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Meta-analysis of *Lamiaceae* and *Euphorbiaceae* medicinal plants inoculated with arbuscular mycorrhizal fungi

Sonivaldo Ruzzene Beltrame[1,2], Rayane Monique Sete da Cruz[1], Emerson Luiz Botelho Lourenço[3], Odair Alberton[3*]

[1]Programa de Pós-graduação em Biotecnologia Aplicada à Agricultura; Universidade Paranaense – UNIPAR; Praça Mascarenhas de Moraes; 4282; 87502-210; Umuarama, PR, Brasil
[2]Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso do Sul – IFMS, Campus Dourados; Rua Filinto Müller, 1790; 79833-520; Dourados, MS, Brasil
[3]Programa de Pós-graduação em Plantas Medicinais e Fitoterápicos na Atenção Básica. Laboratório de Pesquisa Pré Clínica de Produtos Naturais e Bioativos, Universidade Paranaense, Umuarama, PR, Brasil

***Corresponding author: odair@prof.unipar.br; oalberton@yahoo.com.br (Alberton O.).**

Abstract

Lamiaceae and *Euphorbiaceae* are two families of plants grown as medicinal, aromatic, food and ornamental crops, and are of great commercial importance. The cultivation of these species depends on soil quality and availability of soil resources. Arbuscular mycorrhizal fungi (AMF) usually penetrates plants through their roots, supplying plants with water and nutrients and receiving photosynthesis products in return. These types of symbiosis benefit the development and production of crops. To analyze the effects of AMF inoculation on the production of plants of these families, a meta - analysis was performed using 183 data of *Lamiaceae* plants and 68 data of *Euphorbiaceae* plants. Meta-analysis consists of compiling data from literature to obtain the response ratio, calculated by the mean of the experimental group divided by the mean of the control group. The response variables were shoot dry mass (SDM), total dry mass (TDM), plant height and accumulation of phosphorus in the plant shoot (P-shoot). Results showed an increased mean production of AMF-inoculated plants with a 96% and 97% increase of SDM in *Lamiaceae* and *Euphorbiaceae*, respectively. Increases of 91% in TDM and 248% in P accumulated in the plant shoot were observed for *Lamiaceae*; values that were higher than those of *Euphorbiaceae* at 110% and 675%, respectively. This meta-analysis confirmed the potential of AMF to increase biomass production and P accumulation in medicinal plants of the *Lamiaceae* and *Euphorbiaceae* families.

Keywords: Mycorrhiza, symbiosis, biomass, phosphorus.
Abbreviations: AMF_ arbuscular mycorrhizal fungi; P_ phosphorous; SDM_shoot dry mass; TDM_total dry mass

Introduction

The *Lamiaceae* family includes plants of great representativeness in the planet, totaling approximately 7,000 native species, mostly in the Mediterranean region. In Brazil, there are about 500 species belonging to this family, cultivated as aromatic, medicinal, ornamental and food crops (Mariutti and Bragagnolo, 2007; Oliveira et al., 2016). These species are widely used in the food industry as components of antioxidant substances due to their high level of phenolic compounds, such as rosmarinic acid and caffeic acid (Mariutti and Bragagnolo, 2007). Its antioxidant substances are used in products that act as preservatives to increase the shelf life of foods, avoiding the rapid thermal degradation of vegetable oils and other products rich in fat (Mariutti and Bragagnolo, 2007; Del Ré and Jorge, 2012).
Aromatic *Lamiaceae* plants are characterized by the major presence of flavonoids and terpenes. The species of this family are economically exploited for the extraction of essential oils to produce pharmaceuticals, perfumes and cosmetics (Agostini et al., 2009; Rehan 2014). *Lamiaceae* is one of the most important families for the medicinal area. Many of its species have bioactive substances beneficial for the treatment of gastric, respiratory and nervous diseases, as well as diabetes prevention. They are plants rich in compounds that have antioxidant, antimicrobial, anti-inflammatory, antifungal, antitumor and chemo preventive activities (Agostini et al., 2009; Rehan, 2014; Silva et al., 2016).
Euphorbiaceae is a family of plants composed of approximately 6,300 species, which predominantly are present in tropical regions, especially in the American and African continents (Oliveira, 2013). They have several types of trichomes and very diverse and complex morphology and taxonomy (Secco, 2005). They are among the main families of the Brazilian flora, representing about 1,100 species (Oliveira,

2013) present in practically all types of vegetation (Sátiro and Roque, 2008). They appear as herbs, climbing plants, sub-shrubs, shrubs or trees (Secco, 2005).

