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SUBSTITUTION OF 6-BENZYLAMINOPURINE AND NAPHTHALENE ACETIC ACID BY LEAF EXTRACTS OF *SYMPHYTUM OFFICINALE* AND *LANTANA CAMARA* ON THE *IN VITRO* CULTURE OF *BIOPHYTUM SP* (OXALIDACEAE) AND *PSOROSPERMUM NERVOSUM* (CLUSIACEAE)

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ABSTRACT

To replace the plant growth regulators NAA and BAP on the *in vitro* culture of *Biophytum sp* and *Psorospermum nervosum*, the effects of different concentrations (0.1 to 2.5%) of leaves extracts of *Symphytum officinale* and *Lantana camara* were studied and compared with 0.1% of BAP and 0.1% of NAA. For *Biophytum sp*, the maximum of buds were observed with 1% of leaf extract of *Symphytum officinale* and with 2.5% for the extract of leaves of *Lantana camara*. *In Psorospermum nervosum*, buds were produced on medium with 2.5% of *S. officinale* extract. The maximum shoots elongation of *Biophytum sp* was obtained with 0.5% to 2% of *S. officinale* extract and 2.5% for *L. camara* extract. In *Psorospermum nervosum*, it was observed in the presence of 2.5% of the both extracts. Thus, leaves extracts can replace 0.1% of BAP on the sprouting and growth of these species. The best rooting of *Biophytum sp* was obtained with leaves extracts at 2.5% for *S. officinale* and 0.1% for *L. camara*. As for *Psorospermum nervosum*, the best production and elongation of roots were favored with the same concentration (2.5%) of these extracts of leaves. So the substitution of NAA at 0.1% by these leaves extracts is also possible.

KEYWORDS: *Biophytum sp, Psorospermum nervosm, in vitro culture, leaves extracts, growth regulator, Symphytum officinale, Lantana camara*.

INTRODUCTION

In Madagascar, given the environmental risks caused by nickel and cobalt exploitation in the Ambatovy region, Ambatovy Minerals SA project has developed a biodiversity management program including special conservation techniques for sensitive plant species (Phillipson and *al*., 2010). These species exist only on the footprint of the mine or only one or two other sites in Madagascar (Dickinson and Berner, 2010; http://I). The *Biophytum sp* (OXALIDACEAE) and *Psorospermum nervosum* (CLUSIACEAE) are belong to these endemic and endangered plant species of Ambatovy mine site in Madagascar.

In vitro culture Biotechnology was undertaken for the *ex situ* conservation of the two species. This technique uses a cell, tissue, fragments of organ called "explant". These explants must found in the culture medium all nutriments they need to survive grow and multiply. Thus, in addition to the nutrients, growth regulators or plant hormones may also be added to orient the growth and morphogenesis of explants cultured *in vitro* (http://2). Moreover, many plant extracts have been used in *in vitro* culture. These natural products have been reported as supplements to the culture medium or as substituents of phytohormones (Rahelivololona, 2005; Ramanamidona, 1998; Dinaharilala, 2012). According to research on orchid species, it was shown that the application of organic extracts of green coconut, banana, pineapple has positive effects on the *in vitro* culture of *Aeranthes glandiflora*, *Aeranthes antenophora* and *Bulbophyllum peyrotti* (Ramanampamonjy, 2004; Dinaharimalala, 2011).

Because of the high cost of synthetic growth regulators and local non-availability of these products, research of other organic substances that can substitute these phytohormones is a very interesting alternative to undertake. The effects of leaves extracts of *Lantana camara* and *Symphytum officinale* have been the subject of many studies. It has been shown that these extracts were used as biological liquid fertilizer. They can restore soil fertility and thus improve agricultural production (http:// 3; Rasoanaivo. 1995). So, the effects of the leaves extracts and the synthetic growth regulators on growth and rooting *in vitro* of *Biophytum sp* and *Psorospermum nervosum* have been studied.

The general objective of this study focuses on the intensive multiplication of these species in order to preserve them and to their *in situ* reintroduction. The specific objective is to optimize

the concentrations of *Lantana camara* and *Symphytum officinale* leaf extracts that can replace synthetic plant growth regulators on *vitroculture* of *Biophytum sp* and *Psorospermum nervosum.*

MATERIALS AND METHODS

1.1. Collect site for explants

The area where the mother plants of *Biophtytum sp.*and *Psorospermum nervosum* were collected is the dense humid forest of Ambatovy in Madagascar. This area is geographically bounded by the coordinates 18 ° 49 '0.12" south latitude and 48 ° 18' 00" of longitude (http:// 4).

1.2. Collect site for the leaves of Lantana camara and Symphutum officinale

The place of harvest of these species is close to the campus of the University of Antananarivo Madagascar. *Lantana camara* was from a population in the wild and *Symphutum officinale* from a horticultural garden.

1.3. Plant materials

For this study, plant materials used were constituted of:

- Leaves of *Lantana camara* and *Symphytum officinale,* for the extraction ;
- *Micro-cuttings (nodal segment) from vitroculture of Biophytum sp* and *Psorospermum nervosum*, for the effects of leaf extract study.

