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PROPAGATION AND CULTIVATION OF *MIMULUS GUTTATUS* DC IN THE DISTRICT OF SOFIA – FEATURES AND PROSPECTS

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Abstract

The options to optimize the production of plants and the lifespan and phenological characteristics of *Mimulus guttatus* DC in the climate of Sofia were studied. The seedlings were grown in a greenhouse for 8-10 weeks, at a temperature of 12-17ºC, intensity of natural light – 1300-18500 lx and air humidity 63-74%. The young plants had the following biometric characteristics: a rosette structure, 5 pairs of leaves with length of 7.60 ± 1.37 and 8.11 ± 1.61 cm for the first and second pairs of leaves and respectively average width of 2.39 ± 0.54 and 2.66 ± 0.70; average height of the stem: 16.06 ± 3.6 cm. A link between the amount of precipitation during the growing season in the field and the morphology and lifespan of the individuals was sought, with a view of assessing the risk of self-sowing of *Mimulus guttatus* potential performance as invasive species in the park areas of the city. With precipitation over and around the normal for Sofia, on a soil substrate with unfavourable structure and low availability of nitrogen and potassium and average availability of phosphorus, two experimental options were tested: with none and with a one-time fertilization. *Mimulus guttatus* plants bloomed around 1.5 months and performed as annuals, with no evidence of self-sowing or overwintering, with no danger of invasion or threat of uncontrolled proliferation.

Keywords: *Mimulus guttatus*, lifespan, self-sowing, invasive, propagation.

INTRODUCTION

The ornamental qualities of genus *Mimulus* DC species have long been appreciated in the world floricultural practice. In recent years, in our country, different species of *Mimulus* DC and cultivars of them are being imported or produced, although in limited quantities.

However, there is still no clarity on the lifespan of plants as outdoor ornamentals in park areas, as well as on the relationship between the duration of flowering period and the climatic conditions during the growing season.

Mimulus guttatus DC originates from North America but recent studies have shown secondary distribution in Wales, Northern England, Scotland, Poland, Germany and totally in 16 European countries and New Zealand (Roberts, 1964; Truscott et al., 2008; Van Kleunen and Fischer, 2008; Stace, 2010; Tokarska-Guzik and Dajdok, 2010 – by Vallejo-Marín, 2012). Outside it's natural range, in wet habitats, the species is described even as invasive. In our country, *Mimulus guttatus* DC is defined as adventitious (Assyov et al., 2006), with a habitat in Sofia region.

The record is based on Petrova observations (1976, unpublished data) on a field of *M. gutattus* on wet meadows, near the village of Kazichane (Sofia District). Plants in floral arrangements, located in the nearby park Vrana are supposedly its source.

At the same time, there are data distribution and reports for vegetative reproduction of the populations of three and polyploid sterile hybrid forms with the participation of M. guttatus, described as invasive in the UK (*Mimulus x robertsii* (*M. guttatus x M. luteus*), by Vallejo-Marín, 2012; Vallejo-Marín et al., 2013).

Naturalized populations of sterile hybrids have been observed in Britain at least since 1872 (Silverside, 1990; Rreston et al., 2002; Stace, 2010, BSBI 2011 – by Vallejo-Marín, 2012).

The aim of this study is to characterize the duration of the ornamental performance of individuals of *Mimulus guttatus* DC at the meteorological conditions of 2014 summer in the city of Sofia, Bulgaria, and assessment of the risk of self-sowing and the rupture as invasive species in the park areas of the city.

The opportunities to optimize the seed propagation and production are also researched.

The interest was shown for *M. guttatus* as an ornamental plant, and the direction of our study was determined by the following facts:

- The studied species is extremely flexible. It is typical for taxonomic complex within its kind, bringing together species with similar ecological requirements, namely: *M. guttatus* is presented with populations spread throughout regions on sea level up to 3,050 m above it. (Grant, 1924; Vickery, 1978; Beardsley et al., 2004). The species can be found in open meadows, forests, deserts, streams, bogs, alpine meadows and screes, coastal cliffs and dunes, on a geological basis of basalt cliffs, extreme soil conditions, even the periphery of geysers (at water temperature to 80ºC in the western United States). The species of this complex have shown tolerance to anthropogenic soils, toxically laden with copper and nickel; abandoned mines and pits (Tilstone and Macnair, 1997).

- In the range of habitats, a trend for long drought periods and variable values of soil moisture occurs, and the ecological flexibility is correlated with the flowering period, plant morphology and lifespan (Kiang and Hamrick, 1978). Wu, et al. 2010 define the soil moisture as a key factor determining the distribution and abundance of the species. The authors observed some morphological, physiological and phenological adaptations in the *M. guttatus* complex in conditions of water deficit.