Among the angiosperms, it is one of the most economically important plant families, being widely used by the food, wood and medicinal sectors (Secco, 2005; Sátiro and Roque, 2008; Oliveira, 2013). They are plants rich in latex (Oliveira, 2013), have high concentration of volatile oils in the leaves and produce compounds of antiulcerogenic, anti-inflammatory and antitumor action (Rodrigues and Carvalho, 2001; Ribeiro et al., 2004; Souza and Lorenzi, 2008).

The *Euphorbiaceae* family is of great importance for human health and food (Souza and Lorenzi, 2005; Oliveira, 2013). The roots are edible and contain vitamin B, phosphorus and iron. Examples of use of these plants are cassava flour, very common in the menu of Brazilians, and castor oil, extracted from the castor bean seed (Oliveira, 2013). *Euphorbiaceae* products are also economically used in the manufacture of alcohol, paints and plastics; in heating and electrical insulation; in the production of molasses and chewing gum; and in the formulation of cosmetics and pharmaceuticals (Trindade and Lameira, 2014).

The plant's development and production capacity are directly related to the availability of soil resources, more precisely, organic matter and microbial biomass (Cudlin et al., 2007; Kaschuk et al., 2010; Aleixo et al., 2014; Lermen et al., 2015a; 2017). Arbuscular mycorrhizal fungi (AMF) contribute to the life and development of plants forming a plant-soil-mycorrhizal system (Santos et al., 2013; Lermen et al., 2015a; 2017, Urcoviche et al., 2015). This system represents a symbiotic mutualistic association that establishes in the roots of plants, where the mycorrhiza transfers water and nutrients to the plant and the plant returns carbohydrates necessary for fungus growth (Smith and Read, 2008).

AMF association results in greater development of the plants as it contributes to nutrient cycling, soil structuring and better absorption of nutrients (Truber and Fernandes, 2014). In addition to promoting plant growth, mycorrhization by AMFs contributes to increase the production of active ingredients and therapeutic substances in medicinal species (Lermen et al., 2015b; 2017, Urcoviche et al., 2015).

Mycorrhization improves plant access to minerals present in soil, among them, phosphorus (Augé et al., 2014), one of the three macronutrients essential for plants. The phosphorous level (P) in plant shoot is relevant to investigate plant development and growth (Cruz et al., 2017) since phosphorous participates of plant life processes such as photosynthesis, cell division and growth and synthesis of ATP (Schumacher et al., 2003; Cruz et al., 2017). Therefore, phosphorous contributes to plant vigor (Silva et al., 2010), early root growth, seed and fruit formation and plant biomass production (Cruz et al., 2017; Saldanha et al., 2017).

Meta-analysis consists of compiling data from literature to obtain the response ratio, calculated by the mean of the experimental group divided by the mean of the control group (Alberton et al., 2005; 2014). It is a powerful instrument of statistical analysis and synthesis that measures the impact of a phenomenon on the object of study, extrapolating individual results for general analysis (Gurevitch and Hedges, 2001). Meta-analysis studies have been increasingly performed in scientific studies, in which a percentage index represents the response ratio that provides a quantitative comparison of the experimental group with the control group. Such ability to generalize and size a given object of study is of great relevance for the development of science, as it gives a confirmation of the general applicability of research results (Gurevitch and Hedges, 2001).

In practical terms, the meta-analysis benefits the farmer by providing a statistical parameter (%) of how much the inoculation with AMFs alters the production of *Lamiaceae* and *Euphorbiaceae* species. Kivlin et al. (2013) used meta-analysis to investigate the effect of symbiosis between plants and AMFs in plants subjected to four types of stress (global warming) that can change the plant development (presence of $CO_{2,}$ dry conditions, heating and N disposition). It was observed that symbiont fungi increased the plant biomass production and the N applied to plants potentiated the increase in biomass.

He et al. (2014) performed meta-analysis to investigate the effects of *Glomeraceae* and *non- Glomeraceae* AMFs in plants cultivated in soils with and without heavy metals. Both fungi groups increased the biomass production of plants cultivated in the three levels of heavy metals. The *Glomeraceae* group presented more biomass production in plants with higher levels of heavy metals while the *non- Glomeraceae* group presented more biomass production in plants cultivated without heavy metals.