1.4. Methods

1.4.1 Aqueous extraction by maceration

20g of fresh leaves of each of these species are ground in 160ml of distilled water (m/v). The ground material was stirred for 3 hours at room temperature. It was macerate at 4°C for 12 h, and then stirred for 30 minutes. The liquid was filtered to recuperate the filtrate which was centrifuged at 6000 rpm/min.

1.4.2. Media and culture conditions

The basic medium used was the Murashige and Skoog, (1962), in half strength (MS/2); supplemented with 0.1 mg/L thiamine-HCl, 0.5mg /L pyridoxine-HCl, 0.5mg / L nicotinic acid, 100 mg / L myo-inositol, and 30g / L sucrose. The pH was adjusted to 5.5 - 5.6. Then, the culture media were solidified with 8 g / L (m/v) agar. The media were sterilized by autoclaving at 120 ° C for 20 minutes.

Vitroplant of *Biophytum sp* and *Psorospermum nervosum* of 4 weeks old were cut into 5mm in length. Cuttings were planted vertically, basal pole in the culture medium. The cultures were incubated in a culture room at a temperature of 25°C under a light intensity of 3000lux and a photoperiod of 16h (light) / 8h (darkness). 13 types of culture media named T, T1, T2, C1, C2, C3, C4, C5, L1, L2, L3, L4, L5, different from their concentration in plant growth regulators and extracts of leaves were tested (Table 1). Each treatment was repeated 3 times.

The tested plant growth regulators are a cytokinin compounds: 6-Benzylaminopurine (BAP) (1mg/L) and an auxin: Naphthalene Acetic Acid (NAA) (1mg/L). Their concentrations used were those suitable for the *in vitro* growth and rooting of *Biophytum sp* and *Psorospermum nervosum*.

The average number of bud, size of shoots produced per micro cutting; the average number and length of roots per vitroplant were considered to evaluate the effects of growth regulators and the leaf extracts on the vitroculture of *Biophytum sp* et de *Psorospermum nervosum*.

Table 1: Composition of culture medium according to the concentrations of NAA, BAP, and leaf extracts.

Types of medium	Treatments			
	Naphthalene Acetic Acid (NAA) (mg/l)	6-Benzylaminopurine BAP (mg/l)	Leaf extract of *Symphytum officinale* (mg/l)	Leaf extract of *Lantana camara* (mg/l)
T	-	-	-	
T1	1	-	-	
T2	-	1	-	
C1	-	-	1	
C2	-	-	5	
C3	-	-	10	
C4	-	-	20	
C5	-	-	25	
L1				1
L2				5
L3				10
L4				20
L5				25

I.4.3. Expressions of results

For data analysis, analysis of variance (ANOVA) and comparison of means were performed using the software "STAT-ITCF" Version 4. The separation of homogeneous groups

observed between several medium is made following the Newman and Keuls Test (probability threshold of 5%).

RESULTS

I. Influences of leaf extracts and synthetic plant growth regulators on shoots regeneration of *Biophytum sp* and *Psorospermum nervosum*

Figures (1, 2) show the average number of newly formed shoots after 4 weeks of culture in different treatments.

Analysis of variance in both species showed that there is a significant difference between the data set. Besides, the specific effect of each treatment on the new formation of shoots varied depending on the medium type.

In the *Biophytum sp* (Figure 1), with 1% extract of *Symphytum officinale* (C3) and 2.5% extract of *Lantana camara* (L5) improved shoot production, on average 4.3 per explant (maximum number of shoots). On the other hand, the lowest shoot production was observed in the control medium (T0). In the presence of the extract of *Lantana camara* leaf, average number of shoots formed increased with the concentration of extract (L1 to L5). It respectively ranged from 2 to 4 per explant.

Numbers of shoots produced on the culture media supplemented with NAA and BAP were lower (2 to 3.5 shoots per explant) to that produced with leaf extracts of (maximum up to 4.3 per explant). However, similarity of the effect of 0.1% of BAP (T2) and leaf extracts of the *Symphytum officinale* (1%) and *Lantana camara* (2.5%) was found.

