# English Version

## Biomass production and essential oil of *Plectranthus neochilus* Schlechter cultured in field under increasing doses of organic manure

*Louise Ferreira Rosal[1]; José Eduardo Brasil Pereira Pinto[2]; Renata da Silva Brant[3].*


## Abstract

With the purpose of evaluate increasing doses of organic manure in *Plectranthus neochilus* Schlechter plants, this work studied dry biomass production from vegetal material, content and essential oil yield. The experiment was conducted in an experimental field at the Federal University of Lavras (UFLA) in 2006. The scions, after been acclimatized were taken to field and transplanted to beds. It was used randomized complete block design with three replications of five parcels each. The parcels were made of 16 plants; the four central ones were considered the useful parcel. The treatment had 5 cattle manure doses: 0.0; 2.5; 5.0; 7.5; 10.0 kg $m^{-2}$. The plants were harvested at 180 days old, part of the fresh leaves were for essential oil extraction and the rest of the vegetal material were sent to an oven to dry until they had a constant mass for the dry biomass verification. The essential oil content and yield calculation were done from the mass from the oil extraction. The increasing doses of cattle manure tested gave a linear increase in biomass production and essential oil yield in *Plectranthus neochilus* plants; it was not observed the maximum curve in the studied period of time. The different cattle manure dosages used did not modify the volatile oil content.


**Key words:** *Plectranthus neochilus* Schlechter; medicinal plant; fertilization; secondary metabolites.

## Introduction

The species *Plectranthus neochilus* Schlechter is known for being aromatic and having a considerable beauty, which makes it appreciated by the ornamental cultivation in gardens and orchards. It is also a medicinal plant, popularly used for the treatment of hepatic insufficiency and dyspepsia (DUARTE and LOPES, 2007). According to Lorenzi and Matos (2002) and Couto (2006), this species is herbaceous; it has small leaves, almost triangular, arranged compactly, a little bitter, with a strong smell, violet racemose inflorescence. Still according to the same authors, this species may be planted in any period of the year.

There is not enough information about *Plectranthus neochilus* Schlechter in literature, even more restrict when related to cultivation and fertilization. It is known that the quality of medicinal and aromatic plants is obtained during all the productive process. Facing this question, fertilization is one of the main factors which influence the yield of bioactive substances and, consequently, the quality of medicinal and aromatic plants (MARCHESE and FIGUEIRA, 2005).

The organic fertilizer has the function of soil fertilizer, corrective, improver or conditioner. It acts as fertilizer, although with low concentration, therefore it is necessary to use it in larger quantity, since it contains Nitrogen, Calcium, Phosphorus, Potassium, Magnesium and Sulfur. It is a corrective because it corrects the soil composition, combining with Manganese, Aluminium and Iron, for instance, reducing or neutralizing the toxic effects of these elements, when in excess, over these plants. It is also conditioner by the form it acts over soil, improving its physical, chemical and biological characteristics, as water retention, aggregation, porosity, increase in the capacity of cation chance, facilitating the plant development and nutrition (MIYASAKA et al., 1997).

The vegetal development and, especially, the production of essential oils in aromatic plants are influenced by several environmental factors, including

1 Professor Colaboradora*, Departament of Agronomy, Unicentro (State University of Midwest of Paraná), Rua Simeão Camargo Varela de Sá, nº 3. Vila Carli. 85.040-080. Guarapuava, PR, Brasil. louise_rosal@yahoo.com.br. Author for correspondence

2 Professor* Titular, Departament of Crop Science, Federal University of Lavras, Lavras, MG, Brasil.

3 Researcher with partnership CNPq DTI-1, Departament of Agronomy, State University of Montes Claros, Janaúba, MG, Brasil.

* Brazilian academic degree

soil conditions.

Studies about the application of fertilization associated or not to liming in *Hyptis marrubioides* Epl. plants pointed highly positive influence of the organic fertilization in the production of dry biomass, content and accumulation of essential oil, independent on the presence or not of Limestone (SALES et al., 2009). These results are in accordance to the attributes which are available to organic fertilizers, which, besides potential providers of nutrients to plants, are soil conditioners and correctives (KIEHL, 1985).

