PROPAGATION OF LEMON BALM (Melissa officinalis L.) BY SEEDS AND CUTTINGS ON DIFFERENT SUBSTRATES

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Abstract

Lemon balm (Melissa officinalis L.) is a herb species with many benefits for health. Lemon balm is effective in reducing anxiety, stress, improving insomnia, treating viruses and some digestive diseases.... This species can be propagated by sowing seeds or cuttings or dividing branches from the root. In this study, the propagation experiments using seeds and cuttings were conducted to find a suitable propagation method for lemon balm in Vietnam. The results showed that lemon balm seeds germinated faster and better when treated with warm, moist stratification in a zip-lock bag (97.7%) compared to in a box without lid. The rates of standard seedlings formed when sowing seeds on a substrate containing 70% coco peat + 30% rice husk (85.67%) and a substrate containing 50% coco peat + 30% rice husk (84.33%) were better than the substrate containing 70% soil + 30% coco peat (68.67%). Time for seedlings to meet standards by sowing seeds was longer than 30 days. The rooting rates of cuttings when using rooting stimulant NAA 1000ppm and NAA 3000 ppm were not significant different from the control (no using rooting stimulant), the rates of rooting cuttings reached 96%, 94% and 93.3%, respectively. The appropriate time to propagate by cuttings was in late spring and early summer. It took about 2 weeks for the cuttings to root. Thus, lemon balm can be propagated by both seeding and cuttings methods for obtaining qualified seedlings.

1. INTRODUCTION

Lemon balm (M. officinalis) belongs to the Lamiaceae family and originates mainly from Southern Europe (a traditional European medicinal herb) but today it is grown in countries around the world (McGimpsey J., 1993; The Herb Society of America, 2007). Lemon balm is not only a medicinal herb but also a spice plant with lemon mint scent that is widely used in the pharmaceutical, food, cosmetic and beverage industries as well as in the chemical industry (pesticides). Because of its effectiveness and widespread use, in 2007, M. officinalis was voted as “Herb of the Year” by the International Herb Association.

Lemon balm is considered to have medicinal properties and is used to treat various ailments such as insomnia, cramps, headaches and toothaches, especially stomach and nervous diseases. It is often used in herbal mixtures to mask the unpleasant taste of some other herbs. Lemon balm is used as a medicinal herb more than as a spice. However, because it has a fresh and pure lemon flavor, it becomes a perfect substitute for fresh lemongrass (Mihajlov L. et al., 2013). Lemon balm also contains vitamin C (about 254 mg/100 mL) and Thiamin (B vitamin) (Franke W., 1978). It’s the essential oil content of lemon balm is very low (only about 0.14-0.39%) and depends a lot on cultivating conditions, light intensity, type of fertilizer, harvesting conditions, etc. (Turhan M., 2006; Farahani H. A., et al., 2009). The essential oil has a fresh lemon scent and a pale yellow color. Totally 37 and 16 compounds were identified in the essential oil extracted from the leaves of M. officinalis at the pre-flowering and post-flowering stages (Saeb K. and Gholamrezaeae S., 2012). Lemon balm extract and its essential oil are also used to flavor of alcoholic and non-alcoholic beverages, confectionery and prepared foods. The essential oil is also sometimes used as an ingredient in perfumes. Hot water extract of lemon balm (similar to herbal tea) has strong antiviral activity against a range of viral infections including mumps. Additionally, lemon balm essential oil has been reported to have antibacterial, antispasmodic, and antioxidant activities in vitro (Carvalho F. et al., 2021; Ehsani A., et al., 2017).

Lemon balm is usually planted in early spring and the plant can die in winter. It needs to be grown in full sun, fertile and well-drained soil. Lemon balm can be grown from seeds and transferred to the garden when the seedlings are about 2-3cm in the height. They grow quickly forming large clusters. Lemon balm can also be bred by separating clumps of old plants in spring or early autumn or by cuttings, and micropropagation (Gogu G. H., et al., 2005; Meftahizade H., 2010).

In Vietnam, Nguyen Tien Ban et al. (2005) recorded a species belonging to the genus Melissa L. which is Melissa axillaris (Benth.) Bakh.f. (honey flower). This species is widely distributed from Lao Cai, Ha Giang to Kon Tum, Lam Dong. Luu Dam Ngoc Anh et al. (2017) planted experimentally M. officinalis in Hanoi, Vietnam (the seeds were collected from the Central Botanical Garden, Belarusian Academy of Sciences) for evaluation growth, content and quality of essential oils. The results showed that lemon balm grew well in Vietnam’s climate conditions, the essential oil content in the leaves and stems reached 0.36% (according to dry weight). These authors also identified 23 compounds from the essential oil of lemon balm leaves and stems, accounting for 89.42% of the total essential oil content, including main ingredients such as citronellal (42.55%), citral (15.98%), linalool (8.2%), caryophyllene (5.22%) and geraniol (7.01%).

In general, M. officinalis is not yet commonly grown in Vietnam and related research is quite limited. In this study, we experimented some of different methods of propagating to obtain quality lemon balm seedlings as well as shorten the propagating time.