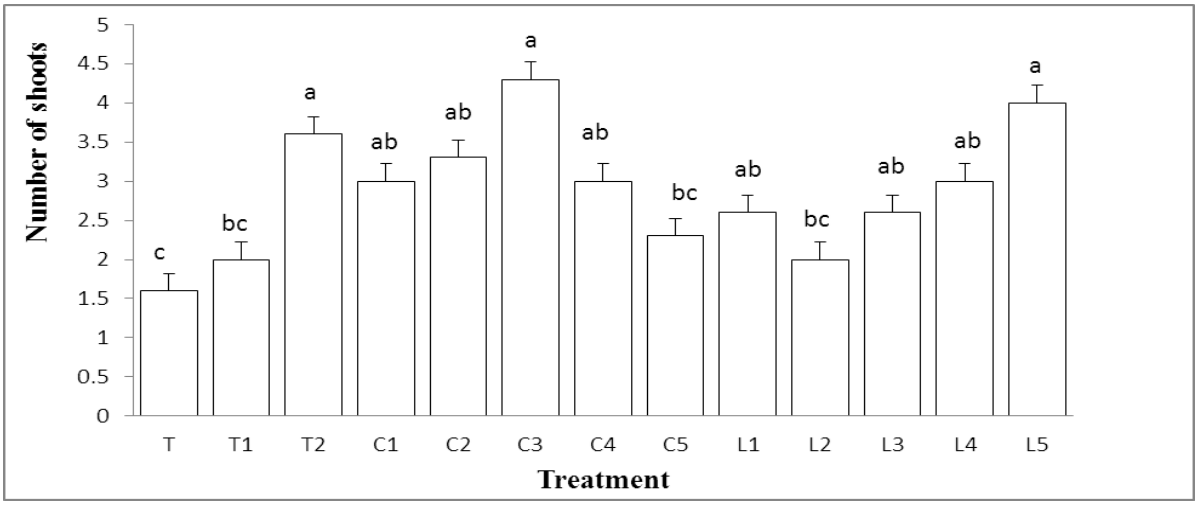

Figure 1: Average number of shoots of *Biophytum sp*. in different treatments after 4 weeks of culture.

T : MS/2 (control), T1 : + 0.1% NAA, T2 : +0.1% BAP, C1 : +0.1% *Symphytum officinale*, C2 : +0.5% *Symphytum officinale*, C3 : +1 % *Symphytum officinale*, C4 : +2% *Symphytum officinale*, C5 : +2.5% *Symphytum officinale;* L1 : +0.1% *Lantana camara*, L2 : +0.5% *Lantana camara*, L3 : +1 % *Lantana camara*, L4 : +2% *Lanatana camara*, L5 : +2.5% *Lantana camara*

The histograms with the same letter are not significantly different according to the Newman and Keuls Test at the 5% threshold.

In *Psorospermum nervosum* (Figure 2), the average number of shoots was high (2.6 shoots per explant) at a concentration of 0.1% BAP (T2) and 2.5% extract of *Symphytum officinale* (C5). The average number of shoots obtained on the culture medium containing 2.5% of *Symphytum officinale* extract (C5) was slightly higher (2.6 per explant) to that produced in the culture medium supplemented with the same concentration of leaf extract of *Lantana camara* (L5) (2.3 buds per explant).

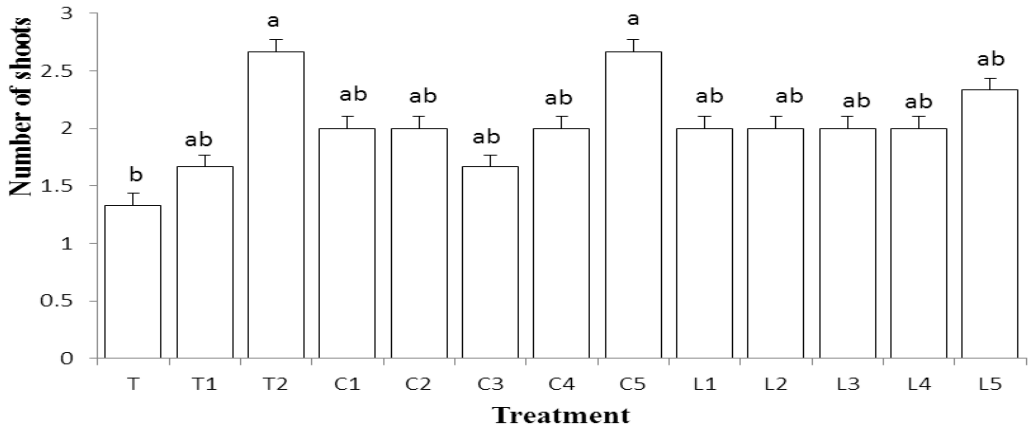

Figure 2: Average number of shoots of *Psorospermum nervosum* according to different treatments after 4 weeks of culture

T : MS/2 (control), T1 : + 0.1% NAA, T2 : +0.1% BAP, C1 : +0.1% *Symphytum officinale*, C2 : +0.5% *Symphytum officinale*, C3 : +1 % *Symphytum officinale*, C4 : +2% *Symphytum officinale*, C5 : +2.5% *Symphytum officinale;* L1 : +0.1% *Lantana camara*, L2 : +0.5% *Lantana camara*, L3 : +1 % *Lantana camara*, L4 : +2% *Lanatana camara*, L5 : +2.5% *Lantana camara.*

The histograms with the same letter are not significantly different according to the Newman and Keuls Test at the 5% threshold.

II. Influences of leaf extracts and synthetic plant growth regulators on the growth of *Biophytum sp* and *Psorospermum nervosum*

In *Biophytum sp* (Figure 3), according to the statistical analysis of data, significant differences were observed on the length of shoots formed.