Due to contradictory results, there are different opinions about the influence of fertilization on the production of substances of interest. There is not in literature an agreement about the responses on oil yield in relation to the use of different kinds of fertilizers and much less of doses.

The goal of this study was to evaluate the effect of the increasing levels of cattle manure in *Plectranthus neochilus* plants grown in field, over the yield of dry biomass of the vegetal material, content and yield of essential oil.

## Material and methods

The experiment was developed in experimental field in the Federal University of Lavras (UFLA) located in the municipality of Lavras, South region of Minas Gerais, at 918 m of altitude, latitude 21° 14' S and longitude 45° 00'W.

The former plant of the species *Plectranthus neochilus* used to begin the experiments was duly identified and incorporated under the number 22858 of the ESAL Herbarium, which is bound to the Biology Department of the Federal University of Lavras (UFLA)

### Preparation of the scions for the installation of the experiments

The vegetal material used for the production of the scions was obtained in the Medicinal Plant Garden of the UFLA. The *Plectranthus neochilus* scions were produced by apical cuttings with approximately 5 cm of length and placed to create roots in polystyrene trays of 72 cells, containing the commercial substrate Plantmax®.

The scions were cultivated in environment protected with 60% shading for thirty days, period sufficient for having them well established and rooted.

### Experiment installation

In March 2006, the scions, after acclimatization, were taken to field and transplanted to beds.

It was used the experiment design with random blocks, with three replications constituted by five plots each. The plots were composed by 16 plants, in which the 4 central were considered useful plot. The treatments were constituted of 5 levels: 0.0; 2.5; 5.0; 7.5; 10.0 kg $m^{-2}$ of cattle manure. The irrigation was performed until the soil achieved the land capacity, three times a week.

At 180 days of cultivation, entire plants were harvested and a part of the fresh leaves was destined to the extraction of essential oil. The time of material picking destined to obtain the oil was strictly the same during the end of the experiment, at 8 o'clock in the morning.

It was calculated the relation between fresh matter and the equivalent value in leave dry matter in order to add those values to the sum of dry biomass in treatments, in which 200 g of fresh matter were equivalent to, in average, 10 g of dry. The remaining plants were separately packed (stems and leaves) in "Kraft" paper bags and conducted to oven with forced air circulation with temperature of 70 °C until it achieves constant mass. Later, it was executed a weight of the vegetal matter in digital weight with sensitivity 0.01 g.

### Extraction of the essential oil

For the extraction of the oil it was used the method of hydrodistillation in device modified of Clevenger, by Wasicky and Akisue (1969). The fresh vegetal matter fractionated (200 g per replication) was transported to a flask of 1,000 mL and it was added a volume of 700 mL of distilled water.

After the beginning of boiling, it was distilled for 2 hours. The hidrolact obtained from each hydrodistillation was submitted to liquid-liquid partition in separating funnel, with 3 portions of 15 mL of dichloromethane (each portion rested for 20 minutes, total 60 minutes for replication). The organic fractions of each replication were joined and dried with Magnesium sulfate anhydrous, hygroscopic

substance to remove any particle of water present in the mixture. The salt was removed by simple filtration and the solvent evaporated at room temperature in fume hood until it reaches constant mass, obtaining the purified essential oil.

Regarding to the obtained mass, it was determined the percentage content of the essential oil of *Plectranthus neochilus* in leaves:

T% = oil mass (g) / 200 g x 100

And the oil yield per plant:

R (g plant$^{-1}$) = oil mass (g) x leaves mass (g) / 200 g

### Statistic analysis

The data obtained were submitted to analysis of variance by F test and, later, to regression analysis.

## Results and discussion

The yield of vegetal matter of *Plectranthus neochilus* plants was influenced by the levels of cattle manure used in this study (Figure 1) and the differences were significant. The results verified for yield present high significance between treatments (Figure 2). Although, the content of essential oil was not influenced by the treatments used (Table 1).

The highest level of supply with organic fertilizer tested (10 kg m$^{-2}$) provided an increment of dry biomass in plants 4.44 times when compared to the control treatment (absence of fertilizer). This result indicates that *Plectranthus neochilus* plants respond satisfactorily to fertilization. Besides this, it was not reached the maximum point in the yield curve for dry biomass in the tested levels.

