STUDIES CONCERNING THE INFLUENCE OF SOME MUTAGEN AGENTS ON THE PRODUCTION OF INFLORESCENCES AT *CALENDULA OFFICINALIS L.* SPECIES

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The economic importance of the pot marigold is increased due to considerable broadening spectrum of raw material use (Flores calendulae, Herba calendulae) in the cosmetics manufacture and in particular the use of the seeds as a source for obtaining the oil used for industrial purposes. In this moment Calendula officinalis L, is in top ten, of the most cultivated herbs in Europe. The aim of this paper was to see the impact of the mutagenic substances on the production of inflorescenses at Calendula officinalis L. in the year 2006 conditions. Applying the treatments on the top of the vegetation have led to obtain variables production of inflorescenses, close to some treatment variants by the production obtained on untreated witness sample.

The analysis of the results obtained on the year 2006 conditions, has allowed us to say that, the differences of the production that we obtained were due on the one hand for the different concentrations used, but also due to the implementation of the treatment and a different time of action on the material used in the experiment. The productions were significant as increasing the concentration in the case of colchicine but decreased in light of the treatment implementation and the extent of increasing the time of action.

***Key words**: Calendula officinalis L, mutagen agents, productions, medicinal plants.*

Calendula officinalis L. is an important species of medicinal plants in many countries such as Germany, Great Britain, Netherlands, USA, Spain, India, Romania, France, Russia, recognized for the special phytotherapeutic virtues of the basic material used in the preparation of many cosmetic products, and for the technological and biological characteristics to capitalize a wide variety of pedoclimatic conditions. [8] The culture is treasured for the capacity of adaptability on these conditions, its requirements being very modest. [7] The inflorescences, harvested almost entirely by the hand, are widely used in scientific and traditional medicine, haling the wounds. [2, 1]

The inflorescences are collected on the extent of flowering, which takes a long time: 60-90 days and sometimes over 100 days. According to the studies,

made to the Resort of Research and Development for Medicinal and Aromatic Plants Fundulea, on fertilization of pot marigold areas, it have been obtained productions of dried inflorescences by 11,1 q/ha to the witness, up to 14,5 q/ha due to the application of N_{45} K_{45} [4]. In terms of Romania conditions in the years of the '90 were obtained 6-8 q/ha dried inflorescences [7]. Besides density, an important factor for obtaining high yields of inflorescences and seeds is also the nutrition area of plants and the distance between rows. The highest production of inflorescences in the Romania conditions, were obtained at densities of 70 plants/m^2. Under the conditions of Romania the production potential varies between 10-12 q/ha [10], 10-15 q/ha [3, 5], 10-20 q/ha [9], 18-20 q/ha [6].

MATERIAL AND METHOD

In the experiment we used as mutagenic substances colchicine, 2.4D acid and etidium bromide. The substances had 4 concentrations and the treatment were made on the top of the vegetation, when the plants have reached the stage of 2-4 leaves, and on the seeds with tow different time of action: 3 and 6 hours. The collections of the inflorescenses start in the middle of June at an interval of 3-4 days.

Each variant of treatment had four repetitions, the results representing the average. For determining the causes which led to differences on the productions we used the analysis of variance.

RESULTS AND DISCUSSION

The production of inflorescences obtained from *Calendula officinalis* L. in 2006, has been varied depending on the months when the flowers were harvested, how the treatments were made, by the substances used and their concentrations.

Thus, if the treatments made on the top of the vegetation, in June, there has been little, insignificant variation of the yields, between variants accounting the fact that the number of collection was reduced. In the case of treatment made whit colchicine, can be seen that we increasing the concentration of the substance, the production was increased to. At the 0.01%, 0.02% and 0.03% concentrations, the yield are recorded much lower relative to the witness. The variant treated with 0.04% achieved the same production as in the witness case. The biggest production, in June, was obtained in the etidium bromide case at 0.01% (1905 kg/ha) recording an increase of the production relative to the witness by 150 kg/ha.

At the 0.02% and 0.03% concentrations the yields obtained were approximately equal, but they recorded an excess approximately whit 125 and 105 kg/ha against the witness. The lowest production of inflorescenses in this substance case, even lower relative to the witness, has been obtained from variant treated whit 0,04% (870 Kg/ha). In the etidium bromide case, can be seen that increasing the concentration the inflorescenses production falls. For the 2.4 D acid, the highest production values were obtained at lowest concentrations respectively 0.01% and 0.02%, exceeding in these cases the witness and starting from 0.03% concentration to record lowest values of production. At the 0.04% concentration was obtained the lowest production value, in June, even compared with the other treatment options.

