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# DEVELOPMENT AND PRODUCTION OF FRESH AND DRIED LEAF BIOMASS OF LEMON BALM (*Melissa officinalis* L.) UNDER ORGANIC FERTILIZERS TREATMENT

**Plamen ZOROVSKI, Vladislav POPOV, Tonya GEORGIEVA**

Agriculture University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: plivz@abv.bg

***Abstract***

*The study was conducted at the Demonstration Center for Organic Agriculture at the Agricultural University - Plovdiv, Bulgaria during the 2015-2018 period. A field trial has been set up in three replications with a plot size of 25 $m^2$ in Lemon balm (Melissa officinalis L.). The aim is to determine the impact of Amalgerol, Lithovit and Tryven organic fertilizers on the growth, development and productivity of Lemon balm. The treatment with the organic fertilizers leads to an increase in values of the researched indicators. Fresh biomass weight when treated with Tryven goes to 250.05 g, when the control variant is only 143 g. Fresh leafs weight reaches higher values at all fertilized variants. Amalgerol treatment leads to - 31.07 g for dry leafs weight and Tryven to 37.72 g, compared to the non treated variant - 22.73 g. Statistically proven - highest yield of green fresh biomass was obtained in the variants treated with Tryven - 989.2 kg/da (7.5% above the control) and Amalgerol - 980 kg/da (6.5% above control), respectively. The yield of the fresh leafs increase with 21.1% compared to the control (up to 541.6 kg/da) when treated with Lithovit and 12.3% (to 502.2 kg/da) when treated with Amalgerol. Highest dry leafs yield was obtained when Tryven was applied - 149.2 kg/da (2.1% above the control).*

***Key words****: organic fertilizers, fresh biomass, dried leaf biomass, Melissa officinalis L.*

## INTRODUCTION

The biological form of agriculture helps to maintain ecological balance and optimization of biological processes in agriculture. The biological system (agroecosystem) is considered to be a living organism having mutually connected components in dynamic interrelations (Vlahova, 2015). Soil and environmental protection is a fundamental task of organic farming. Lemon balm (*Melissa officinalis* L.) is a valuable essential oil and medicinal plant, with 450,000 kg of leaves and branches of the uterus being produced and exported annually in Bulgaria. The areas in the country are increasing, with a total of 518 tonnes being produced in 2016, of which 21 tonnes are in the biological control system. The leaves and tips of the stem in dry form, etc. Herb (Herba and Folia melissae) is the most commonly used part of humans for tea, extracts, sweets and more. Vegetable mass contains up to 0.3% essential oil, tannins, phenolic acids, triterpenic acids (oleanolic, ursolic), vitamin C. The essential oil of *M. officinalis* L., obtained from fresh or dried plants, leaves or tips of the plant, is characterized by a fresh lemon odor and light yellow color. Its viscosity is lighter than that of water (Anonymous, 2003). It is desirable that this value should not be less than 0.05% (Baytop, 1984). Janina M. (2003) reported that most essential oil (0.14%) was obtained from plants harvested at the beginning of flowering grown in Canakkale under ecological conditions. According to Lin et al., (2012) the lemon balm contains antioxidants, also geranium and neral (Masakova et al., 1979). According to Weiss (1974), lemon balm has a well-pronounced sedative and antispasmodic action, anti-inflammatory (Bounihi et al. 2013), mild sleeping pills (Braun et al., 1974) and anti-diabetic effects (Chung et al., 2010). Substances having antiviral activity have been found in the lemon balm (Allahverdiyev et al., 2004). Fresh leaves are torn off, shaded or dried at 35-40°C. When dried in the sun, the leaves darken and deteriorate. Weed control is an important element of cultivation technology (Tonev et al., 2019). The best time to harvest the above ground mass is before flowering, when the leaves are well developed, because at

a later stage the content of the active ingredients decreases. Crushing the raw material causes a sharp deterioration in the quality of the drug. Drying the leaves at 30 and 45°C leads to a 16% and 23% loss of essential oil, respectively, while drying at higher temperatures of 60°C causes significant losses (65%) (Argyropoulos et al., 2014). The application of leaf fertilizers affects the growth of the plant as well as the quality of the essential oil (Keshavarz et al., 2018). The purpose of the study is to monitor the vegetative development and productivity of fresh and dry leaf mass of *Melissa officinalis* L. when treated with leaf fertilizers for organic farming.