Concerning the effect of extracts of leaves, the maximum average length of shoots was obtained on the culture medium supplemented with 0.5 to 2% extract of *Symphytum officinale* (C3) and 2.5% of extract of *Lantana camara*. On these media, the average size of shoots varied between 10 to 12mm. The size has increased in parallel with the concentration of leaf extracts of *Lantana camara* (L1 to L5), reaching 10mm at a dose of 2.5%. Contrariwise, the shortest size (3.3mm) was observed on the control medium. The effect of leaf extracts of *Symphytum officinale* (C3) was higher than the plant growth regulators (NAA and BAP) (T1 and T2). Concerning these plant growth regulators, the highest shoots (10.3mm) were observed in the presence of 0.1% of BAP (T2).

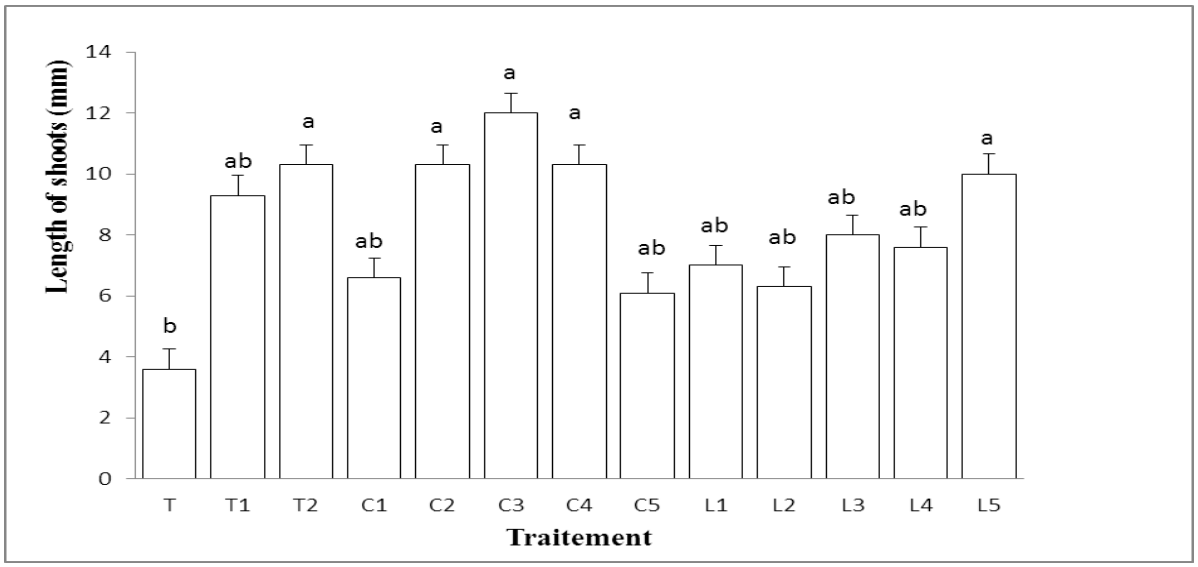

Figure 3: Average length of shoots of *Biophytum sp*. according to different treatments after 4 weeks of culture

T : MS/2 (control), T1 : + 0.1% NAA, T2 : +0.1% BAP, C1 : +0.1% *Symphytum officinale*, C2 : +0.5% *Symphytum officinale*, C3 : +1 % *Symphytum officinale*, C4 : +2% *Symphytum officinale*, C5 : +2.5% *Symphytum officinale;* L1 : +0.1% *Lantana camara*, L2 : +0.5% *Lantana camara*, L3 : +1 % *Lantana camara*, L4 : +2% *Lanatana camara*, L5 : +2.5% *Lantana camara*

The histograms with the same letter are not significantly different according to the Newman and Keuls Test at the 5% threshold.

For *Psorospermum nervosum* (Figure 4), the treatments performed showed significant differences. The longest shoots (16.6mm) were observed in media containing 2.5% of *Symphytum officinale* leaf extract (C5).

On the other hand, NAA and BAP act differently on the growth of shoots. Compared with the control medium (T), this difference was significant according to the test of Newman Keuls and 5%. The maximum length of the shoots (11mm) was observed on the medium containing 0.1% of BAP (T2). The superiority of the effect of leaf extracts from that BAP and NAA was observed.