Rúa et al. (2016) used meta-analysis to examine how adaptive some plant-soil-AMF systems are. The authors verified that the totally sympatric system caused more biomass production than the totally allopatric system; the soil-plant sympatric relation but allopatric to the fungus produced more biomass than the totally allopatric system; and the soil-fungus sympatric relation but allopatric to the plant caused no effect in biomass production, indicating poor adaptation of fungus to soil. These results suggested that geographic origin of plants and fungi is a relevant factor influencing biomass production and that local adaptation is a power factor to create fungus-soil-plant systems.

This study aims at evaluating the influence of AMF inoculation in the growth and biomass of medicinal plants belonging to the *Lamiaceae* and *Euphorbiaceae* family by examining variations in biomass production and level of phosphorous (P) accumulated in the shoot of these plants. Considering that mycorrhizal inoculated plants have their development and production favored, meta-analysis is the method chosen to estimate variations in biomass production and P accumulated in the shoot of *Lamiaceae* and *Euphorbiaceae* plants inoculated with AMF. Unpublished and relevant information on such biomass production, especially for the producers of plants of these families, are discussed in this work.

Results and Discussion

Meta-analysis general information

This meta-analysis was based on studies of AMF inoculation influencing biomass production of plants belonging to *Lamiaceae* and *Euphorbiaceae.* Data on biomass production and P level on plant shoot were searched in 43 published papers about *Lamiaceae* and 13 published papers on

Euphorbiaceae, totaling 183 and 68 references respectively. Results are presented in Table 1.

Considering all variables investigated in the meta-analysis for *Lamiaceae* (n=143), it was observed that AMF inoculation positively increased biomass production at 106%. Plant height and biomass production (n=143) increased together at 79%. Each single variable increased at 17% (height, n=23), 96% (SDM, n=84), 91% (TDM, n=36), 248% (P-shoot, n=40) (Figure 1).

Considering all the variables examined for the AMF inoculation effects on *Euphorbiaceae* (n=68), an increase of 62% in biomass production was observed. Plant height and biomass production (n=45) increased together at 81%. Each single variable increased at 13% (height, n=1), 97% (SDM, n=34), 43% (TDM, n=10), 32% (P-shoot, n=23) (Fig 2).

Significant increases in plant height and biomass production above the root were similar in both families of plants. Meta-analysis results for SDM are expressive because values double with AMF inoculation, which can increase the market value of production and profits for the producer. These results confirm the findings of Jayne and Quigley (2013), who investigated by meta-analysis the influence of AMF inoculation in plants subjected to water stress and concluded that these plants had their biomass increased by the presence of these fungi on their roots. The AMF-inoculated plants of both families had their height increased (Figure 3). However, only a single study was found for *Euphorbiaceae*. The plant height was the last criterion used to evaluate biomass for *Euphorbiaceae*; therefore, it does not interfere drastically with the results. The AMF potential to promote biomass production in *Lamiaceae* is confirmed by an 17% increase in height of the mycorrhizal plants and these results agree with those of Smith and Read (2008). Cuenca et al. (1990) also verified that AMF inoculation increases the height and biomass production in cocoa plants.TDM production also increased in both families (Figure3). However, *Lamiaceae* presented a 91% increase in TDM, which was 110% higher than *Euphorbiaceae*. It suggests that AMF inoculation for *Lamiaceae* crops is potentially more beneficial. Increases in total biomass production with AMF inoculation verified in this study confirm the fungi's ability to promote better yields for these crops (Alberton et al., 2014) with sustainability. According to Urcoviche et al. (2015), the mycorrhization practice reduce the use of defensives, helping the environment preservation and bringing benefits to human health. SDM values (96% for both families) and TDM values (Figure 3) indicate that the root biomass of *Lamiaceae* plants is greater than *Euphorbiaceae,* evidencing that AMF inoculation is potentially more beneficial for *Lamiaceae* crops, especially in those species whose metabolites are concentrated in roots.

This finding is in line with the results of Nell et al. (2009), who verified that root biomasses were greater than shoot biomasses of *Salvia officinalis* L. inoculated with AMFs. Souza et al. (2013) verified that increased levels of P raised biomass production of Mentha *piperita* L. Although the increased P availability to the plant is a common condition in mycorrhizal plants (Chandrasekaran et al., 2016), Souza et al. (2013) also observed that the highest levels of P caused decreasing increment in shoot biomass in contrast to increasing increment in root biomass.