## MATERIALS AND METHODS

The study was conducted at the Demonstration Center for Organic Agriculture at the Agricultural University - Plovdiv, Bulgaria during the 2015-2018 period. A field trial has been set up in three replications with a plot size of 25 $m^2$ in lemon balm (*Melissa officinalis* L.). Factors of reading: Factor A: Vegetation year, A1 - 2015-2016, A2 - 2016-2017. A3 - 2017-2018; Factor B: Organic fertilizers: B1 - Control - no application of foliar fertilizers, B2 - Amalgerol - at a dose of 300 ml/da, once in the beginning of May, B3 - Lithovit - at a dose of 300 g/da once in the beginning of May, B4 - Tryven - at a dose of 300 ml/100 l once in the beginning of May. Statistical processing of the data was carried out with the program SPSS for Microsoft Windows (SAS Institute Inc. 1999). The mowing was done before flowering began. The leaves were removed manually, drying was carried out at room temperature in the shade to avoid darkening and deterioration of the quality of the leaves. The following indicators were monitored: phenological development; growth of plants in height - on the 7th and 14th days after application of organic fertilizers; number of stems per plant (rhizome) - measured 10 plants from each plot in three replications for each variant; number of branches per stem; number of leaves per plant - lists all the leaves of all the stems of a single plant; weight of fresh mass of one plant, g - fresh mass of 10 plants of each variant and repetition; weight of fresh leaf from a plant, g - manual separation of leaves from each plant; weight of the dry leaf mass of the plant, g - drying of the fresh leaf mass of each plant at room temperature in the shade; yield of fresh and dry leaves, kg/da. Organic leaf fertilizers used: Lithovit - nanotechnological product. Increases yield, quality and storage properties. Contains 79.19% - $CaCO_3$, 4.62% $MgCO_3$, 1.31% Fe; Amalgerol® is rich in hydrocarbons and natural plant growth hormones. Contains seaweed extracts, distilled paraffin oil, vegetable oils, distilled herbal extracts. Stimulates plant growth, improves the quality and quantity of production in oilseeds, etc.; Tryven - contains N total 24.4%, ammonium nitrogen (N) 2.60%, nitrate nitrogen < 0.01%, urea nitrogen 4.47%, organic nitrogen 17.3%, $P_2O_5$, water-soluble 17.2%, $K_2O$ water-soluble 7.42%. Designed for use by foliar nourishment, especially for crops with large foliage in order to achieve great vegetative growth.

## RESULTS AND DISCUSSIONS

### 1. Analysis of agrometeorological conditions during the study period.

The soils in the area of the experiment are alluvial-meadow. They have low availability with N and P and good with K (Popova et al., 2010). Meteorological conditions during the study period are relatively favorable for the development of the lemon balm (Figure 1). The 2015-2016 growing season is characterized by being warm and well stocked with moisture. Long-term rainfall was reported in October (70.3 mm/$m^2$). Precipitation values in May (64.7 mm/$m^2$) are twice as long as the long term (32 mm/$m^2$). These conditions, in turn, allow for a good yield of fresh vegetable mass.

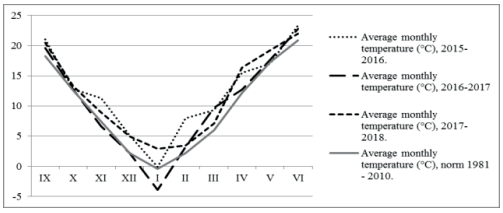


Figure 1. Daily average air temperatures of ten days (°C) for the period 2015-2018

The growing season 2016-2017 is relatively warm with uneven rainfall (Figure 2). In May,

when the growth is active, temperature values are in the norm 17.6°C and are accompanied by precipitation above those for the long term of 52.7 mm/m$^2$. The hot month of June combined with low rainfall causes stress in plants.