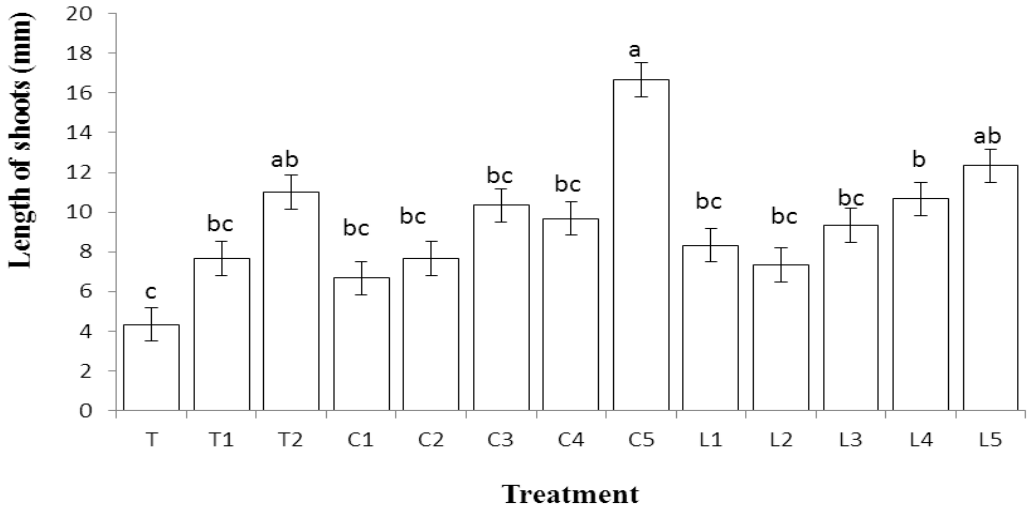

Figure 4: Average length of shoots of *Psorospermum nervosum* according to different treatments after 4 weeks of culture

T : MS/2 (control), T1 : + 0.1% NAA, T2 : +0.1% BAP, C1 : +0.1% *Symphytum officinale*, C2 : +0.5% *Symphytum officinale*, C3 : +1 % *Symphytum officinale*, C4 : +2% *Symphytum officinale*, C5 : +2.5% *Symphytum officinale;* L1 : +0.1% *Lantana camara*, L2 : +0.5% *Lantana camara*, L3 : +1 % *Lantana camara*, L4 : +2% *Lanatana camara*, L5 : +2.5% *Lantana camara*

The histograms with the same letter are not significantly different according to the Newman and Keuls Test at the 5% threshold.

III. Influences of leaf extracts and synthetic growth regulators on rooting of *Biophytum sp* and *Psorospermum nervosum*

After 4 weeks of culture, the explants have produced roots. The number and length of roots varied depending on the type of culture medium and according to the species. From the

statistical analysis, the results were significantly indifferent between all treatments. But the specific effect of each treatment (type of culture medium used) was noted.

Concerning of the effects of leaf extracts and synthetic growth regulators on number of roots, for *Biophytum sp* (Figure 5), the maximum root production (4 per vitroplant) was observed with the highest concentration (2.5%) of *Symphytum officinale* leaf extract (C5), but with the lower dose (0.1%) for *Lantana camara* leaf extract (L1). Besides, the number of roots decreased (3.6 to 2.5 roots per explant) when the concentration of *Lantana camara* leaf extract increased (0.1 to 2.5%). Moreover, there was a significantly difference between the effect of NAA and the leaf extracts on the rooting of this species. Thus, adding 0.1% of NAA in the culture medium led to the maximum rhizogenesis (an average of 5.6 roots per vitroplant).

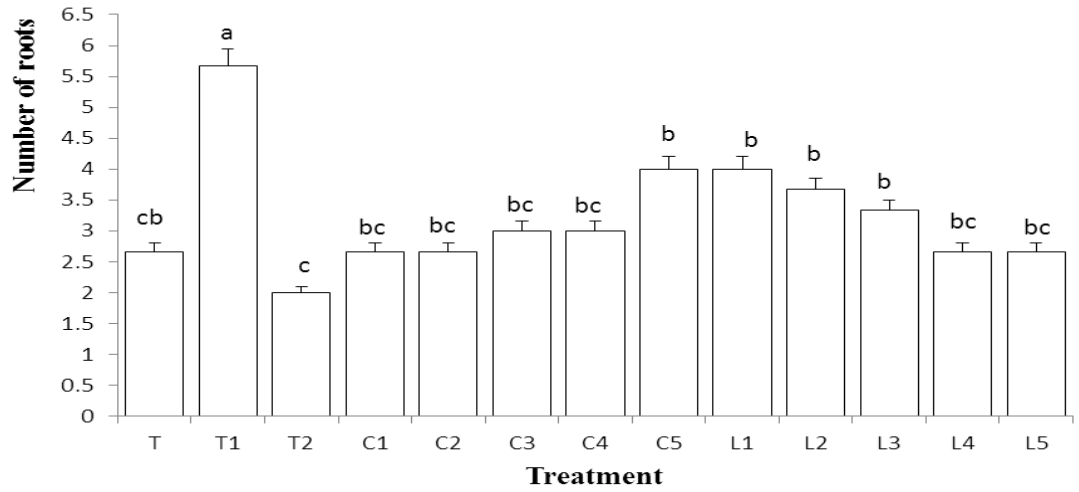

Figure 5: Mean number of roots of *Biophytum sp.* according to different treatments after 4 weeks of culture

T : MS/2 (control), T1 : + 0.1% NAA, T2 : +0.1% BAP, C1 : +0.1% *Symphytum officinale*, C2 : +0.5% *Symphytum officinale*, C3 : +1 % *Symphytum officinale*, C4 : +2% *Symphytum officinale*, C5 : +2.5% *Symphytum officinale;* L1 : +0.1% *Lantana camara*, L2 : +0.5% *Lantana camara*, L3 : +1 % *Lantana camara*, L4 : +2% *Lanatana camara*, L5 : +2.5% *Lantana camara*

The histograms with the same letter are not significantly different according to the Newman and Keuls Test at the 5% threshold.