Like the TDM results, AMF inoculation increased the P accumulated in the plant shoot of both families (Figure 3). In *Lamiaceae* this increase (248%) was 675% higher than *Euphorbiaceae*, again demonstrating that AMF inoculation is potentially more beneficial for *Lamiaceae* crops as P is an essential nutrient to plant growth (Cruz et al., 2017). It is important that plants accumulate P because this nutrient plays several roles in cell metabolism (Schumacher et al., 2003; Silva et al. 2010; Cruz et al., 2017), constituting nucleic acids or biomembranes and acting in primary and secondary metabolisms (Nell et al., 2009). In addition, a higher amount of P accumulated by inoculated plants indicates that their production was increased by the presence of P (Augé et al., 2014). Chandrasekaran et al. (2016) affirm that AMF-inoculated plants can uptake more P and other soil nutrients, improving their development conditions and biomass production (Silva et al., 2010; Cruz et al., 2017).Like this study, biomass production related to increased P levels on plant shoot was verified in the literature. Meta-analysis investigated the responses of leguminous species to rhizobia and AMFs, the interactive effects of plant microbial symbionts and the influence of AMF inoculation on plants grown in saline soils (Kaschuk et al., 2010; Larimer et al., 2010; Chandrasekaran et al., 2016). Increases in plant growth, biomass production and nutrient uptake have been proven, suggesting that AMFs help plants supply their demand for P and improve their survival and production conditions (Chandrasekaran et al., 2016).

Lamiaceae responses

The mycorrhizal species most used in *Lamiaceae* inoculation were *Funneliformis mossae* (n=54), *Rhizophagus intraradices* (n=29), *R. irregularis* (n=20), *Claroideoglomus etunicatum* (n=17) and *R. fasciculatus* (n=16) (Figure 4). It is observed that *F. mossae* was used in 1/3 of the essays. About 6% of the essays used combinations of different fungi. Assays were also done with *Glomus versiforme*, *G. rosea, Rhizophagus clarus, Gigaspora margarita*, *Septoglomus viscosum*, *Acaulospora* sp., coded-commercial and native fungi from regions where some studies were performed.The major *Lamiaceae* species used in studies to identify the effect of AMF inoculation on biomass production are *Mentha* sp. (n=49), *Oscimum basilicum* L. (n=26), *Lavandula spica* L. (n=24), *Origanum* sp. (n=22) and *Salvia officinalis* L. (n=16) (Figura 5). Further studies on AMF inoculation were found for *Pogostemon* sp., *Satureja macrostema, Rosemarinus officinalis* L., *Coleus forskohlii, Thymus* sp., *Teucrium scorodonia* and *Glechoma heredaceae.* Considering the great representativeness of Lamiaceae in the global flora, its vast occurrence on continents and its potential for medicinal use (Mariutti and Bragagnollo, 2007; Oliveira et al., 2016; Silva et al., 2016), the amount of studied species is small. Only 12 types of *Lamiaceae* in this meta-analysis study was found.

Euphorbiaceae responses

The AMF species most used in *Euphorbiaceae* inoculation were *Rhizophagus fasciculatus* (n=10), *R. irregularis* (n=06) and *R. intraradices* (n=4) (Figure 6). It is observed that combinations of different fungi were used in 1/3 of the

Table 1. Meta-analysis for shoot dry mass (SDM), total dry mass (TDM), plant height and P level in the shoot (P-shoot) of AMF-inoculated *Lamiaceae* and *Euphorbiaceae* plants

Family	Variables	R	95% CI	n
Lamiaceae	Height	1.17	1.06 – 1. 28	23
	SDM	1.96	1.69 – 2.27	84
	TDM	1.91	1.62 – 2.24	36
	Height + TDM + SDM	1.79	1.61 – 1.97	143
	P-shoot	3.48	2.39 – 5.06	40
	Height + TDM + SDM + P-shoot	2.06	1.84 – 2.32	183
Euphorbiaceae	Height	1.13	0.94 – 1.32	01
	SDM	1.97	1.50 – 2.58	34
	TDM	1.43	1.05 – 2.07	10
	Height + TDM + SDM	1.81	1.45 – 2.26	45
	P-shoot	1.32	1.19 – 1.43	23
	Height + TDM + SDM + P-shoot	1.62	1.38 – 1.92	68

R: response ratio; CI: confidence interval; n: number of observations.