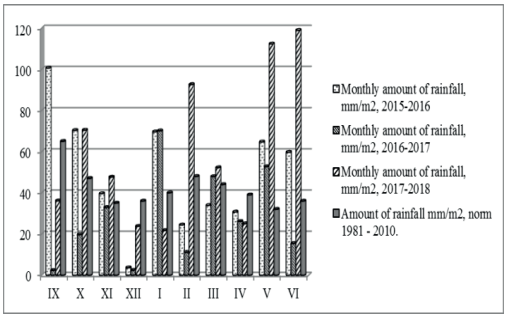


Figure 2. Rainfall sum (mm/m$^2$) during the study period 2015-2018

The 2017-2018 growing season is characterized as warm and moist. In May, heavy rainfall of 112.3 mm/m$^2$ was registered with long-term values of 32 mm/m$^2$.

## 2. Phenological development

Insufficient soil moisture and uneven distribution of rainfall during the growing season have the risk of loss of foliage and hence lower yields. In the absence of soil moisture, the plants form a small number of stems with small leaves. Yields decline sharply. In extreme rainfall, the plants become susceptible to fungal diseases. Irrigation was performed to reduce losses. During the study period, the beginning of the growing season begins in March (20-25 November). In the initial stages, especially in the first year after transplanting, the plants develop very slowly. Phenophase third - fourth leaf occurs in the period 7-10.IV. During this period, the plants grow slowly, and the weeds quickly, which carries the risk of strong weeding, and for this purpose weeding and digging were carried out. The studied leaf fertilizers were applied once at doses of 300 ml/da + 40 l water/da, in the beginning of May (03-05.V.), according to the scheme in three replications. Phenophase budding occurs in the period 09 -21.VI. During the study period, no differences were observed in the occurrence of the different phenological phases between the studied variants of foliar fertilization compared to the control.

## 3. Biometric indicators

3.1. Plant height on days 7 and 14 after application of organic fertilizers

In the first year of development, lemon balm forms a smaller number of stems up to 50 cm in height. Simultaneously with the formation of the stems, rhizome growth begins. Early in the spring of the second year, development progressed faster. The rhizomes have a greater branching ability, which allows for the formation of more stems and hence a larger number of leaves. When measuring the height 7 days after application of the fertilizers studied, no significant differences were observed in the treated plants relative to the control, but a percentage increase was more pronounced after Tryven treatment - (1.8% above the control - 44.73 cm) (Table 1).

Table 1. Plant height on days 7 and 14 after application of leaf fertilizers, averaged over the study period

| Day / Variant | 7th day | | 14th day | |
|---|---|---|---|---|
| | Height, cm | % compared to control | Height, cm | % compared to control |
| Control | 43.95 a | 100 | 51.19 a | 100 |
| Amalgerol | 44.70 a | 101.7 | 52.35 a | 102,.3 |
| Lithovit | 44.66 a | 101.6 | 52.42 a | 102.4 |
| Tryven | 44.73 a | 101.8 | 48.37 a | 94.5 |

Duncan's Multiply Range Test ($P < 0.05$)

Increases in the value of the indicator were also reported when treated with Lithovit - 44.6 cm, and Amalgerol - 44.70 cm relative to the untreated control (43.95 cm), but the differences are not statistically proven. The application of leaf fertilizers complements plant nutrition and also affects the quality of production. 14 days after application of leaf fertilizers, higher percentages were observed compared to controls in the variants treated with Amalgerol and Lithovit, respectively, by 2.3% for Amalgerol and 2.4% for Lithovit. A value close to the control is reported in the variant treated with Tryven.

### 3.2. Number of stems per plant (rhizome)

According to the indicator the number of stems per plant (rhizome) - from the data in Table 2, it can be seen that in the variant treated with Tryven we have proven a greater number of formed plant stems - 11.6 compared to the control (9). Higher control values were also reported when treated with Lithovit and

Amalgerol, but these were not proven. In terms of the number of branches and number of leaves per stem, the treated variants with organic fertilizers show higher values than the control.

