L⁻¹ (Fig. 3A). Oxidation observed in these explants were a lot browning points at the base stem.

The gibberellins are involved in the growth of buds and meristems; moreover, they promote also the break of bud dormancy (Machado *et al.*, 2011). In the present study, the GA₃ had beneficial effect for the number and percentage of lateral shoots (Fig. 3B and D). Similar results were found in *Zantedeschia aethiopica*, wherein the largest shoot formation was promoted with GA₃, nevertheless, this was associated to the addition of larger sucrose concentration (Ribeiro *et al.*, 2009). The shoot percentage was not influenced by GA₃ in *Nidularium innocentii* and *Nidularium procerum*, nevertheless, the root number was significantly affected by GA₃, in *Nidularium innocentii* the absence of GA₃ showed best results (3.4 shoot per explant), and for *Nidularium procerum* the best results was found at 8.2µM GA₃ (3.4 shoot per explant) (Silva *et al.*, 2012).

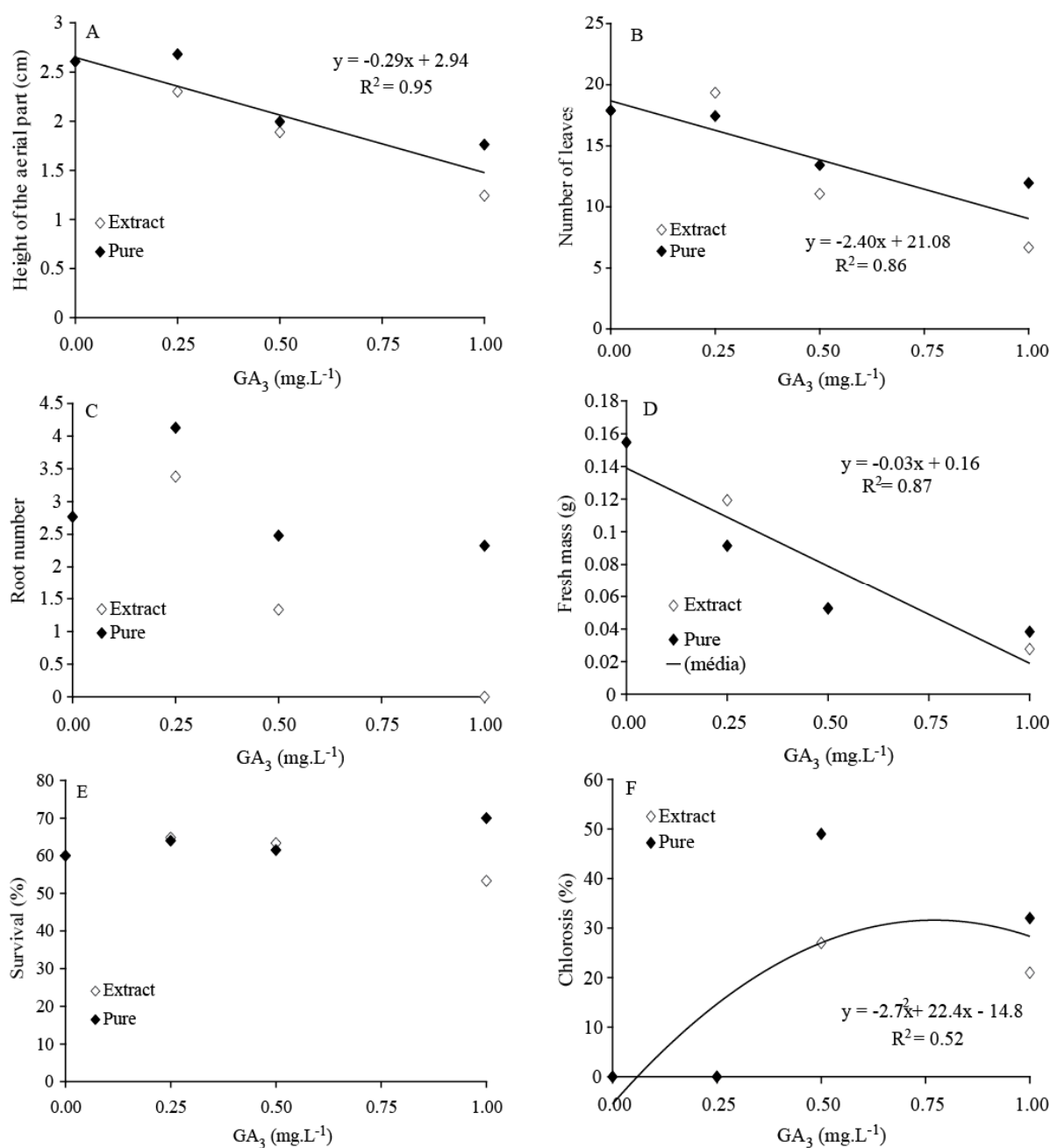


Fig. 2. *In vitro* effects of different sources and levels of Gibberellic acid (GA₃) in common lavender (*Lavandula angustifolia* Mill) after 85 days. (A) Height of the aerial part (cm), (B) Number of leaves, (C) Root number, (D) Fresh mass (g), (E) Percentage of survival and (F) Percentage of chlorosis.

In order to adjust regression equations for the root percentage, two positive quadratic equations were adjusted due to this variable has interaction between the two factors studied (Fig. 3C). The 0.25 mg.L⁻¹ GA₃ promoted the best results, approximately 70% rooting, nevertheless, the fermented extract inhibits root formation at higher levels (1.0 mg.L⁻¹), this effect is probably caused by other compounds found in fermented extract (Fig. 1B).

Acclimatization: *Ex vitro* culture of *L. angustifolia* plantlets had demonstrated residual effects of GA₃