2. MATERIALS AND METHODS

2.1. Materials

Seeds of M. officinalis was purchased from Hat giong the gioi Company, Vietnam (originated from America).
Materials of substrates for sowing seeds include soil, manure, coco peat and husk. The details of the substrate formulas were as follows: S1 (70% coco peat + 30% husk); S2 (50% coco peat + 50% husk); S3 (70% soil + 30% coco peat). Each formula was added 5% manure.

Materials of substrates for cutting: 50% coco peat and 50% sand.

Rooting stimulants: NAA 98% (India) was diluted with water to get the concentrations of 1000 ppm and 3000 ppm.

Plastic sowing and nursery trays.

2.2. Methods

Sowing: Seeds of lemon balm were soaked in warm water (about 40°C) for 4-6 hours. There were two formulas for sowing experiments: F1 included 100 seeds were spread evenly on damp non-woven fabric and fold, incubating in a zip-top plastic bag, no watering; F2 included 100 seeds were spread evenly on damp non-woven fabric and fold, putting in a plastic box without lid, daily spraying water to maintain humidity 70%. After the seeds sprouted, sowing them in the plastic trays containing different substrates (formulas S1, S2 and S3), watering daily (maintaining humidity about 70%). Monitoring the ratio of formation seedlings (the standard of seedlings: having at least 2 pairs of real leaves, about 3-4cm in height).

Propagating by cuttings: Putting the substrate on the nursery tray. Cutting tops of lemon balm branches into 10 cm long pieces (usually containing 3 pairs of leaves). Removing two-thirds of the leaves at the base of each cutting and pinching off tips of the leaves to prevent wilting. The cuttings were dipped in rooting stimulants including NAA 1000 ppm, NAA 3000 ppm (dipping in NAA for 3-5 seconds) and the control was the cuttings without dipping rooting stimulant. Then the cuttings were inserted into the substrate on the trays with 50 holes, one cutting/one hole, 50 cuttings/formula. Watering daily (maintaining humidity about 70%). Monitoring the rooting of the cuttings.

Statistical Analysis: Data were analyzed by Excel 2010 and Iristat 5.0 software.

3. RESULTS AND DISCUSSION

3.1. The effect of incubation methods on seed germination of lemon balm

The seeds of lemon balm are very small in size. When they were soaked in warm water, forming a mucous membrane around the seeds. After being soaked in water for 4-6 hours, the seeds were incubated with non-woven fabric and placed in two different conditions including placing in a zip-lock plastic bag (F1) and placing in an uncovered box (F2). The results of observations showed that seeds in formula F1 germinated faster (34 hours) than seeds in formula F2 (47 hours). After 47 hours, the germination rate of seeds in formula F1 was also higher (97.7%) than that in formula F2 (88.3%) (Table 1).

<table>
<thead>
<tr>
<th>Seed incubation conditions</th>
<th>Time for sprouting (hours)</th>
<th>Germinating rate after 48 hours (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 (zip-lock plastic bag)</td>
<td>35.3 ± 1.53^a</td>
<td>97.7 ± 2.08^a</td>
</tr>
<tr>
<td>F2 (uncovered box)</td>
<td>47.0 ± 1.73^a</td>
<td>88.3 ± 4.04^a</td>
</tr>
<tr>
<td>LSD5%</td>
<td>9.64</td>
<td>8.55</td>
</tr>
</tbody>
</table>

*Note: The different letters in the same column indicated a statistically significant difference, p-value < 0.05.*

Seeds germinate well when they absorb enough water to reach the necessary humidity and at the suitable temperature. The warm temperature is required for increasing activity of seeds, promoting embryonic development. The seed stratification is the process of simulating the natural conditions of seeds to speed up and improve germination. Methods of the cold, moist stratification and the warm, moist stratification can be used for different types of seeds (Luna T. et al., 2015). For the cold, moist stratification, the best germination rate of *M. officinalis* (95%) was obtained with seeds chilled for 15 days at 15/25°C alternation under the 12 hours photoperiod (Póvoa O. and Monteiro A., 2009).

In this experiment, we applied the warm, moist stratification method (soaking the seeds in warm water or keeping them in a moist environment (using a damp paper/fabric) and placing them in a plastic zippered bag for several days for maintaining moisture of the seeds and creating a warmer environment. In this way, the germination rate of lemon balm seeds was higher than that in the box without the lid. The warm, moist treatment also shortened the time of germination (shortened about 12 days).

3.2. The effect of different substrates on the ratio of seedling formation of lemon balm

In this experiment, the ratio of formatting seedling was determined on three different substrate formulas. The results showed that formula S1 including 70% coco peat + 30% husk and formula S2 including 50% coco peat + 50% husk had the ratio of seedling formation greater than formula S3 including 70% soil + 30% coco peat (85.67%, 84.33% and 68.67%, respectively). The difference between formulas S1, S2 compared to S3 was statistically significant with p-value < 0.05. The rates of seedling formation in S1 and S2 were similar. Actual observations also showed that the seedlings in formula S1 and S2 grew faster than in formula S3. The process of monitoring the experiment showed that it took about 30 days for the seedlings to reach a height of 3-4 cm and have two pairs of true leaves (as mentioned in the method section).

