Study of trifluralin influence on effective material of *Mentha pulegium* L.

Faramarz Mir, Seyed Kamal Kazemi Tabar

*Faculty of Farming Sciences of Sari Agricultural Sciences and Natural Resources University, Sari, Iran (faramarz_mir@yahoo.com)*

**Abstract**

The essential oil of *Mentha pulegium* L., also known as Pennyroyal, is shining with a blue glaze due to presence of an element in it named "azulene". According to published results, any increase in *ploidy* level is usually associated with an increase in dry material of plant and secondary metabolites. Therefore, in this study, an investigation has been conducted to analyze variations in effective materials of *pennyroyal* with respect to mutant chemical materials influence such as *trifluralin*. The research was done as a factorial experiment through a completely randomized sampling with 6 repeats in which *trifluralin* was used with concentrations of 1.7, 3.4 and 5 milligrams/liter during periods of 6, 12 and 18 hours in comparison with observer (without using chemical inducer materials). Considering amount of influenced samples' oil with method of titration, difference between various levels of inducers as well as mutual noticeable effect of concentration and time exhibited level of 5 milligrams/liter in the period of 12 hours as the most variation. Generally, *trifluralin* caused alteration of 66.66% in *ploidy* level of aroma *pennyroyal* L.

**Keyword:** pennyroyal L, essential oil, polyploidy, trifluralin

**Introduction**

Secondary plant metabolites are organic combinations which are not involved directly in growth and reproduction of plant. These combinations have more complicated chemical structures than those of primary metabolites (e.g. *amino acids*) which are essential for cell health. *Alkaloids* (*morphine*, *codeine*, and *atropine*), *terpenoids*, *flavonoids*, dyes and *tannins* are of the principal combinations among them (Beigi, 1997).

Considering chemical combinations of *Pennyroyal* L. (*Mentha pulegium* L.), Parviz Babakhanloo (Babakhanloo, 1998) reported the principal combinations of *pennyroyal* *L.* essential oil as follows: *pulegone* (66.5%), *menthone* (24.5%), *menthoruran* (4.2%).

Many investigations have been conducted related to cytochemical inducers. Most of these attempts indicated that in a large number of medical varieties, *polyploidy* induction would result to increase sizes of cells. Therefore, organs containing effective material become larger compared to parental *diploids* organs and finally production of medical compounds increases (Brown et al., 1987) and (Majidi et al., 2005).

Zheljazkoo et al. (1996) stated that *polyploidy* motivation is successful and efficient as a modifying technique in raising the production of plants having essential oil. Through experiments done with *polyploidy* induction, they succeeded to produce new brands in which production contents were superior in comparison with parental *diploid* plants in the case of *azine* and essential oil (Croteau and Gershenzon, 1994). Using *polyploidy* has been validated with numerous investigations as an efficient modifying method in modification of medical plants as well as those with essential oil.

The effect of ultraviolet rays on *Pennyroyal* L. growth is now under investigation (Adams, 2007). Maffei and Scannerini announced a reduction of 40% in *pennyroyal* L. essential oil through consideration of blue and white light (Maffei and Scannerini, 1999).

Increase of medical compound and secondary metabolites is possible in treated plants.
through ploidy inducers and making artificial polyploidy. Therefore, it is possible to increase plant's effective material with increasing plant genome as much as several orders, (Dhawan and Lavania, 1996).

The aim of this study is to investigate the effect of chemical inducers such as "Trifluralin" on the Pennyroyal L. and its relation to production percentage of secondary metabolites in plant.

There are two main types of Pennyroyal L. variety: one has thin stems lying on the ground in a way that its branches touch the ground easily and consequently grow up to 10 centimeters and at the same time propagate on the ground through rooting (samples under consideration in this investigation).

Another variety has stems perpendicular to the ground growing vertically. Hence, their propagation is more difficult while harvesting the crop is easier.

Depending upon the type of plant from which pennyroyal L. essential oil has been resulted by organs distillation, it exhibits relatively different characteristics where pulegone is one of its principal effective materials (Zargari, 1989).

Materials and Methods

The experimental design intended for this purpose is a factorial test in the form of complete random pattern including six repeats. To this aim, three different contents of trifluralin consisting 1.7, 3.4 and 5 milligrams per liter were considered. In addition, three time spans of 6, 12 and 18 hours were used to consider the samples treated.

The material used for increasing the ploidy level, trifluralin, as the Trif factor was supplied in three levels in study compared to the observer (0).

Trifluralin was prepared as much as 0.1 weight of its molecular mass separately and was solved in 2 CC of DMSO solvent. Then, according to concentration, pure water was added. Trifluralin as the Trif factor was applied having the concentration of 0 (observer), 1.7, 3.4 and 5 milligrams per liter. Young shoots which had roots, after timing of interest for treatment (6, 12 and 18 hours) were taken out of the ploidy inducer material and were put in the environment after being washed with flowing water along with 30 grams per liter of sucrose. Then, they were transferred to wet soil environment to pursue the growth process. After about 35 days from growth initiation, sampling was performed in order to extract the essential oil and also to determine ploidy level. The stages performed in the experiment are as follows:

Firstly, the required samples in the form of root-included stem with 2-4 leaves and ending shoots were selected and dispersed in trifluralin solution and in pure water in the case of observer for 6, 12 and 18 hours. After taking samples out of solution, they were washed once with pure water and twice with ordinary water. For following the growth, ordinary water with 30 grams per liter of sucrose (saccharose) was used. Essential oil was extracted when the samples acquired enough growth. The whole process lasted for 45 days.

Essential oil extraction:

For performing essential oil extraction, two methods were utilized.