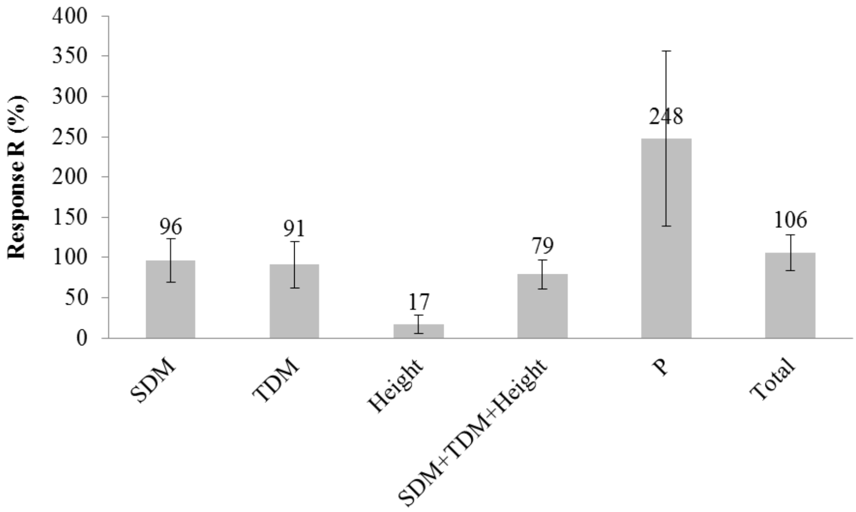

Fig 1. Percent increase (Bars: 95% confidence interval) of the production in *Lamiaceae* plants inoculated with AMFs. SDM: shoot dry mass. TDM: total dry mass. P: phosphorus.

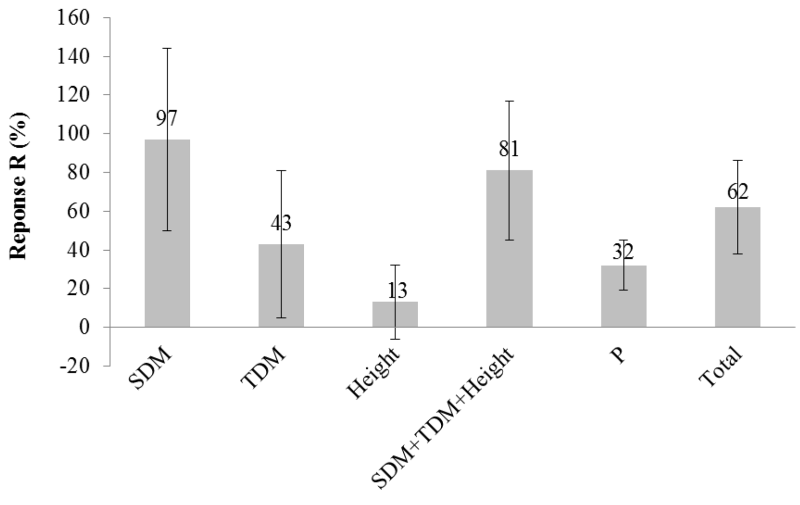

Fig 2. Percent increase (Bars: 95% confidence interval) of the production in *Euphobiaceae* plants inoculated with AMFs. SDM: shoot dry mass. TDM: total dry mass. P: phosphorus.

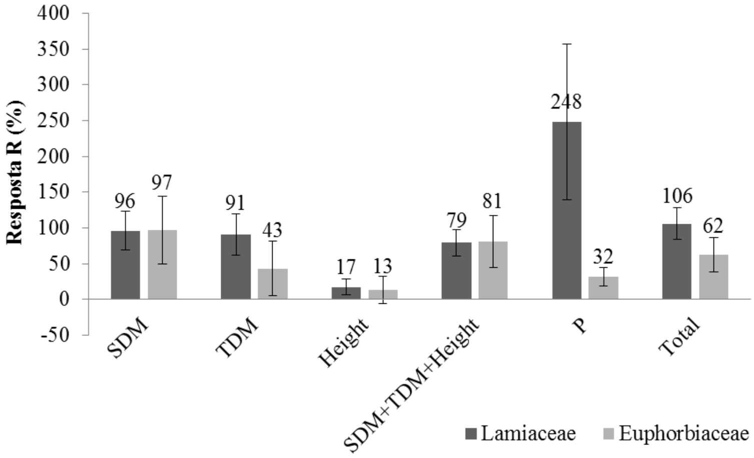

Fig 3. Increases in biomass production (Bars: 95% confidence interval) of *Lamiaceae* (Dark Gray Bar) and *Euphorbiaceae* (Light Gray Bar) plants inoculated with AMFs. SDM: shoot dry mass. TDM: total dry mass. P: phosphorus.