originated from *In vitro* culture. Many growth parameters were influenced by these residual effects during acclimatization (Table 2). The best results for survival percentage were found in the treatments: control, 0.25 and 0.5 mg.L⁻¹ GA₃ (pure) and 0.25 mg.L⁻¹ GA₃ (extract), varying from 85.7 to 93.7% survival (Table 2). The best result for number of leaves was observed at 0.25 mg.L⁻¹ GA₃ (extract) with 29.8 leaves per plant. The lowest results for number of leaves were found at 1.0 mg.L⁻¹ GA₃ (pure) and 0.5 mg.L⁻¹ GA₃ (extract), with 15.5 and 17.8 leaves per plant, respectively. The number of leaves

seems to be the only variable related with survival percentage, whereas plants from treatments with number of leaves above 21.8 did not show statistical differences in survival percentage (Table 2). In many cases, the culture medium influence survival rate during acclimatization as observed in micropropagated plants of *Eucalyptus globulus* cultured on media with different IBA levels (Bennett *et al.*, 2003/4). On the other hand, some species were not influenced as demonstrated in *Nidularium innocentii* and *Nidularium procerum*, which plantlets cultured on 4.1 and 8.2 μM GA₃ and on medium free of GA₃ did not promote differences in survival rate (Silva *et*

al., 2012). However, it is possible that a minimum number of roots be indispensable to a suitable survival rate of *L. angustifolia*, as observed in present study, the plantlets cultured on 1.0 mg.L⁻¹ GA₃ (pure) and 0.5 mg.L⁻¹ GA₃ (extract) had 4.5 and 2.3 roots per plantlet, respectively. These plantlets obtained the lowest survival rate (Table 2). Plantlets with a minimum of 5.1 roots per plantlet had a suitable survival rate (Table 2). Similar results were found in *Eucalyptus globulus*, which plantlets with a minimum of 6 roots showed 100% survival (Bennett *et al.*, 2003/4).