- Within the species, two types of populations are distinguished: intercontinental populations that often are found in western North America. Plants grow as small annual plants with early spring blooming and rapid decaying of stems and leaves. The second type: coastal populations are formed by later blooming ecotype *M. guttatus ssp. grandis* Greene. They are spread in wetland habitats (Hall et al., 2006), distinguished by a longer and later flowering season. In a study Wu, et al. 2010 found that individuals of *M. guttatus*, that inhabit coastal areas with lack of moisture, abloom too late and fail to form seeds.

- In specialized literature, for the production of the natural species, two options for seed propagation are recommended: the spring-winter sowing in unheated greenhouses or autumn sowing in an open bed. For the perennial species and their hybrids is recommended vegetative propagation as follows: with a division of the roots; with soft cuttings; with detaching the stems to the soil. The duration of production by cuttings is between 6 and 12 weeks.
(http://www.mimulusevolution.org), by Department of Energy's Joint Genome Institute. (JGI; http://www.jgi.doe.gov;http://www.ncbi.nlm .nih.gov)

- According to recent studies, there is a tendency to reduce care for watering grass and flowers in the park areas in our country, particularly in Sofia, where to a large extent the water needs of plants are met only by the quantity of the natural precipitation (Lozanova et al., 2013). This problem particularly occurs in spaces between residential areas, where the maintenance of the plants is unfavorable and the use of drought-resistant, oligotrophic species is preferred. (Rangelov, 1990; Rangelov, 2016). Recent studies suggest opportunities use *Mimulus* species for the purposes of reclamation (Petrova et al., 2015) and prove the phytotherapeutic effect of the plants in modern medical practices, making them suitable for use in urban areas in the context of elimination of the unhealthy energy impacts the recreation areas. (Shahanov, 2012).

MATERIALS AND METHODS

For laboratory and soil germination, a seed sample of *Mimulus guttatus*, received by Viote, Alpine Bot. Garden (Monte Bondone, Trento, Italy) was tested. Due to the extremely small size of the seeds (22 000-30 000 units/g. (Kabatliyska, 2005), the listing of the seeds is with accuracy up to ± 5%. The applied laboratory germination test complied with the rules of ISTA: testing on filter paper, temperature range 20-30ºC; storing in the refrigerator at 0-5^{o}C and first/final report accordingly 7/21 day, set of 4 samples; 100 seeds per sample.

The sowing and germination test in greenhouse took place on 17 and 18 March 2014 in round containers with dimensions: d = 13/4,5 cm, no coverage of soil; set of two samples, 400 seeds per sample. Seedlings were replanted 22 days after sowing (April 8.) in phase cotyledons and first leaf, in plates for seedlings FR50N, 54x28x8.5, with a beveled bottom. Replanting of a total of 30 plants at an open bed was carried out on 11 June in 3 furrows, with a spacing of 25 cm.

Soil conditions. Enriched peat substrate with the following characteristics: pH: 5,5 to 6,5; organic matter more than 80%; a medium specific structure of the fibers; NPK: 1,5 kg/m^3; Ms/cm 1,0:1,5 (origin from Lithuania) was used for sowing and planting of the seedlings in the greenhouse. The soil in the outdoor beds was analyzed in the Central Laboratory of University of Forestry as follows: particle-size distribution (sieve analysis); mechanical structure (%) – by the method of *Kaczyński*; bulk density (g/cm3) - method of the rings; humus content (%) – method of Turin; total nitrogen (%) – Kjeldahlmethod; pH (H_2O) - potentiometric; mobile forms of phosphorus (mg/100g) - acetate- lactate method of P. Ivanov. Fertilization of plants during the growing period was applied only once with Osmocote granulate (NPK): 18/09/10 amounting to 3g/1m^3 superficially, before planting. For the samples of fertilization and the control (nonfertilized) plants, after this in the text are used the following abbreviations: (F) and (C).Because of the alleged link between lifespan and flowering performance with the weather conditions, the experimental set included detailed monitoring of environmental conditions as follows: in a laboratory, greenhouse and outdoors.

Lighting conditions. In the laboratory, lighting conditions were monitored by lux-meter (Model "YU116"), at 50 cm from the window. The intensity of illumination (lx) was recorded with reports in 9, 12 and 15 h. In greenhouse conditions, the light mode was monitored by the above-mentioned device with records in the same hours, every day. The intensity of illumination (lx) and density of the photosynthetic photon flux (PPFD, $\mu mol.m^{-2}.s^{-1}$ -average, absolute minimal and maximal values) were traced to the period of March-June 2014. At the outdoor beds the natural day length for Sofia was considered in the period of the study (from June to September 2014- http: //www.nao-rozhen). Plants were planted in direct sunshine.