In conclusion in June, the largest production has been registered for variant treated with the etidium bromide at the 0.01%, and the lowest production has been obtained at the same substance on 0.04%.

For July, the number of collection was 7, at an interval of 3-4 days, achieving the highest production in the same case of treatments with etidium bromide at 0.01% concentration (4210 Kg/ha), and the lowest of 0.04% concentration. As in June, in the colchicine case, increasing the concentration has as result an increased production, the greatest amount of inflorescenses being achieved at 0.04% (3205 kg/ha), this amount representing a percentage of 119.14%. In the case of 2.4 D acid, it is maintained the same rule, that by the extent of increasing concentration, the production register decreases. Nevertheless, in the case of the three substances used in the experiment we obtained very significant differences relative to the witness. If we make an analysis of the results for the august, very significant production is the one obtained at the etidium bromide to the 0.01% concentration (4670 kg/ha) in which case the witness was passed in a percentage of 113.90%. From an analysis of variants treated with colchicine, we can see a very significant difference, but the values are negative, compared with those obtained in the case of the witness. At the etidium bromide, the variants of treatment 0.01% and 0.02%, have exceeded the witness, but in the second case, the difference of 90 kg / ha is classified as DL> 5% and she is insignificant. The other two variants of treatment have obtained lower values relative to the untreated witness. Regarding the production of inflorescenses obtained in September this is declining toward the one obtained in august, but here are two other factors that have interfered. On aside the number of the harvests decreased, and on the other hand it has reduced the number of flowers/plant and increased the number of flowers forming seeds.

A significant production has been obtained in the case of the treatment with etidium bromide (0.01%) and the lowest production has been obtained at the 0.04% of the same substance. Nevertheless, the productions recorded on the treatments variants were quite high. The treatment with colchicine applied on the top of the vegetation, recorded the highest production in august but these productions have not succeeded in overcoming the witness in June July and September. They may find that, in the case of treatment with colchicine was kept the same rule, that increasing the concentrations, to register and increases of the yields. The variants treated with the etidium bromide have managed to overcome the witness at the 0.01% and 0.02% concentration throughout the season. At the 0.03% concentration this was achieved only in June and September and a value close enough has been reported for the august. This time, when the concentration was increased, we obtained lowest productions. This can be observed also in the case of 2,4D acid where the witness was exceeded only in July. The productions obtained in the case of the treatment made on seeds with 3 time of action, in June are between 1755 kg/ha (witness) and 1020 kg/ha at 0,01% colchicine concentration , being also the maximum and minimum registered for this month. The 0.01%, 0.02% and 0.03% colchicine treatment, registered in June very significant differences compared with

the witness. The negative differences were:-755 kg/ha (colchicine 0.01%), -665 kg/ha (colchicine 0.02%) and -590 kg/ha (colchicine 0.03%). The 0.04% concentration has achieved a significantly distinct value of the production of -555 kg/ha relative to he witness. In this case, the treatment with colchicine led obtaining increased production in the extent of increased concentrations. Compared with June, in the case of the treatment made on the top of the vegetation, the productions were lowest. The etidium bromide treatment have also registered lower values so, at the 0.01% the difference of -230 kg / ha is considered following the statistical calculations as insignificant. A very significant difference has been obtained at the 0.04% where production was 1115 kg / ha. In the case of treatments made with 2,4D acid the productions registered values between 1525 kg/ha at the 0.01% and 1135 kg/ha at the 0.04% with a very significant difference of -620 kg / ha compared with the witness. Significant differences were obtained at the 0.02% (-350 kg/ha) and 0.03% (-390 kg/ha. It also may find that, we increasing the concentrations, the production are decreasing. Compared with the same month, in the case of the treatment made on the top of the vegetation, the yields obtained are much lower. For the august, the witness registered on this case a production that exceeded very significantly the yields obtained after the treatment. The etidium bromide treatment, recorded a deficit relative to the witness and the values are between 3240 kg/ha at the 0,01% and 2665 kg/ha at the 0,04%, the differences being particularly significant. The results analysis highlights for august a low level of the productions obtained after the treatments.

The significantly effect of the treatment, could be observed at increased concentrations of substances used in the experiment, where it seems that the yields obtained were much lower relative to the untreated witness. The values obtained were also lower than the productions obtained on the treatment made on the top of the vegetation. For September, the productions of inflorescenses in all variants of treatment decreased do to reducing the number of collection and the number of flowers/plant. The reductions of the yield in all variants of treatment, were not very significant, the differences between them being 475 kg / ha recorded at 0.04% concentration, at the etidium bromide substance and 80 kg/ha at 2.4D 0.01%. The production decreased to 88.70% at the colchicine treatment 0.01%, reaching up to 93, 94% at the 0,04% concentration, with a difference of -185 kg / ha against untreated witness. In the case of etidium bromide treatments the highest difference of production (-475 kg/ha), has been obtained from the 0.04% concentration. On these variations of the treatment the yields obtained decreased relative to the witness, on the extent of increasing concentrations, being low relative to the yields obtained in the same month in the case of the treatment made on the top of the vegetation. Following the results in the case of 2.4D acid, we can see that, the productions have made the following decreases: 97.38% at the 0.01%, approximately the same value at the 0.02%, reaching out to 95.58% at the 0.04%.