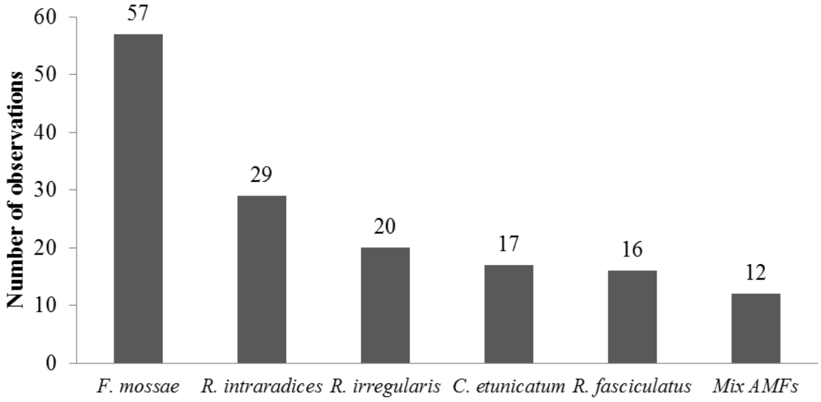

Fig 4. Number of observations of AMF species inoculated in *Lamiaceae* plants (*F. mossae*: *Funneliformis mossae*. *R. intraradices*: *Rhizophagus intraradices*. *R. irregularis*: *Rhizophagus irregulars*. *C. etunicatum*: *Claroideoglomus etunicatum*. *R. fasciculatus*: *Rhizophagus fasciculatus*).

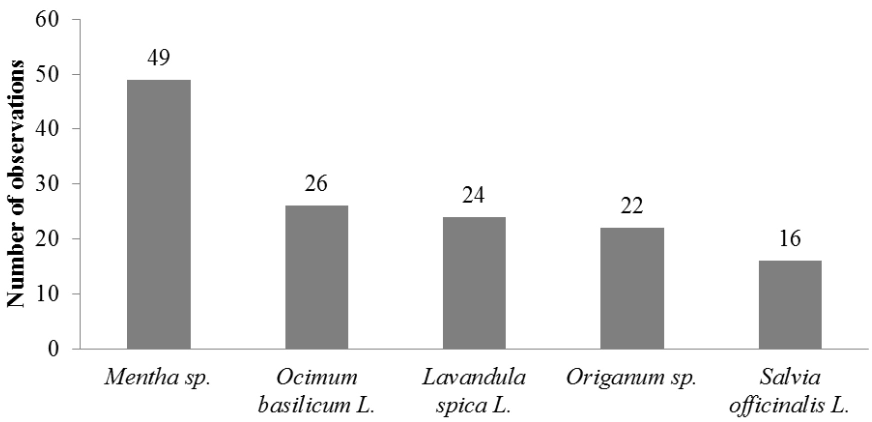

Figure 5. Number of observations of *Lamiaceae* plants inoculated with AMFs.

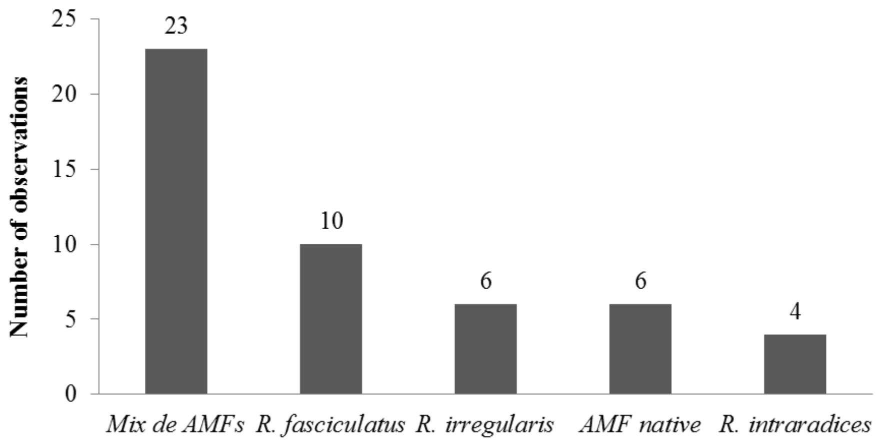

Fig 6. Number of observations of AMF species inoculated in *Euphorbiaceae* plants (*R. fasciculatus*: *Rhizophagus fasciculatus*. *R. irregularis*: *Rizhophagus irregularis*. *R. intraradices*: *Rizhophagus intraradices*).