Temperature and air humidity. In the laboratory, temperature measurements were made with a term thermometer. In greenhouse conditions, the temperature was monitored every six hours by an electronic wireless device TFA Dostmann 433 MHz – Temperature station. Data were automatically transferred for processing in Excell. The average diurnal temperature was calculated using the formula (Klimatichen spravochnik, 1983):

$$t_{avg} = \frac{t^{7h} + t^{14h} + 2t^{21h}}{4}$$

Humidity was measured with the same periodicity (in %). (Klimatichen spravochnik, 1979):

$$r_{avg} = \frac{r^{7h} + r^{14h} + r^{21h}}{3}$$

Outdoor, temperature data were summarized by the newsletters of National Institute of Meteorology and Hydrology of Bulgarian Academy of Science for 2014 (June-August).

The data of air temperature, relative humidity and precipitation were compared to the 1961-1990 period, adopted by the World Meteorological Organization as the climatic normal.

Soil moisture. In a greenhouse, after sowing, the containers were initially capillary watered to achieve the full soaking. The second irrigation took place after the appearance of the cotyledons. Under field conditions, intensive care with watering of the seedlings was applied only during the time of planting (10-15 June). Watering was done in the morning, 3 times a week with a quantity of 500 ml per plant. After stabilization of growth, the plants were grown only in naturally fallen precipitation amounts for the period (data from NIMH of BAS, 2014, June-August). To assess the conditions of atmospheric drought, De Marton index (J) was applied, attached an indicator characterizing atmospheric conditions of drought:

$$i = \frac{12p}{t+10}$$

where ***p*** is the monthly rainfall (mm), and ***t*** – average month temperature (°C). Drought conditions are determined when the values of the index *(i)* are less than 30, and values less than 20 are indicative for high drought. (Raev et al., 2003; Koleva and Alexandrov, 2008)

Seedlings were measured on 10. 06. 2014, before being planted outdoors. Twenty plants (10 for each sample) were measured, taking into account: the length and the number of rosette leaves; length and number of pairs of leaves; stem length and diameter.

The flowering plants were measured as follows: during full blossom (07. 07. 2014), ten individuals in total, randomly selected from each option (F and C), taking into account the number of flowers in the inflorescence and the inflorescence axes length; at the end of flowering (18. 08. 2014): total number of flowers and the percentage of withered away flowers and formation of seeds.

RESULTS AND DISCUSSION

The percentage of laboratory germination of seeds at temperature: min-max 17-23ºC accordingly and intensity of natural lighting 3200-40000 lx was 98.In greenhouse conditions at 12-17ºC (March-April) (Figure 1), intensity of natural lighting 1300-18500 lx (PPFD between 20.41-340.23 $\mu mol.m^{-2}.s^{-1}$) (Table 1) and air humidity 63-74% (Fig. 2), seeds sprout for 5-7 days with germination energy (on 7th day) 83%, and final report on the 21-th day of the beginning the experiment: 95% (± 5%).