Both of formulas S1 and S2 contained mostly coco peat substrate (a type of substrate that has the ability to retain moisture, retain fertilizer, and easily drained to prevent the plant from being waterlogged), while formula S3 only contained mostly soil, so this may be the reason the seedlings in formulas S1 and S2 were formed and grew better than in formula S3. Furthermore, formulas S1 and S2 were also added husk, which increased the porosity and ventilation of the growing medium.
The germination ratio of lemon balm depend on the variety of the seed and the sowing medium (Krystyna Winiarczyk et al., 2015). It was reported that lemon balm seedlings were formed at a rate ranging from 87-91% depending on the variety when sown on peat substrate (Winiarczyk K. et al., 2016).

**Table 2. The ratio of seedling formation of lemon balm sown on different substrates**

<table>
<thead>
<tr>
<th>Substrate formulas</th>
<th>The seedling rate (%)</th>
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</thead>
<tbody>
<tr>
<td>S1 (70% coconut peat + 30% husk)</td>
<td>85.67 ± 3.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>S2 (50% coconut peat + 50% husk)</td>
<td>84.33 ± 3.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>S3 (70% soil + 30% coconut peat)</td>
<td>68.67 ± 3.51&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD5%</td>
<td>8.55</td>
</tr>
</tbody>
</table>

*Note: The different letters in the same column indicated a statistically significant difference, p-value < 0.05.*

**Figure 1. Germination and seedling formation of lemon balm**

### 3.3. The effect of rooting stimulants on the rooting of the cuttings

There are many ways to propagate lemon balm. The propagation from seeds is so easy that propagation by other methods may not be necessary (The Herb Society of America, 2007). However, the lemon balm seeds usually germinate after 5-9 days under suitable temperature conditions of 65-70°F. The seedlings from sowing seeds take a long time to grow before they are qualified for planting (more than 30 days, even 6-8 weeks). Therefore, the method of propagation by division or cuttings is still commonly applied to this plant species. In this experiment, we propagated lemon balm by cuttings using different solutions of NAA rooting stimulants. This experiment was implemented two periods of the year in order to evaluate which time was suitable for propagating lemon balm by cuttings: late spring to early summer (April) and autumn (September). The substrate for cuttings was mixed with sand (1:1) to further improve the conditions for the cuttings. An environment with high humidity was maintained to create easily condition for the cuttings to form new roots. The result was shown in table 3.

**Table 3. The effect of rooting stimulants on the rooting ratio of the lemon balm cuttings**

<table>
<thead>
<tr>
<th>Formulas</th>
<th>April</th>
<th>September</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA1000ppm</td>
<td>96.0 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.3 ± 8.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NAA3000ppm</td>
<td>94.0 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.0 ± 6.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>93.3 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.0 ± 4.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5%LSD</td>
<td>5.12</td>
<td>11.49</td>
</tr>
</tbody>
</table>

*Note: The different letters in the same column indicated a statistically significant difference, p-value < 0.05.*

According to table 3, the cuttings in April (late spring and early summer) gave a rate of rooting cuttings higher (93.3-96%) than the cuttings in September (48-55.3%). This may be due to the temperature and light conditions in April are more suitable for propagating and planting lemon balm than in September. Therefore, the lemon balm cuttings at this time achieved better quality with more vitality as well as the higher rate of taking root. This also corresponds to the study of Póvoa and Monteiro (2009): lemon balm was propagated by cuttings with 4 cm in length in March, the successful rooting rate achieved 80% at 15 °C and the successful rooting rate achieved 100% at 25 °C.

**Figure 2. Propagation of lemon balm by the cutting**
When the cuttings were dipped in NAA rooting stimulant (formulas NAA 1000 ppm, NAA 3000 ppm), the rate of rooting cuttings in these two formulas was not significantly different from the control (the rates of rooting cuttings reached 96%; 94% and 93.3%, respectively) (table 3). Thus, the propagation lemon balm by cuttings is quite easy and does not even require using of rooting stimulants. This is one of the favorable physiological characteristics of lemon balm can be applied in cases there are no seeds or seeds are scarce. On the other hand, during the monitoring of the cuttings experiment, the time for the cuttings taking root only lasted about 2 weeks. Meanwhile, the propagating by seed sowing method took at least more than 30 days to form standard seedlings. Therefore, the result of propagation by cuttings method have great significance for the lemon balm cultivation process.

4. CONCLUSION

Lemon balm seeds germinated better in moist, warm stratification condition in a zip-lock bag. When the seeds were sown on the substrate containing 70% coco peat + 30% husk or 50% coco peat + 50% husk, the rate of qualified seedlings was higher than the substrate containing 70% soil + 30% coco peat (84.67%, 85.33% and 68.67%, respectively). Lemon balm could be easily propagated by cuttings, even without using rooting stimulants (the rate of rooting cuttings reached 93.3-96%). The most suitable time of year for cuttings was late spring and early summer.

REFERENCES