From an analysis of the results obtained in 2006 (*fig.1*) in the case of treatments made on the seeds with 3 time hours of action, we can extract the following conclusions. All variants of treatment recorded low production relative

to the witness (11600 kg/ha). The differences are very significant in all variants of treatment with colchicine, differences between being -3065 kg/ha (colchicine 0.01%) and 2110 kg/ha (colchicine 0.01%). In the case of two other substances the yields decreasing on increasing the concentrations. The two substances have obtained the highest production, at the de 0.01%.

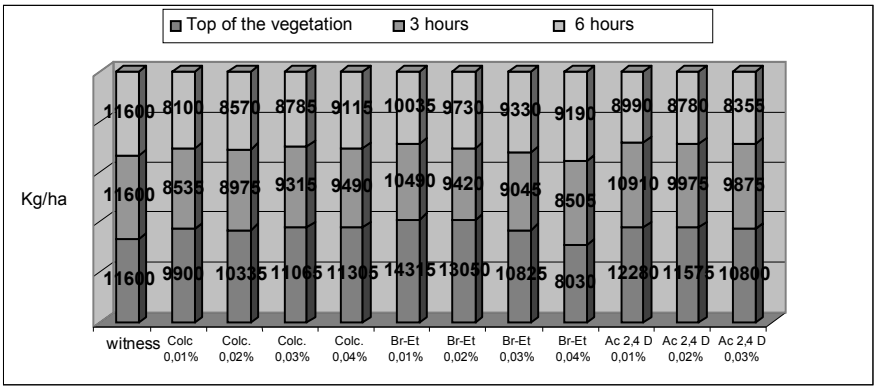

Figura 1 **The production obtained to *Calendula officinalis* L. depending on the application of the treatment in 2006**

If we do an analysis of the results obtained in the case when the treatment was made on the seeds with 6 hours time of action, we can see that the recorded values varied between maximum of 1755 kg/ha at the witness and minimum of 1025 kg/ha at 0,04 (2,4D acid). All variants of treatment recorded low production differences being very significant. The biggest production at the colchicine treatments has been obtained from the concentration of 0.04% (1355 kg / ha). The production obtained, if the treatment was made with etidium bromide have seen a very significant difference over the witness. Reviewing the results of 2,4D acid we can see that, here were obtained productions between 1450 kg/ha to 0.01% and 1025 kg/ha to 0.04%. As with the other two modes of treatment presented earlier, were registered increased productions with increasing concentration in the case of colchicine, and a reverse situation in the case of other two substances.

Quantitative the productions were lower than those obtained for the same month in the case of treatment made on the top of the vegetation and thou made on seed with 3 hours time of action. In July, the yields obtained after treatment, have not succeeded in overcoming the production obtained by the witness, the production deficit recorded being one very significant. The 2,4D acid 0.01%, recorded the highest percentage of production by 95.35% followed by the 0.02%, 0.03% and the lowest value was registered at 0.04%. August brought changes regarding the maximum of the inflorescenses production which was obtained in the case of etidium bromide 0.01% (3245 kg/ha), and the lowest production being achieved at 0.04% by the 2.4 D acid. As is notes in this month the witness has exceeded all variants of treatment. In September, regarding the fact that the collection of flower was reduced and ware made at greater intervals of time, the yields have achieved significant reductions.

CONCLUSIONS

The treatments with colchicine had the highest production when the treatments were made on the top of the vegetation, the greatest value being achieved at the 0.04% concentration (11305 kg/ha). It can be seen that the yields increase as the concentration is increased, but they fall depending on the implementation of treatment. Increasing the time of action determined a decreased amount of inflorescenses. The treatments made on the top of the vegetation, have registered the maximum of the production at the 0.01% etidium bromide, and the lowest in the case of 0.04% concentration (8030 kg/ha). The 2.4D acid obtained at the four concentrations large productions, by applying the treatments on the top of the vegetation, on the second place were the one performed at the level of seeds with 3 hours time of action and on the last place where the treatment with substances which have operated 6 hours on the seeds. In the last case the lowest production was carried out at 0.04% (7600 kg/ha).

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