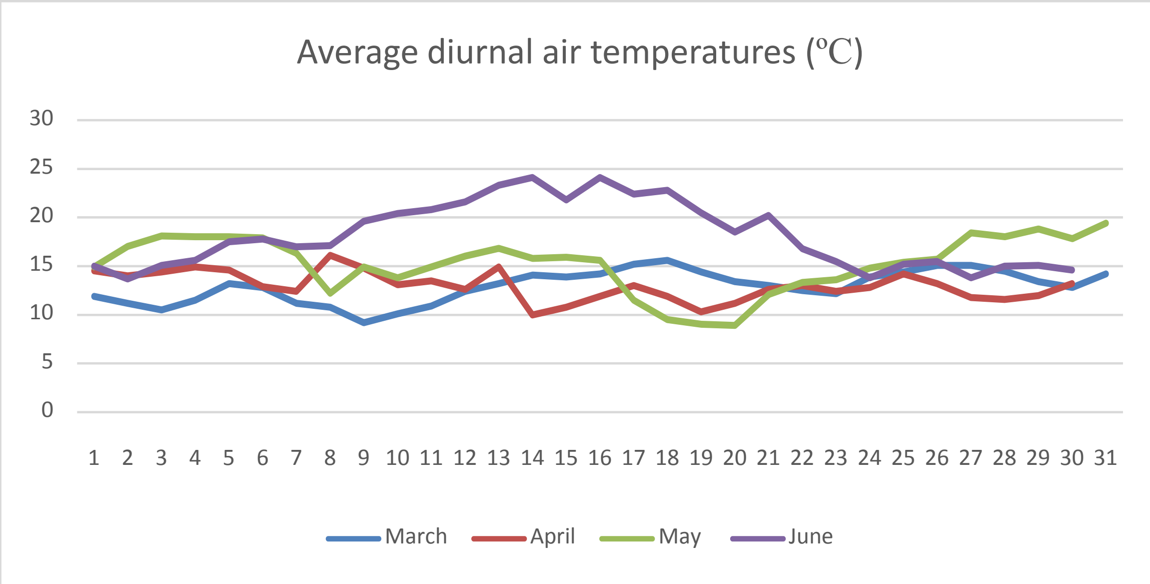

Fig. 1. *Air temperatures in a greenhouse(Cº) March, April, May, and June, 2014*

Seedlings were grown at: average diurnal temperature 8 ºC (May) – 23 ºC (June); intensity of natural lighting 2800-23000 lx (PPFD between 50,18-420,55 μmol.m^{-2}.s^{-1}) (Table 1) and an average air humidity of 69-83% (Fig. 2).

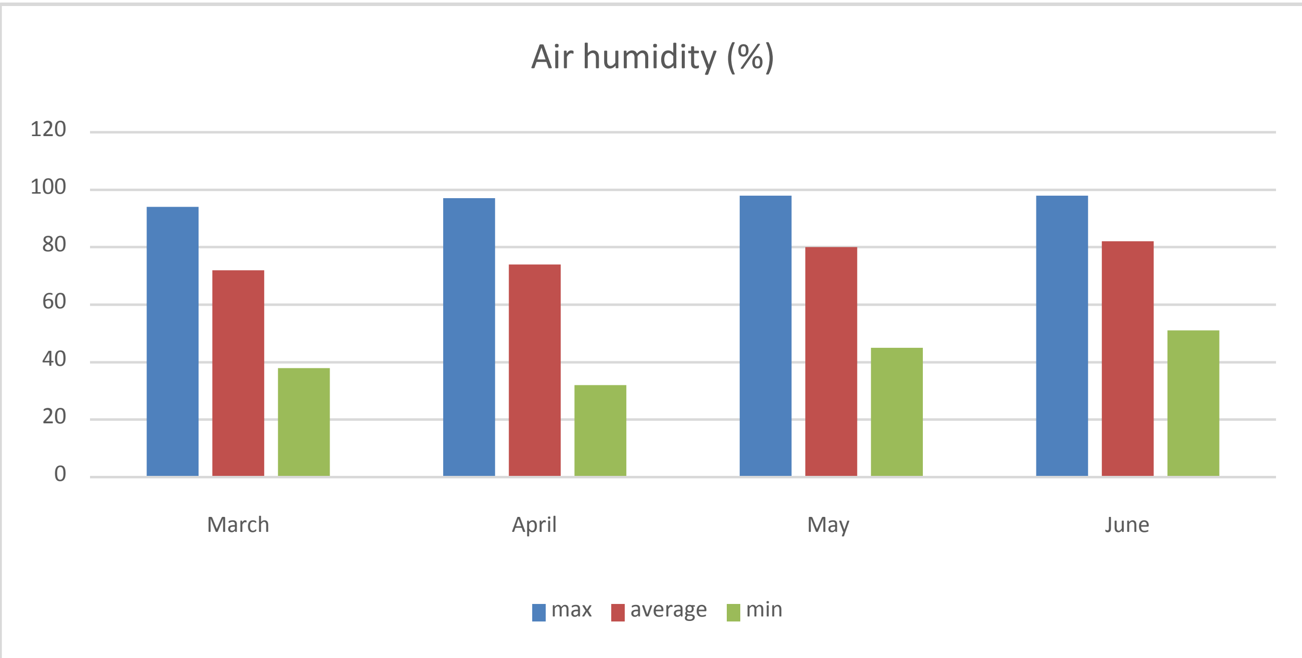

Fig. 2. *Air humidity in a greenhouse (%) March, April, May, and June 2014*

Table 1. The intensity of illumination (lx) and density of the photosynthetic photon flux (PPFD, $\mu mol.m^{-2}.s^{-1}$ in the greenhouse - average, absolute minimum and maximum values, March-June 2014)