Similar results were obtained by Ferreira et al. (2004) and Chagas (2007). The first authors tested doses of cattle manure (0; 320; 640; 960 g plant$^{-1}$) in order to evaluate the yield of *Catharanthus roseus* plants and observed that the total dry matter increased linearly with the increase of the level of manure applied. The second author researched *Mentha arvensis* L. plants submitted to the same levels of fertilization tested in the present study

**Table 1.** Essential oil content in *Plectranthus neochilus* Schlechter plants in function of increasing levels of fertilization with cattle manure. Federal University of Lavras, Lavras, MG, 2008.

| Doses (kg m$^{-2}$) | Oil content (%) |
|---|---|
| 0.0 | 0.0060 |
| 2.5 | 0.0048 |
| 5.0 | 0.0050 |
| 7.5 | 0.0056 |
| 10.0 | 0.0056 |

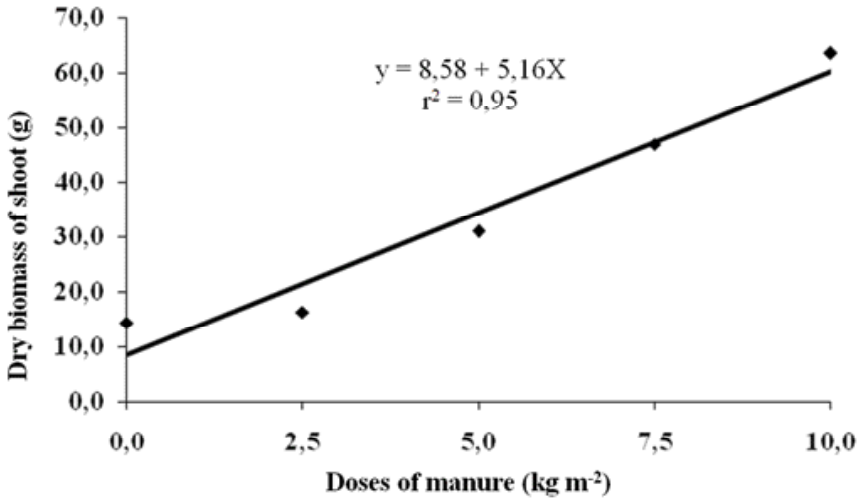


**Figure 1.** Biomass dry matter yield in *Plectranthus neochilus* Schlechter plants in function of increasing levels of fertilization with cattle manure. Federal University of Lavras, Lavras, MG, 2008.

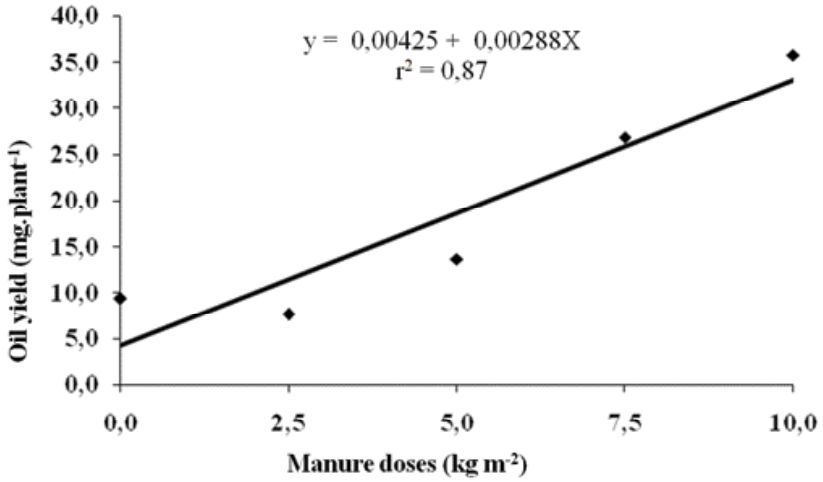


**Figure 2.** Essential oil yield in *Plectranthus neochilus* Schlechter plants in function of increasing levels of fertilization with cattle manure. Federal University of Lavras, Lavras, MG, 2008.