Table 2. Biometric indicators of a plant averaged over the study period

| Variant | Number of stems | Number of branches per stem | Number of leaves | % leaves compared to control |
|---|---|---|---|---|
| Control | 9.0 b | 4.9 b | 109.0 b | 100 |
| Amalgerol | 9.7 b | 7.1 a | 179.0 a | 164.2 |
| Lithovit | 9.3 b | 5.3 b | 119.0 b | 109.2 |
| Tryven | 11.6 a | 5.1 b | 112.0 b | 102.7 |

Duncan's Multiply Range Test ($P < 0.05$)

Amalgerol treatment has been shown to increase the number of branches and number of leaves relative to the untreated control. At a value of 7.1 branches per stem, 179 leaves were formed on average over the period. Amalgerol treated variants are followed by Lithovit treated versions with 5.3 branches and 119 leaves and Tryven - 5.1 branches and 112 leaves. Here, too, the values of the indicator are higher than the control but are not statistically proven (Table 2).

It can be said that the number of stems per plant increases only when treated with Tryven. According to the indicator the number of branches per stem and the number of leaves per plant, the application of the studied preparations leads to values greater than those of the control. Amalgerol has been shown to increase the values of both indicators relative to controls. The fertilizers studied led to an increase in the values of indicators - fresh mass (leaves + stems), fresh leaf mass and dry leaf mass (herb), compared to those of the untreated control (Table 3).

Table 3. Weight of fresh plant mass, fresh leaves mass and dry leaves mass of plant by variants averaged over the study period, g

| Variant | Fresh biomass weight | % | Fresh leaves weight | % | Dry leaves weight | % | % of dry leaves of fresh leaves mass |
|---|---|---|---|---|---|---|---|
| Control | 143.21b | 100 | 69.62b | 100 | 22.73c | 100 | 32.65 |
| Amalgerol | 239.66ab | 167.3 | 122.82a | 176.4 | 31.07b | 136.7 | 25.29 |
| Lithovit | 198.54b | 138.6 | 117.08a | 168.2 | 31.83ab | 140.0 | 27.19 |
| Tryven | 250.05a | 174.6 | 107.89a | 154.9 | 37.72a | 165.9 | 34.96 |

Duncan's Multiply Range Test ($P<0.05$)

In the indicator weight fresh mass of the whole plant, the highest value was shown in the variant treated with Tryven - 250.05 g, which is 74.6% more than the control (143.21 g). Amalgerol treated variants with 67.3% and Lithovit 38.6% also have higher values, but these differences are not proven.

The weight of the fresh leaf mass is strongly influenced by the applied leaf fertilizers and is proven to be higher than the controls in all three types of fertilizers. Amalgerol-treated plants have a fresh leaf mass of 122.82 g per plant weight, which is 76.4% above control. The implementation of Litovit increases the values of the indicator by 68.2% and Triven by 54.9% above the control. When measuring the weight of the dry leaf mass, the variants treated with Amalgerol - 31.07 g and Lithovit - 31.83 g were approximately the same values. They are proven to exceed the control variant - 22.73 g. The strongest influence on the weight of the dry leaf mass was observed in the variant treated with Tryven - 37.72 g, which is 65.9% above the control. It is statistically proven that the fertilizers studied lead to an increase in the values of the indicators compared to the untreated control. The data in Table 4 shows that, on average, for the study period, Triven and Amalgerol have been shown to increase yields of fresh mass (leaves + stems) per decare to a different degree than controls. The highest yield of fresh mass was obtained with the Tryven treated version - 989.2 kg/da, which was 7.5% above the yield of the control variant. Amalgerol, increased fresh weight yield by 6.5% (980 kg/da) above control (920 kg/da). The values for treatment with Lithovit are close to the control variant. In terms of fresh leaf yield, Lithovit has proven to increase the weight of fresh leaves by 21.1% over control - 541.6 kg/da.