In *Psorospermum nervosum* (Figure 6), the maximum number of roots (3.6 per vitroplant) was observed in the culture media containing the same concentration (2.5%) of *Symphytum officinale* and *Lantana camara* leaf extracts (C5, L5). On the other hand, in the control medium (T) and in the medium containing 0.1% extract of *Lantana camara*, only a few roots was produced, in this case, there was an average of 0.6 roots produced per vitroplant.

0.1% of NAA appears to have a similar effect to the leaf extracts of *Symphytum officinale* and *Lantana camara* at 2.5%. In these media, an average of 3.3 roots per explant was produced.

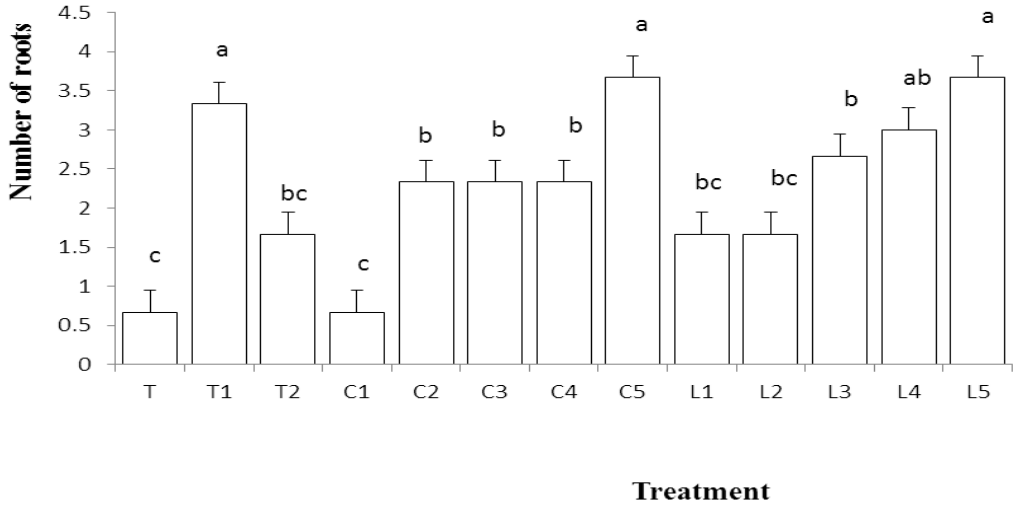

Figure 6: Mean number of roots of *Psorospermum nervosum* according to different treatments after 4 weeks of culture

T : MS/2 (control), T1 : + 0.1% NAA, T2 : +0.1% BAP, C1 : +0.1% *Symphytum officinale*, C2 : +0.5% *Symphytum officinale*, C3 : +1 % *Symphytum officinale*, C4 : +2% *Symphytum officinale*, C5 : +2.5% *Symphytum officinale;* L1 : +0.1% *Lantana camara*, L2 : +0.5% *Lantana camara*, L3 : +1 % *Lantana camara*, L4 : +2% *Lanatana camara*, L5 : +2.5% *Lantana camara*

The histograms with the same letter are not significantly different according to the Newman and Keuls Test at the 5% threshold.

As for the effects of leaf extracts and synthetic growth regulators on the length of newly formed roots, in *Biophytum sp*, (Figure 7), the maximum root elongation was observed in the presence of the highest concentration (2.5%) of *Symphytum officinale* leaf extract (C5) and of the lowest concentration (0.1%) of *Lantana camara* (L1). In these culture media, the roots

were respectively 7mm and 8mm. The shorter roots (3.3mm) were produced in the control medium (T). However, 0.1% of ANA (T1) induced the maximum root growth compared with BAP and leaf extracts. The mean length of the roots was 9.3mm.

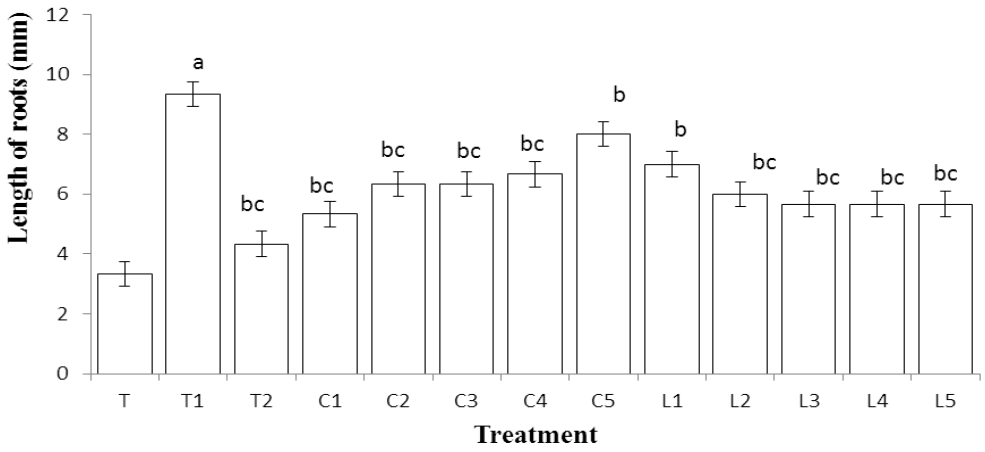

Figure 7: Average length of roots of *Biophytum sp.* according to different treatments after 4 weeks of culture