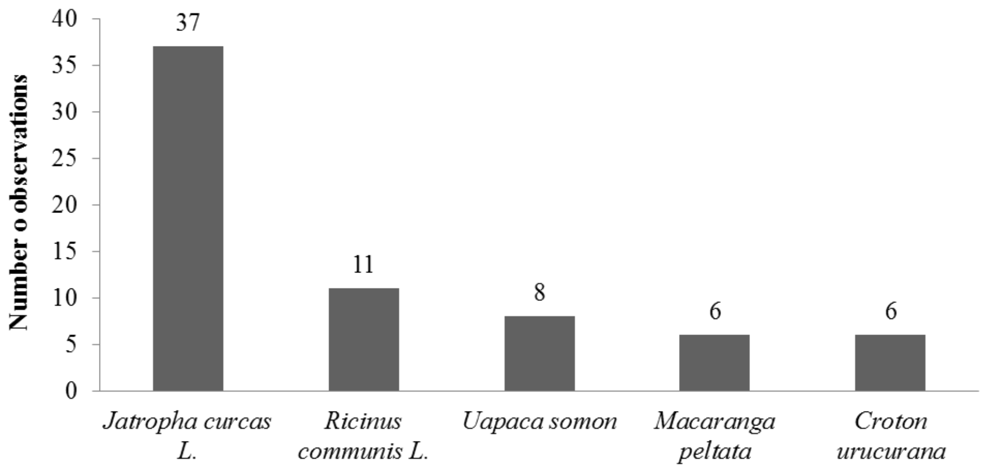

Fig 7. Number of observations of *Euphorbiaceae* plants inoculated with AMFs.

essays. About 8% of the essays used native fungi. Assays were also done with *Acauplospora* sp. and coded-commercial fungi.

The *Euphorbiaceae* species used in studies to identify the effect of AMF inoculation on biomass production are *Jatropha curcas* L. (n=37), *Ricinus communis* L. (n=11), *Uapaca somon* (n=8), *Macaranga petalta* (n=6) and *Croton urucurama* (n=6) (Figure 7).

There are few studies on AMF inoculation in *Euphorbiaceae.* Only five species among about six thousand were investigated (Oliveira, 2013) and considering all data of this meta-analysis, more than a half of the studies (54%) are about AMF inoculation on *Jatropha curcas* L., because this species is much used by the fuel industry to produce biodiesel (Koh and Gahzi, 2011).

Such studies also collaborate with the medical field because *Jatropha curcas* L. has potential for composing medicinal drugs. Its substances are used as antibiotics, anticancer, anti-HIV, in influenza treatment (Prasad et al., 2012) and as anti-inflammatories. The substances of *Jatropha curcas* L. also have a healing effect and may be used in the treatment of dermatitis, malaria, schistosomiasis and control of dengue vectors (Santos et al., 2008).

In this meta-analysis, about one in thirteen trials were performed in the field. Of the total field trials, six trials were on inoculation of AMF in *Lamiaceae* (3.3%) and fourteen on inoculation of AMF in *Euphobiaceae* (20.6%). The increased biomass production verified in AMF-inoculated plants, cultivated either in open field or in controlled environments, agree with the meta-analysis results on AMF inoculation performance in response to AMF abundance, legume species and water deficit (Lekberg and Koide, 2005; Kaschuk et al., 2010; Jayne and Quigley, 2013). Nell et al. (2009) affirm that AMF-inoculated plants are more resistant to pathogens that theoretically increase the production of field-cultivated plants.

This meta-analysis confirmed the study's hypothesis: "The AMF inoculation contributes to increase the production of biomass and accumulation of phosphorous in the shoot of *Lamiaceae* and *Euphorbiaceae* plants". Such contribution adds market value to crops since mycorrhization reduces the use of defensives, making crops more sustainable. Therefore, further field studies should be performed to confirm the AMF response to open field cultivation, especially of *Lamiaceae* plants. Considering that tests with

Lamiaceae is 170% more numerous than tests with *Euphobiaceae,* further research with *Euphobiaceae* is recommended to confirm the results of this meta-analysis, since this family is of great importance to the value chain of herbal medicines.