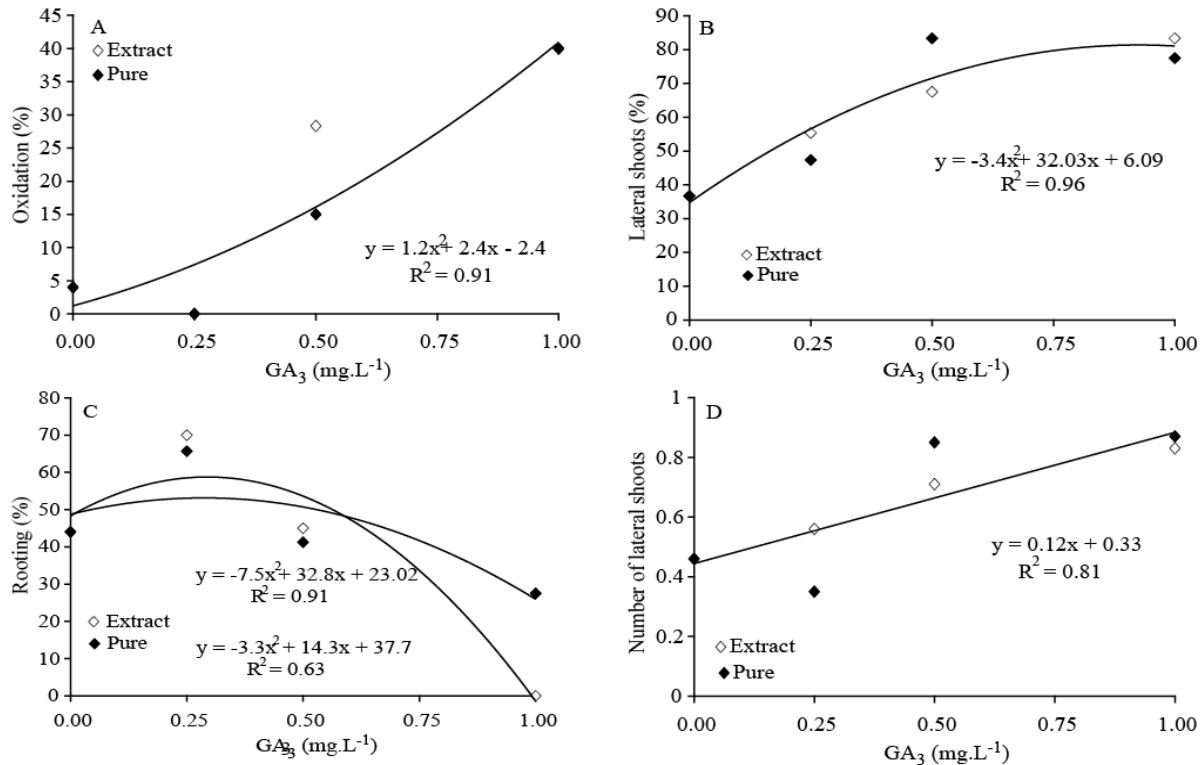


Fig. 3. *In vitro* effects of different sources and levels of Gibberellic acid (GA₃) in common lavender (*Lavandula angustifolia* Mill) after 85 days. (A) Percentage of oxidation, (B) Percentage of lateral shoots, (C) Percentage of rooting and (D) Number of lateral shoots.

The best result for height of the aerial part was observed at 0.25 mg.L⁻¹ GA₃ (extract), which reached 5.68cm height, followed by control, which reached 4.16cm height, others treatments had lower height of the aerial part, varying from 2.92 to 3.57cm (Table 2). These results were in accordance with results obtained in *In vitro* culture in present study, which GA₃ was not beneficial to increase height of the aerial part (Fig. 2A). Nevertheless, this GA₃ level (0.25 mg.L⁻¹) is the more close to control and in *In vitro* culture presented similar result with control, however this same level performed with pure source of GA₃ showed a lowest height comparing with the results presented by fermented extract at this same level (0.25 mg.L⁻¹), what suggests influence of others substances in fermented extract.

Although the fermented extract was partially purified by precipitation and clean by an adsorption column of activated charcoal, some substances are yet presents. These

substances were not completely identified. But this present study suggests a positive influence in *L. angustifolia* (during acclimatization) when lower levels are used, up to 0.25 mg.L⁻¹ GA₃ and 7.8mg.L⁻¹ fermented extract, quantities above this level seems to be not influence at this stage. These others substances present in fermented extract (0.25 mg.L⁻¹ GA₃) also maintained residual effects in plantlets from *In vitro* culture. These residual effects also were observed in others growth parameters, such as, fresh mass of the aerial part, total fresh mass, leaf number, root dry mass and percentage of the lateral shoots (Table 2). These results demonstrated that 7.8mg.L⁻¹ fermented extract content 0.25 mg.L⁻¹ GA₃ was suitable to produce acclimatized plantlets of *L. angustifolia*, with plantlets more vigorous than other treatments.