(0.0; 2.5; 5.0; 7.5; 10.0 kg $m^{-2}$), and the results are in accordance with the responses presented by *P. neochilus,* either to the variable dry biomass or to the oil content and yield.

According to Kiehl (1985), organic fertilizers applied to the soil always provide positive response over the crop yield, matching or even overcoming the effects of the chemical fertilizers. Although, Costa et al. (2008), when cultivating *Ocimum selloi* under different dosage of cattle (0-12 kg $m^{-2}$) and poultry (0-6 kg $m^{-2}$) manure, verified that the species responded until a certain limit to both sources of fertilizer – 9.7 kg $m^{-2}$ and 4.3 kg $m^{-2}$, respectively – and, from this values, the dry biomass yield was not increased.

Unlike what some authors suggest, there are also situations in which plants do not respond to organic fertilization concerning yield, i.e., the supply with organic fertilization does not influence significantly the yield of vegetal material and essential oil. This verification was reported by Bezerra et al. (2006) and Santos and Innecco (2004), who cite that, after applying increasing dosed of fertilizers, there were no differences in the results for these characteristics in *Justicia pectoralis* and *Ocimum gratissimum* plants, respectively.

In the case of *P. neochilus*, as the other contents were maintained constant, the yield of the volatile oil was function of the increase of vegetal biomass yield. The organic fertilization may not influence the content of essential oil, but it is highly compensated by the biomass yield which increases the active principles yield per plant (SILVA et al., 2006).

According to Silva et al. (2006), increases in the essential oil yield may be reached with higher availability of nutrients for the plants. The yield of essential oil is function of its concentration in the tissue and vegetal matter yield (OLIVEIRA JÚNIOR et al., 2005). This increase in the vegetal matter yield with the use of organic fertilizer may me linked to the properties which are assigned to this input, as: nutrient provider, soil corrective and conditioner (RODRIGUES e SUMIOKA, 2003), which benefits all the plant.

In the work of Silva (2005) with *Aloysia triphylla* plants submitted to increasing doses of cattle manure (0; 3; 6; 9; 12 kg $m^{-2}$), the oil content had a cubic adjustment, occurring an increase until the dose of 9 kg $m^{-2}$ and, in dose 12 kg $m^{-2}$, there was a lower content of essential oil. Maia (2006) submitted *Hyptis suaveolens* plants to those of same dosage previously cited and the percentage of oil of this species was superior with the application of the highest dose (12 kg $m^{-2}$).

It can be verified, therefore, that there is no consensus referent to the behavior of medicinal and aromatic plants facing the use of different levels of organic manure, which signals the need of more

research in this area due to the increasing interest for information about cultivation of plants producers of secondary metabolites.

## Conclusion

The increase of the cattle manure levels tested provides linear increase in the biomass production and yield of essential oil in *Plectranthus neochilus* plants.

The use of different doses of cattle manure evaluated in this work does not modify the content of volatile oil.

## Acknowledgments

We thank the Coordination of Improvement of Higher Education (CAPES), the National Council for Scientific and Technological Development (CNPq) and the Foundation for Research Support of Minas Gerais (FAPEMIG) for the financial support.

## References

BEZERRA, A.M.E., NASCIMENTO JÚNIOR, F.T., LEAL, F.R, CARNEIRO, J.G.M. Rendimento de biomassa, óleo essencial, teores de fósforo e potássio de chambá em resposta à adubação orgânica e mineral. **Revista Ciência Agronômica**, v.37, n.2, p.124-129, 2006.

CHAGAS, J.H. **Propagação, adubação orgânica, níveis de irradiância, idade e época de colheita e armazenamento na produção de biomassa seca e teor de óleo essencial em plantas de *Mentha arvensis* L.** 135p. Dissertação (Mestrado em Fitotecnia), Universidade Federal de Lavras – UFLA, Lavras. 2007.

COUTO, M.E.O. **Coleção de plantas medicinais, aromáticas e condimentares**. Brasília, DF: Embrapa, 2006. 91p. (Documentos CPACT/Embrapa, 157).