Months	Hour	*Average*(lx)	*Average* ($\mu mol.m^{-2}.s^{-1}$)	*Absolute minimum* (lx)	*Absolute maximum* (lx)	*Absolute minimum* $\mu mol.m^{-2}.s^{-1}$	*Absolute maximum* $\mu mol.m^{-2}.s^{-1}$
March	9:00	4027	69,581	1300,00	11500,00	20,41	210,28
	12:00	6017	104,02	1500,00	18500,00	20,78	340,23
	15:00	6017	104,03	1800,00	17500,00	30,33	320,38
April	9:00	10208	176,41	8000,00	18000,00	140,80	330,30
	12:00	10052	173,78	8000,00	13000,00	140,80	240,05
	15:00	7043	121,71	1700,00	13000,00	30,15	240,05
May	9:00	10133	175,19	6000,00	20500,00	110,10	370,93
	12:00	10468	180,95	2800,00	21800,00	50,18	400,33
	15:00	10473	181,05	7500,00	23000,00	130,88	420,55
June	9:00	10248	177,17	10000,00	15000,00	180,50	270,75
	12:00	10375	179,31	11000,00	17000,00	200,35	310,45
	15:00	10238	176,90	9000,00	17000,00	160,65	310,45

After two and a half months of growth, the seedlings of *Mimulus guttatus* had the following biometric characteristics (Table 2).

Table 2. Biometry of seedlings of Mimulus guttatus DC. before replanting outdoor. (10.06.2014)

Feat ures	Lenght of rosette leaves (cm)			Lenght of stem leaves (cm)										Leng- of stem (cm)	Diam. of stem
	1	2	3	I pair of leaves		II pair of leaves		III pair of leaves		IV pair of leaves		V pair of leaves			
				L.	W.	L.	W.	L.	W.	L.	W.	L.	W.		
Aver.	1.9	3.5	5.7	7.60	2.39	8.11	2.66	7.22	2.57	5.92	2.65	4.33	1.99	16.06	3.48
Max.	3.7	4.9	7.6	9.5	3.2	10.5	3.8	9.7	3.6	8.9	4.9	6.2	2.8	21	4.5
Min.	0.8	2.4	4.9	5	1.5	5	1.7	4.3	1.7	3.4	1.6	2.5	1.2	9.8	2
St. Dev.	0.8	0.8	0.8	1.37	0.54	1.61	0.70	1.56	0.63	1.49	0.87	1.01	0.50	3.60	0.78

L. - lenght; W. - Width

At the base, there were about 3 rosette leaves with an average length between 1,9 ± 0,8 and 5,7 ± 0,8 cm. Each developed 5 pairs of leaves and the average length of them decreased from base to top as follows: 7,60 ± 1,37 and 8,11 ± 1,61 cm (first and second pair of leaves) to 4,33 ± 1,01 cm (fifth pair).

Similarly, the average width of the leaves ranged from 2,39 ± 0,54 and 2,66 ± 0,70 to 1,99 ± 0,50 cm with the same pairs of leaves. The analysis of the values of the standard deviation of the measured parameters outlined uniformity of the propagating material. More heterogeneous are the plants, concerning the stems height (average 16,06 ± 3,6 cm; min 9,8 cm – max. 21 cm), which is explained by the specific pattern of the individuals in containers for planting. Assessed by the average diameter of the stem (3,48 ± 0,78 mm) the seedlings were defined as a well-developed with no traces of etiolating, so and the intensity of light can be determined as sufficient for the optimal growth of the young plants.

The properties of the soil for growing the plants outdoors are as follows: adverse lumpy soil structure with high bulk density - 1,5 g/cm³; medium sandy-clay mechanical structure - physical clay content of 34%; slightly acidic soil solution - pH 6,3; low humus content – 1,26%; low availability of nitrogen – 0,134%; average availability of phosphorus - 7,5 mg/100 g and low availability of potassium - 12 mg / 100 g.

The length of the day during the growing period ranged as follows (June: 15,06 to 15,17 hours; July: from 15,16 to 14,31 hours; August: 14,29 to 13,13 hours and September 13,10 to 11,48 hours). The number of gloomy days during the three summer months ranged from 1 to 13 per month.

The analysis of the above-mentioned weather data showed that the period of cultivation of the individuals of *Mimulus guttatus* exceeded the average month number of gloomy days.

The temperatures from June to August ranged as follows (fig. 3, 4, 5): the average meanings between 18,4 ºC (June) and 21,6 ºC (August) with minimum temperature for these months: 6,2 and 8,4 ºC and maximum: 29,8 and 33,0 ºC.