An increase in the yield of fresh leaves was also reported when treated with Amalgerol - 502.2 kg/da (12.3% above control).

Table 4. Average yield of lemon balm kg/da by fertilizer variants

| Variant | Yield of fresh biomass (leaves + stems) | | Yield of fresh leaves | | Yield of dry leaves mass | |
|---|---|---|---|---|---|---|
| Control | kg/da | % | kg/da | % | kg/da | % |
| | 920.00c | 100.0 | 447.23 c | 100.0 | 146.14 b | 100.0 |
| Amalgerol | 980.00 b | 106.5 | 502.23 b | 112.3 | 127.00 c | 86.9 |
| Lithovit | 918.46 c | 99.8 | 541.60 a | 121.1 | 147.25 b | 100.8 |
| Tryven | 989.23 a | 107.5 | 426.80 d | 95.4 | 149.21 a | 102.1 |

Duncan's Multiply Range Test ($P<0.05$)

In the Tryven treated variant, despite the higher yield of fresh mass, no increase in the yield of fresh leaves was observed relative to the control. These differences between the fertilizer variants are due to the different composition of the fertilizers being studied, which also gives us a more accurate idea of their influence on lemon balm. After drying, the yield of dry leaves in the treated variants differs from the control.

Proven higher yields of dry leaves were reported with the Tryven treatment variant - 149.21 kg/da, which is 2.1% above control. A slight percentage increase in yield was also reported for the variant treated with Lithovit - 147.25 kg/da, but this difference from the control is not proven.Treatment with Tryven and Amalgerol increases the yield of fresh plant mass, but the yield of fresh leaves per decare is smaller than the control when treated with Tryven. This is due to the composition of the different fertilizers and the directing of nutrients to different organs of the plant. A similar variation of values is also observed in the dry leaf mass (herb).

When treated with Amalgerol, the yield of fresh leaves mass is increased, but after drying, the yield of dry leaves mass is less than that of the control.

The possibility of forming more leaves, but smaller in size, and the amount of dry matter in them, is yet to be explored. The data can be used when choosing a leaf fertilizer, depending on which part of the plant we want to influence according to the use direction - to increase the yield of fresh plant mass, for fresh leaves or for dry leaf mass (herb).

## CONCLUSIONS

No differences in the occurrence of the phenological phases of the crop as a result of treatment with the leaf fertilizers compared to the control are taken into account.

A percentage increase in the values of the plant height indicator on the 7th and 14th days after treatment, more pronounced on the 14th day, was reported, but differences in control were not proved.

Treatment with Tryven leads to the formation of more stems per plant - 11.6 pieces compared to the control (9 pieces). This effect has not been demonstrated when treated with Lithovit and Amalgerol. Amalgerol treatment has been shown to increase the number of branches and number of leaves (7.1 number of branches per stem - 179 pieces) compared to the control (4.9 number of branches and 109 leaves). An increase in the values of the indicator was reported after the application of Lithovit and Tryven, but the difference from the control is not proved.

Tryven increased the weight of fresh mass of plant - 250.05 g, which is 74.6% above the control (143.21 g). The weight of the fresh leaf mass was strongly influenced by the applied products and was proven to be higher with Amalgerol -122.82 g (76.4% above control), Lithovit - with 68.2% and Tryven with 54.9% above control. Given the weight of the dry leaf mass, the variants treated with Amalgerol - 31.07 g and Lithovit - 31.83 g were approximately the same values. They are proven to exceed the control untreated variant (22.73 g). The weight of the dry leaf mass after Tryven administration increased also - 37.72 g, (65.9% above control), followed by the variants treated with Amalgerol - 31.07 g and Lithovit - 31.83 g.

Highest yield of fresh mass/da obtained after Tryven treatment - 989.2 kg/da and Amalgerol – 980 kg/da, from fresh leaves after treatment with Lithovit (541.6 kg/da) and Amalgerol (502.2 kg/da) and from dry leaves (herb) after Tryven treatment - 149.21 kg/da. Values exceed those of control and are statistically proven.

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