Materials and Methods

Data collection, processing and analysis followed the methodology described by Alberton et al. (2014), with adaptations for this study as presented below:

Data collection

Data were gathered from Scientific Eletronic Library Online - Scopus, Scielo and Pubmed, with indexed keywords Mycorrhiza; *Lamiaceae*; Medicinal Plants to search papers on *Lamiaceae* family and Mycorrhiza; *Euphorbiaceae*; Medicinal Plants to search papers on *Euphorbiaceae* family. The collection period was from January 2 to May 26, 2017. Data collection from Google Scholar was limited to the first 150 papers on each plant family, published between the search date and the year 2012, whereas Google uses the PageRank algorithm to display the search results in descending order of relevance (Brin and Page, 2012).

Variables

Data were collected on shoot dry mass (SDM), total dry mass (TDM), height (h) of the plant and phosphorus content on the plant shoot (P-shoot).
The target variables of this study, mentioned above, are characteristics that indicate the production of *Lamiaceae* and *Euphobiaceae* plants commercially cultivated. The amount of biomass, plant height and P level in the shoot are equally important variables, since they are directly related to the survival and development capacity of the plants.
Studies that did not present the target variables were disregarded. When the target variable was measured more than once in the same study, data was prioritized according to the order of importance previously defined (according to next item below). Only the last sampling date was used for meta-analysis.

Order of importance for variable choice

To avoid bias and maintain the independence of data, studies were selected for only one measurement variable since multiple variables in the same study transgress the presumption of data independence (Gurevitch and Hedges, 2001).
The order of priority determined to classify the responses of AMF-inoculated plants was: shoot dry mass (SDM), total dry mass (TDM), plant height (h) and level of P in the plant shoot (P-shoot).

Statistical analysis

The papers' data were separated into two groups: experimental (AMF-inoculated plants) and control (non-inoculated plants), considering their standard deviation (SD) and replicate number (*n*). Relevant observations were typed in specific fields. Preliminarily, the data collected from literature were recorded in Microsoft Excel worksheets. Subsequently on June 1, 2017, calculations were performed using MetaWin® 2.0 in the same worksheets.
The reported measurement units were not considered since the calculation response ratios are dimensionless. Data presented into graphics had values estimated to entry. Data collected for standard error (SE), variance (S^2) or coefficient of variation (CV) had their values converted to standard deviation (SD). They were converted to SD according to the equations:
Equation 1: $SD = SE.\sqrt{n}$
Equation 2: $SD = (CV/100).\bar{x}$
Equation 3: $SD = \sqrt{S^2}$
where: SD is the standard deviation, SE is the standard error, *n* is the replicate number, CV is the coefficient of variation, $\bar{x}$ is the mean and S^2 is the variance (Pimentel-Gomes, 2009).
The response ratio is calculated by the mean of the experimental group (AMF-inoculated plants) divided by the mean of the control group (non-inoculated plants). Results were reported by the weighted mean response ration (*R*), the 95% confidence interval for R (CI), and the number of observations (*n*). A response proportion of one (1) indicates no effect.
The ratio response calculation according to Gurevitch and Hedges (2001) is simplified:

1) the response ratio "r" obtained in each test is calculated by dividing the mean of the experimental group by the mean of the control group;
2) the natural logarithm of "r" (ln r) for each test is calculated;
3) the mean of natural logarithms is calculated, obtaining the "R" response ratio of the experimental group in relation to the control group;
4) the weighted variance, the standard deviation and the 95% confidence interval for the mean "R" are calculated;
5) statistical inference is made, and the result obtained for the "R" response ratio is discussed, comparing these results with previously published results on the research topic.

Transformation of each "r" response ratio by calculating "R" from each natural logarithm of "r" is necessary because this is the procedure indicated for analyzing data in the biology field. This conversion reduces the risk of occurrence of statistical errors that may cause bias in the meta-analysis results (Gurevitch and Hedges, 2001).
A random effect model was used when the value of pooled within-class variance (S^2) (item 4 described above) was higher than zero, and a fixed effect model was used when the quantity was equal or lower than zero.
Response variable means (R) were considered significantly positive if the lower endpoint of the 95% CI was higher than one.

Conclusion

Inoculation of *Lamiaceae* and *Euphorbiaceae* plants with AMF is a sustainable alternative of production since it increased height, P-shoot, SDM and TDM of plants belonging to these families.

The AMF-inoculated *Lamiaceae* and *Euphorbiaceae* plants presented similar increases in SDM production (about 96%) and plant height (about 15%).

Increases of 91% in TDM and 248% in P accumulated in the plant shoot were observed for *Lamiaceae*; values higher than those of *Euphorbiaceae* at 110% and 675%, respectively.

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