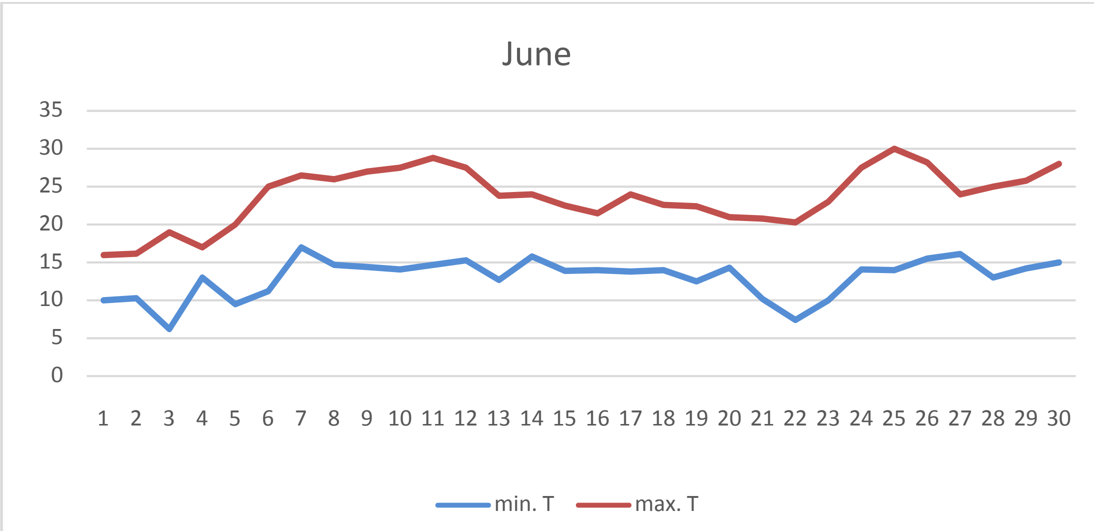

Fig. 3. *Air temperature(Cº), June 2014, city of Sofia*

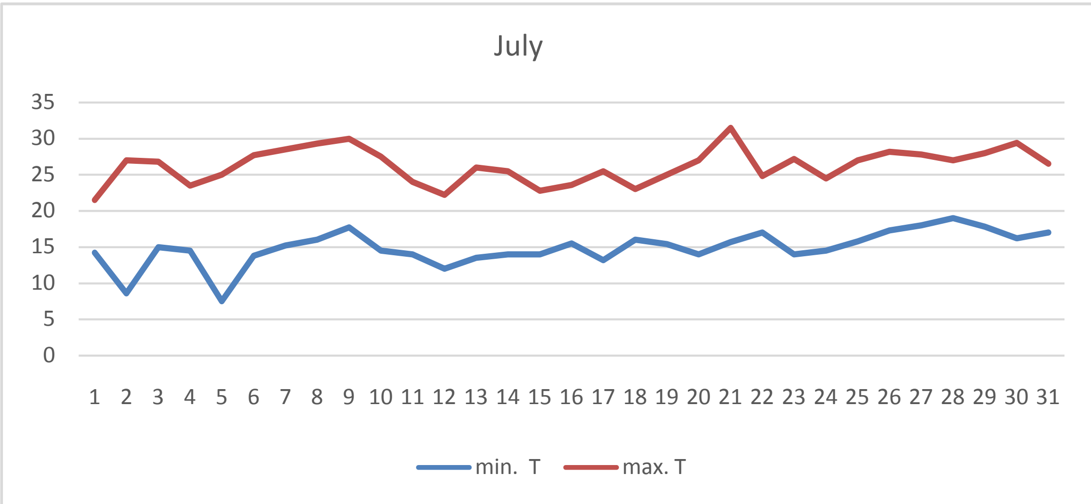

Fig. 4. *Air temperature(Cº), July 2014, city of Sofia*

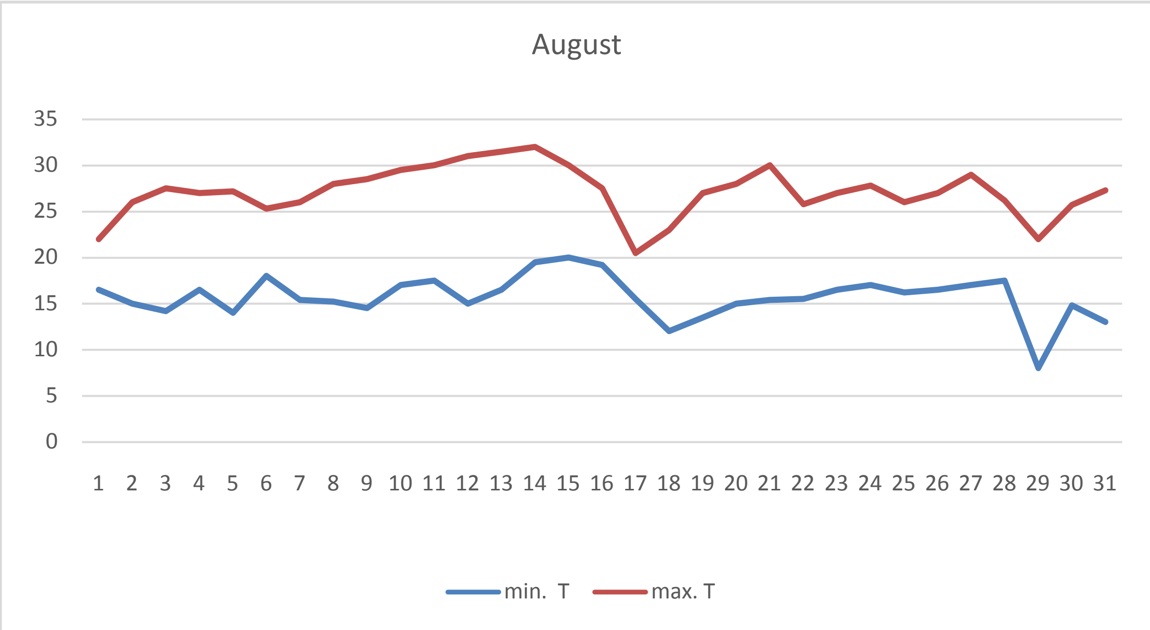

Fig. 5. *Air temperature (Cº), August 2014, city of Sofia*

The values of the indicator δT, determining the deviation of the monthly rate of temperature ranged between 0,6 and 2,1 ºC, and the deviation is with a positive sign.

During the growing season had been observed diurnal temperature amplitudes, the maximum of which reached 15-20 ºC.

The rainfall amounts for the months of June and July were abundant (NIMH of BAS newsletter for June, July and August 2014 in Sofia), respectively 115 and 118 mm for June and July and 77 mm for August.

For all months, the values of Q/Qnratio (%), representing the ratio of monthly precipitation amount compared to the normal was over 150, which defines the 2014 summer as humid.

The values of de Marton Index (J) for the months of June, July and August were respectively 48,6; 46,1 and 29,2 that exceeds the limit, defying drought and extreme drought conditions (Koleva et al., 2008; Raev et al., 2003).

The data for the number of days with rainfall exceeding 1 mm indicated that during the months of active vegetation and full blossoming (June and July) in 1/3 of the days of the month such rainfall had seen observed (Table 3).

The largest amounts of rain fell consistently in the days between June 15 to 21 and July 9 to 29, which coincided with the period of the beginning and full blossoming (Fig. 6).

Table 3. Meteorological data for Sofia, 2014 (The newsletter of NIMH, BAS, 2014)

Months	Air temperatutes (Cº)						Precipitation (mm)				Days with:			
	T avg.	δT	T max	Day	Tmin	Day	Amount	Q/Qn (%)	Max	Day	Precip. (mm) ≥1	Precip. (mm) ≥10	Wind (m/s)	Thunderstor (ms)
June	18.4	0.6	29.8	24	6.2	3	115	153	22	16	11	4	2	9
July	20.7	0.9	30.6	21	8.8	2	118	188	24	9	13	4	1	14
Aug.	21.6	2.1	33.0	14	8.4	29	77	151	58	1	6	1	0	6