T : MS/2 (control), T1 : + 0.1% NAA, T2 : +0.1% BAP, C1 : +0.1% *Symphytum officinale*, C2 : +0.5% *Symphytum officinale*, C3 : +1 % *Symphytum officinale*, C4 : +2% *Symphytum officinale*, C5 : +2.5% *Symphytum officinale;* L1 : +0.1% *Lantana camara*, L2 : +0.5% *Lantana camara*, L3 : +1 % *Lantana camara*, L4 : +2% *Lanatana camara*, L5 : +2.5% *Lantana camara*

The histograms with the same letter are not significantly different according to the Newman and Keuls Test at the 5% threshold.

In *Psorospermum nervosum*, the Figure 8 shows that the root length increased with the concentration of leaf extracts (C1-C5) and (L1-L5). The maximum length of roots (7.3mm) was observed at 2.5% for *Symphytum officinale* and at 2 to 2.5% for *Lantana camara* leaf extracts (7.6mm). On the other hand, the addition of 0.1% of NAA induced the maximum root elongation (4.6 mm) compared to hormone free medium and to the medium supplemented with 0.1% BAP. Thus, the effect of leaf extracts was stronger than that of ANA.

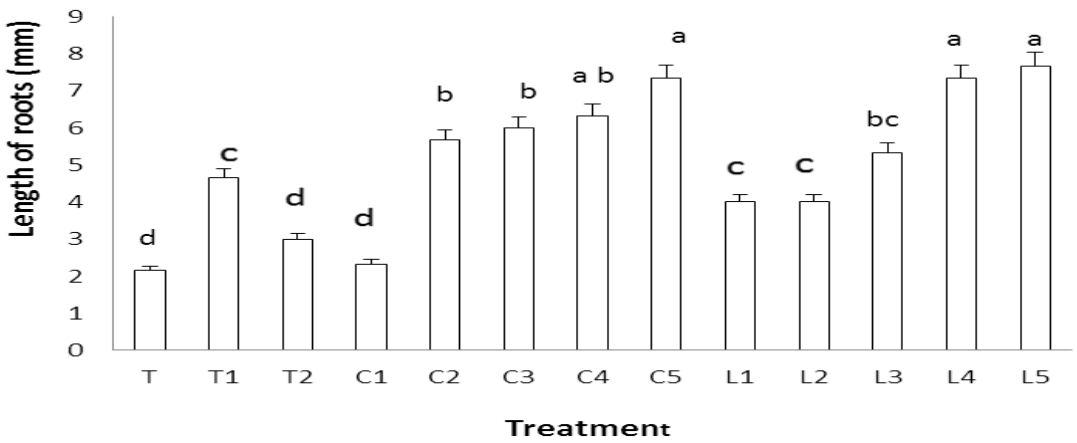

Figure 8: Average length of roots of *Psorospermum nervosum* according to different treatments after 4 weeks of culture

T : MS/2 (control), T1 : + 0.1% NAA, T2 : +0.1% BAP, C1 : +0.1% *Symphytum officinale*, C2 : +0.5% *Symphytum officinale*, C3 : +1 % *Symphytum officinale*, C4 : +2% *Symphytum officinale*, C5 : +2.5% *Symphytum officinale;* L1 : +0.1% *Lantana camara*, L2 : +0.5% *Lantana camara*, L3 : +1 % *Lantana camara*, L4 : +2% *Lanatana camara*, L5 : +2.5% *Lantana camara*

The histograms with the same letter are not significantly different according to the Newman and Keuls Test at the 5% threshold.

DISCUSSION

The specific effects of synthetic growth regulators on the shoot regeneration showed that 0.1% of BAP promoted the maximum production of shoots in both species « *Biophytum sp* » and "*Psorospermum nervosum* ». This result agrees with that obtained by Meftahizade et al, (2010) on *Melissa officinalis* L. because of the property of cytokinin such as the removal of apical dominance and the awakening of dormant axillary buds. As regards of rooting of *Biophytum sp* and *Psorospermum nervosum*, the presence of 0.1% of NAA in the culture medium improved the production and elongation of roots. According Margara (1989), auxins stimulated cell division and differentiation of roots. This result is in agreement with those obtained during the work of Vikrant and Rasmid (2001), on *triticale sp*, showing that the NAA has a great ability to activate rooting.