1. Distillation using water (hydro distillation); in this method, in fact, water and essential oil are distilled together and following that oil is easily derived. Since the essential oil percentage is computed according to dry weight of leaves and plant, therefore, contents of samples' moisture are measured prior to performing the oil extraction.

2. Extraction using solvents; 20 grams of interested organ having dehydrated are picked and milled in a mortar to become in the form of powder. Then, 10 grams of the powder is poured in a tidy beaker and a combination of 5 cc of ammonia, 10 cc of ethanol and 30 cc of ether is added and the whole mixture is whipped in order for the powder to be absorbed entirely with the liquid phase. After that, it was required that the combination of ammonia,
ethanol and ether with the same value is added again to the mixture. Finally, three stages of the mixture mentioned above are added to the dried powder and then the final mixture being totally wet was transferred to a percolator. The powder was influenced with solvents for about 4 hours. Following that, the considered mixture was sifted. Because it is possible that considerable amount of effective materials is left in remainder of mixture, so within two other stages, the same mixture was used and for each time, 45 minutes passed for extraction process. After all, 50 cc of the resultant solution was transferred to a neat burette which was washed with ether.

Since density of ammonia, ethanol and ether which contribute major part of the solvent are 0.77, 0.81 and 0.71 grams per cubic centimeter, respectively, about 100cc of ether was added to this solution. Therefore, the final mixture has density less than that of water.

After sampling, test's results were analyzed using commercial software "SPSS 10.0". Information analysis was performed and analyzed through factorial test in completely random basic pattern and in the form of definite random pattern. Finally, variance analysis and comparison of averages using LSD method were carried out for all tests.

Results and discussion
From 72 samples in the study which were under the trifluralin's influence, in a single case, an increase of 35.37% in effective material was observed and on average, approximately 10.12% enhancement in effective material was indicated in all samples. Concentrations of tested trifluralins and considered time periods for being influenced on percentage of Pennyroyal L. essential oil had considerable effect in probability level of 1% with the most content of essential oil in concentration of 5 milligrams per liter of trifluralin and the least amount in uninfluenced samples (observers). In addition, considering the effect of timing of being influenced on essential oil percentage, it was concluded that time period of 12 hours brought about more essential oil percentage compared to two other periods; i.e. 6 and 18 hours. Differences between various periods of time concerning LSD test were not considerable in level of 1% (Table 2). In the case of various concentrations of trifluralin considered in this study and average values obtained, difference between concentration of 0 and 5 milligrams per liter and also 1.7 and 5 milligrams per liter were noticeable at the probability level of 1% with no noticeable difference between other averages at the level of 1%. However, if the probability level of 5% is considered for average difference of various concentrations of trifluralin, in the case of LSD=0.13, difference between concentrations averages of 0 and 3.4 milligrams per liter as well as 3.4 and 5 milligrams per liter would also be noticeable (Table 1).

Table 1. Comparison of average values of essential oil percentage of aroma pennyroyal L. at various trifluralin concentrations according to LSD test

<table>
<thead>
<tr>
<th>Trif</th>
<th>A0</th>
<th>A1</th>
<th>A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.039ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>0.145ns</td>
<td>0.106ns</td>
<td>0.145ns</td>
</tr>
</tbody>
</table>

** Noticeable difference at probability level of 1% between averages of concentration levels of trifluralin according to LSD test, ns: Absence of noticeable difference at probability level of 1% between averages of concentration levels of trifluralin according to LSD test
A0, A1, A2, A3: Various concentrations of trifluralin at levels of 0, 1.7%, 3.4%, 5%, respectively.
Table 2. Comparison of average percentage of essential oil in *aroma pennyroyal* *L.* in different time periods for samples being influenced according to LSD test

<table>
<thead>
<tr>
<th>Time (T)</th>
<th>T0</th>
<th>T1</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.057ns</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>0.097ns</td>
<td>0.039ns</td>
</tr>
</tbody>
</table>

ns: Absence of noticeable difference at the level of 1% between average percentage of essential oil concerning various time periods of influencing according to LSD test

T0, T1 and T2: Various time periods of treatment under the *trifluralin* influence as long as 6, 12 and 18 hours, respectively.

Produced content of effective material (essential oil) concerning the mutual effect of time and *trifluralin* material is shown in Graph 1. In this graph, the most value equal to 1.8% was resulted from using 5 milligrams per liter of *trifluralin* for 12 hours. Nevertheless, increasing time period at the same level of *trifluralin*, the amount of effective material decreases which indicates negative effects of longer periods of time for using this cytochemical material on the content of effective material.

**Graph 1. Mutual effect of various concentrations of *trifluralin* in different time periods of treatment on the content of *pennyroyal* *L.* essential oil**

**Consideration of *trifluralin* effect on ploidy level of *Aroma pennyroyal* *L.*:**
Observing doubled samples with respect to different concentrations of *trifluralin*, it was concluded that this material totally brings about 66.66% change in ploidy level within samples with the most effect in concentration of 1.7 and 3.4 milligrams per liter for the period of 18 hours (Graph 2).
Conclusions

This study was performed as the factorial test in the form of pure random pattern with six repeats in which effect of trifluralin with concentrations of 1.7, 3.4 and 5 milligrams per liter for time periods of 6, 12 and 18 hours was compared to the uninfluenced samples (without use of chemical inducer materials). Considering content of essential oil in influenced samples using method of titration, differences between various levels of inducers as well as mutual noticeable effect of concentration and time were found considerable with the most change related to the concentration level of 5 milligrams per liter in period of 12 hours. In general, trifluralin brought about change as much as 66.66% in ploidy level of Aroma pennyroyal L.

References