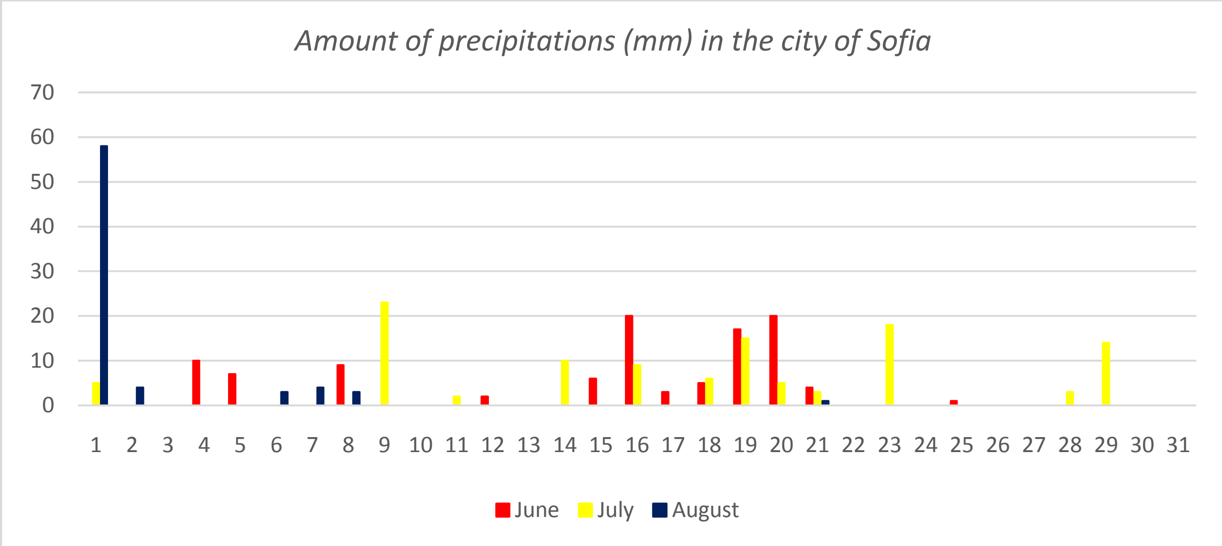

Fig. 6. *An amount of precipitations (mm) city of Sofia, months: June, July, and August 2014*

For the month of August, rainfalls were typical, especially for the first 10 days. This coincided with the end of the flowering season and beginning of ripening and decaying. In version (F), an extending of the flowering period was recorded with a tendency for branching of plants (up to 5 branches per plant with between 4 and 8 flowers) and forming the outlook of a perennial plant with basal shoots. The biometric characteristic of mature plants in phenophase flowering and ripening is presented in Table 4.

The flowering period at (F) and (C) variants began simultaneously, but in the phase of full blossoming in (F) the number of flowers was increased in favour of version (F)- the average number of flowers was 10,2 ±3,91 (F), compared to 9,5 ±3,63 in version (C). The deviation in the length of the inflorescence was similar (5,2 ± 1,23 cm. (F) versus 5,3 ±0,48 cm (C). A significant difference was observed in the percentage of the withered away flowers. At the end of August they were86,19% of the total number of flowers in version (C) and the ripen seed capsules in 90% of the individuals of the same version, while the plants of the version (F) at the same period had only 79,7% withered away flowers and only 20% of them had ripened seed capsules. However no more ripen capsules with seeds were obtained at variant (F) till the end of the experiment. In September, all the plants were withered away. There were no new basal rosettes as an indication of readiness for wintering. In the spring of 2015, neither overwintered individuals nor self-sown were observed.

Table 4. Biometry of Mimulus guttatus DC Inflorescences (Full blossom, 07.07.2014, Defloration 18.08.201 г.)

Features/	Sample with fertilizer (F)				Sample without fertilizer (C)			
	Full blossom (07.07. 2014)		Defloration (18. 08. 2014г)		Full blossom (07.07. 2014)		Defloration (18. 08. 2014г)	
	Number of flowers	Length of Inflorescence axe (cm)	Total number of flowers	Percentage of withered flowers (%)	Number of flowers	Length of Inflorescence axe (cm)	Total number of flowers	Percentage of withered flowers (%)
Average	10.2	5.2	11.5	79.70%	9.5	5.3	10.1	86.19%
Maximum	18	6.5	18	88.89%	19	6.1	23	91.30%
Minimum	5	2.5	5	71.43%	7	4.5	7	71.43%
St. Deviation	3.91	1.23	4.50	6.16	3.63	0.48	4.95	5.44

CONCLUSIONS

1. The global assessment of the data mentioned above and their analysis shows that the period when the individuals of *Mimulus guttatus* were grown can be defined as above the normals, concerning the rainfalls and within normal rates and even over, concerning the number of gloomy days. Regarding the de Martonindex (J) it should be described as a period with high atmospheric humidity, excluding drought conditions.

2. Fertilization of plants contributes to the extension of the ornamental period (in particular the flowering period) and results in increasing the number of flowers in the inflorescence, but does not contribute to prolonging the blooming period.

3. Referred to Sofia weather conditions in 2014, without extra watering (unless the required initial care) and direct sunshine, individuals of *Mimulus guttatus* bloom about a month and a half and perform as annuals. Drought at the end of their vegetation period contributes to the formation and maturation of seeds.

4. The use of individuals of the species in the above-mentioned weather conditions does not imply invasion or threat of uncontrolled proliferation.

5. The recommended in the literature spring planting in late March - beginning of April may contribute to the growth of the vegetative mass of the plants, but the duration of flowering is determined by the weather conditions in the summer months.

6. By seed propagation, ready for planting, the homogenous material is obtained in8-10 weeks, which is commensurate with the duration of propagation from cuttings.

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