Many plant extracts have been used *in vitro* culture. These natural products have been adduced as supplements to the culture medium or as substituents of plant hormones (Rahelivololona, 2005; Ramanamidona, 1998; Dinaharilala, 2012). From the researches on orchid species, it was shown that the application of organic extracts of green coconut, banana,

and pineapple has positive effects on the *in vitro* culture of *Aeranthes glandiflora*, of *Aeranthes anternophora* and *Bulbophyllum peyrotti*, (Ramanampamonjy, 2004; Dinaharimalala, 2011).

The leaf extracts have stimulated the growth of shoots and rooting of *Biophytum.sp* and *Psorospermum nervosum.* Results depend on the type and concentrations of leaf extracts and according to the species studied : 1% of *Symphytum officinale*, and 2.5% of *Lantana camara* leaf extracts have allowed shoots regeneration and roots development in *Biophytum. sp* and *Psorospermum nervosum*. The same result occurred with 0.1% BAP. These results coincide with those obtained in *Hibiscus cannabinus*. L. and in *Psorospermum sp* (Rakotoarisoa, 2013).

Generally, leaf extracts of *Symphytum officinale* and *Lantana camara* have higher effects than those of synthetic plant growth regulators on the growth and development of *Biophytum. sp* and *Psorospermum nervosum.* Indeed, it seems possible that *Symphytum officinale* and *Lantana camara* leaf extracts contain active molecules having similar properties (homologous) to the growth regulators.

Besides, *Symphytum officinale* is a plant rich in organic and inorganic substances such as nitrogen, calcium, potassium, phosphorus, iron, silica, magnesium, copper, potassium, boron , manganese, and zinc (Bernard Bertrand, 1999 ; Joseph Poussé, 2002 ; Rahetlah, 2002 ; http:// 5 ; http:// 6. According to Heller (1949), some macro and microelements play an important role in stimulating the proliferation and growth of *in vitro* plants. These elements contribute to better process of breathing during *in vitro* propagation (Heller, 1949; http://2). Furthermore, *Symphytum officinale* has been grown since 1989 to be used as green manure or liquid manure, as fertilizer and / or insecticide (Rasoanaivo., 1995; http://7)

As for *Lantana camara,* it is very rich in secondary metabolites, some of which are considered as fertilizers agents. (http://8). In addition, the presence of minerals in the extract increase the mineral content in the culture medium, in particular potassium and nitrogen. These substances are favorable to induce *in vitro* organogenesis of plants, include the case of *Feijoa sellowina* (Delvesco and Guerra, 2001) and *Cunila galioide* (Fracaro and Echeverri Garay, 2001).

However, increasing the concentration of *Lantana camara* leaf extract led to a decrease in root length of *Biophytum sp*. This result could be due firstly, to the presence of active

substances pentacyclic triterpenoids in the plant which are responsible for the toxicity of the plant (Barceloux, D.G, 2008). Moreover, *Lantana camara* also excrete chemicals (allelopathy) whose main property is to reduce the growth of surrounding plants by inhibiting germination and elongation of the root.

CONCLUSION

It appears from this work that in *Biophytum sp,* the addition of 1% of *Symphytum officinale* and 2.5% of *Lantana camara* leaf extracts has promoted shoot regeneration. Moreover, 0.5 to 2% of *Symphytum officinale* and 2.5% of *Lantana camara* leaves extracts allowed the maximum regenerated shoot growth. These concentrations have the same effect as 0.1% BAP. Thus, the latter could be replaced by leaf extracts at concentrations above. The best rooting and their maximum elongation were obtained in culture media supplemented with 2.5% of *Symphytum officinale* and 0.1% of *Lantana camara* leaves extracts. This result was almost identical to that observed using 0.1% ANA. As for rhizogenesis, the substitution of ANA by the leaf extracts is also possible.

In *Psorospermum nervosum*, the average number of shoots produced was maximal at a concentration of 2.5% of *Symphytum officinale* leaf extract. This result is similar to that obtained in the culture medium supplemented with 0.1% BA. Regarding to the shoots growth, the maximum was obtained on media with 2.5% of *Symphytum officinale* leaf extract. The effects of 0.1% BAP and 2.5% of *Lantana camara* were similar but slightly lower than those of *Symphytum officinale* leaf extract at 2.5%. Indeed, for the growth of *Psorospermum nervosum*, BAP could be replaced by 2.5% of *Symphytum officinale* leaf extract.

The best rooting was observed in culture media with the same concentration 2.5% of leaf extracts and with 0.1% of NAA. The substitution of NAA with *Symphytum officinale* and *Lantana camara* leaf extracts is therefore recommended.

Indeed, the appropriate culture media for the *in vitro* propagation of "*Biophytum sp"* and "*Psorospermum nervosum",* two species endemic and endangered by mining at Ambatovy Madagascar were optimized. For the *in vitro* growth and development of these species, the comparative study of the effects of leaves extracts and plant growth regulators, showed the possibility of the substitution of *Symphytum officinale* and *Lantana camara* leaves extracts by synthetic plant growth regulators (BAP and ANA). It would be a promising alternative to reduce production costs, to facilitate the availability of products, and to valorize local natural substances.

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