The control and 0.25 mg.L⁻¹ GA₃ (extract) showed the best results for root dry mass, dry mass of the aerial part and total dry mass, these two treatments did not differ

statistically (Table 2). The best results found for shoot number were: 0.25, 0.5 and 1.0 mg.L⁻¹ GA₃ (pure), varying from 1.4 to 1.6 shoots per plantlet, followed by 0.25 mg.L⁻¹ GA₃ (extract) with 1.3 shoot per plantlet; however these treatments were no significant (Table 2). Nevertheless, the best shoot percentage was obtained at 0.25 mg.L⁻¹ GA₃ (extract) reached 93.3% shoots, being statistically superior to others treatments. These results are interesting to potential use of these plantlets as mini-stumps to seedling production. These mini-stumps can be used as donors of mini-cuttings, which can be induced to produce adventitious roots as demonstrated in the hybrid *Eucalyptus benthamii* x *Eucalyptus dunnii*, which mini-

stumps produced shoots that were used as mini-cuttings to produce seedlings (Brondani *et al.*, 2012).

This fermented extract content GA₃ represents a great potential as source of GA₃ to innumerable applications, from agriculture to plant biotechnology with the advantages to be more inexpensive than the GA₃ purified (reagent grade). Moreover, these others substances present in this fermented extract can be useful, with positive effects on the plantlets, nevertheless must used in lower levels. Nowadays, plant biofactories are dispersed in the whole world, and need technologies and alternatives more efficient and cheap to become more competitive.

Table 2. Characteristics observed in the acclimatized plants of common lavender (*Lavandula angustifolia* Mill) after 28 days of *ex vitro* culture. These plants were cultivated *In vitro* on different sources and levels of Gibberellic acid (GA₃). Height of the aerial part (AP cm), root fresh mass (MFR mg), fresh mass of the aerial part (MFPA mg), total fresh mass (MFT mg), root number (NR), number of leaves (NF), root dry mass (MSR mg), dry mass of the aerial part (MSPA mg), total dry mass (MST mg), percentage of lateral shoots (BL %), number of lateral shoots (NB) and percentage of survival (S%).

GA ₃ (mg.L ⁻¹)	AP cm	MFR mg	MFPA mg	MFT mg	NR	NF
0.00	4.16 b ¹	136.9 a	252.4 b	389.3 b	5.1 c	23.8 b
0.25 (Pure)	3.46 c	60.0 b	148.4 c	208.4 c	8.0 a	23.5 b
0.50 (Pure)	2.98 c	24.3 bcd	85.6 d	110.0 d	5.5 bc	21.8 bc
1.00 (Pure)	2.92 c	8.7 d	61.0 d	69.7 d	4.5 c	15.5 d
0.25 (Extract)	5.68 a	132.1 a	318.2 a	450.3 a	6.6 b	29.8 a
0.50 (Extract)	3.57 c	43.5 bc	131.4 c	174.9 c	2.3 d	17.8 cd
CV(%)	10.1	22.8	17.2	16.2	6.9	6.4
GA ₃ (mg.L ⁻¹)	MSR mg	MSPA mg	MST mg	BL %	NB	S %
0.00	13.8 ab	39.7 a	53.6 a	50.0 c	0.6 c	90.9 a
0.25 (Pure)	7.7 bc	26.5 b	34.3 bc	72.7 b	1.4 a	91.6 a
0.50 (Pure)	5.0 c	18.1 bc	23.1 bcd	83.3 b	1.6 a	85.7 a
1.00 (Pure)	2.0 c	12.2 c	14.2 d	75.0 b	1.5 a	66.6 b
0.25 (Extract)	15.6 a	44.8 a	60.4 a	93.3 a	1.3 ab	93.7 a
0.50 (Extract)	5.7 bc	22.0 b	27.8 b	57.1 c	0.7 bc	70.0 b
CV(%)	51.3	25.2	20.4	10.4	12.8	10.2

¹Means within a column followed by the same letter for each parameter are not different at p<0.05 by Duncan's test

Conclusions

The differences between the GA₃ sources (fermented extract and reagent grade) in nodal segments of *Lavandula angustifolia* cultured *In vitro* and the plantlets acclimatized are little. The GA₃ pure increases more the root number than fermented extract. The GA₃ pure promotes highest chlorosis rate than fermented extract. Fermented extract inhibits root formation at higher levels (1.0 mg.L⁻¹). In acclimatization, the fermented extract at 0.25 mg.L⁻¹ GA₃ showed a beneficial residual effect promoting plants more vigorous than other treatments.

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