COSTA, L.C.B., PINTO, J.E.B.P., CASTRO, E.M., BERTOLUCCI, S.K.V., CORRÊA, R.M., REIS, E.S., ALVES, P.B., NICULAU, E.S. Tipos e doses de adubação orgânica no crescimento, no rendimento e na composição química do óleo essencial de elixir paregórico. **Ciência Rural**, v.38, n.8, p.2173-2180, 2008.

DUARTE, M.R., LOPES, J.F. Stem and leaf anatomy of *Plectranthus neochilus* Schltr., Lamiaceae. **Revista Brasileira de Farmacognosia**, v.17, n.4, p.549-556, 2007.

FERREIRA, M.M., MOTTA, M.B., PINTO, J.E.B.P., CASTRO, E.M. Crescimento e alocação de biomassa de plantas de vinca (*Catharanthus roseus* (L.) G. Don) em função da adubação e época de colheita. **Revista Brasileira de Plantas Medicinais**, v. 6, n.2, p.72-76, 2004.

KIEHL, E.J. **Fertilizantes orgânicos**. Piracicaba: Agronômica Ceres, 1985. 492p.

LORENZI, H., MATOS, F.J.A. **Plantas medicinais do Brasil: nativas e exóticas**. Nova Odessa: Plantarum, 2002. 544p.

MAIA, S.S.S. **Propagação, adubação orgânica e níveis de radiação nas características anatômicas e composição do óleo essencial em *Hyptis suaveolens* (l.) POIT. Lamiaceae**. 150p. Tese (Doutorado em Fitotecnia), Universidade Federal de Lavras – UFLA, Lavras. 2006.

MARCHESE, J.A., FIGUEIRA, G.M. O uso de tecnologias pré e pós-colheita e boas práticas agrícolas na produção de plantas medicinais e aromáticas. **Revista Brasileira de Plantas Medicinais**, v.7, n.3, p.86-96, 2005.

MIYASAKA, S., NAKAMURA, Y., OKAMOTO, H. **Agricultura natural**. 2.ed. Cuiabá: Sebrae/MT, 1997. 73p. (Coleção agroindústria).

OLIVEIRA JÚNIOR, A. C., FAQUIN, V., PINTO, J.E.B.P., LIMA SOBRINHO, R.R., BERTOLUCCI, S.K.V. Teor e rendimento de óleo essencial no peso fresco de arnica, em função de calagem e adubação. **Horticultura Brasileira**, v.23, n.3, p.735-739, 2005.

RODRIGUES, E.T., SUMIOKA, A.T. Produção de cará em função de fontes orgânicas de adubação. **Ciência e Agrotecnologia**, v.27, n.4, p.822-828, 2003.

SALES, J.F., PINTO, J.E.B.P., BOTREL, P.P., SILVA, F.G., CORREA, R.M., CARVALHO, J.G. Acúmulo de massa, teor foliar de nutrientes e rendimento de óleo essencial de hortelã-do-campo (*Hyptis marrubioides* epl.) cultivado sob adubação orgânica. **Bioscience Journal**, v.25, n.1, p.60-68, 2009.

SANTOS, M.R.A., INNECCO, R. Adubação orgânica e altura de corte da erva-cidreira brasileira. **Horticultura Brasileira**, v.22, n.2, p.182-185, 2004.

SILVA, F. G., PINTO, J.E.B.P., CARDOSO, M.G., SALES, J.F., MOL, D.J.S., DIVINO, S.P. Influence of manure and fertilizer on *Baccharis trimera* (Less.) D. C. growth and essential oil yield. **Journal of herbs, spices & medicinal plants**, v.12, n.1-2, p.1-11, 2006.

SILVA, R. **Crescimento e teor de óleo essencial de *Aloysia triphylla* (L'Herit) Britton (Verbenaceae), em função da adubação orgânica, sazonalidade, horário de colheita e processamento pós-colheita**. 2005. 66p. Dissertação (Mestrado em Fitotecnia), Universidade Federal de Lavras – UFLA, Lavras. 2005.

WASICKY, R., AKISUE, G. Um aparelho aperfeiçoado para a extração de óleos essenciais. **Revista da Faculdade de Farmácia Bioquímica da Universidade de São Paulo**, v.7, n.2, p.339-405, 1969.