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Populations dynamics in sizes of wild *Melissa officinalis* L. (*Lamiaceae*) during the last decade in Armenia

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Abstract
This paper reports the results of five consecutive years of field studies conducted in Armenia from 2007-2011 to 1) study native populations' habitats physical features, *Melissa officinalis* L., 2) assess the growth pattern and dynamics in populations' sizes 3) identify the impact of certain habitat features on the population growth pattern. Field studies and eco-geographic surveys in respect with Maxted (1995) methodology were conducted to examine *M. officinalis* L. populations' ecology. Researches exposed populations either expanding or dwindling in their sizes in different regions of Armenia. Certain habitat and environmental factors are identified as crucial for a population growth pattern over the study years. The study is essential for assessing *M. officinalis* L. populations' vulnerability towards environmental deterioration and climate change impacts. This research provides a baseline dataset that can be used for the development of further conservation strategies of this important medicinal and culinary species in Armenia.

Keywords: *Melissa officinalis* L., population, dynamic, Armenia, conservation.

1. Introduction

The small mountainous country of Armenia has a rich flora of ca. 3600 species of vascular plants (nearly 50% of the entire Caucasian flora), with both Caucasian and Iranian elements, distributed across desert and semi-desert, steppe, forest and alpine habitats [1]. As one of the first countries to join the Convention on Biological Diversity (CBD), Armenia has a strong interest in examining the biodiversity of native plant species, particularly those with potential or existing economic value (e.g., medicinal plants), and assessing their conservation status [2]. Only limited information, however, is at this time available on the genetic biodiversity, population location, structure and size, and conservation status of most of these species. Anthropogenic threats to this biodiversity, such as overpopulation, deforestation and urbanization have simultaneously hindered research and increased the need for it.

Of the ca. 500 species in the Armenian flora with records of medicinal and/or economic use, around 50 species are commonly used in the folk medicine and include both wild-collected (e.g., *Crataegus* L. sp., *Hypericum perforatum* L., *Melissa officinalis* L., *Origanum vulgare* L.) and cultivated (e.g., *Chamomilla recutita* (L.) Rauschert, *Mentha* x *piperita* L., *Crocus sativus* L.) species [3, 4]. Only limited information on the genetic biodiversity, conservation status, and population location, structure and size, however, are available for these species at this time.

From 2007-2011, field studies and eco -geographic surveys were conducted to study *Melissa officinalis* L. (Lemon Balm, *Lamiaceae*) populations' ecology, habitat characteristics, populations' grows pattern and dynamics over the study time as well as the role of intricate interactions between environment have been observed.

2. Materials and Methods.

Eco-geographic survey was conducted in six regions of Armenia, during the time period of 2007-2011, focusing on the central (Aragatsotn, Kotayk), northern (Tavush, Lori,) and southern regions (Vayoc Dzor, Syunik) to examine wild *M. officinalis* L. populations' ecology following the methodology of Maxted *et al.* (1995) [5]. It has been gathering and synthesizing ecological, geographical and taxonomic information during the field trips. The most fundamental demographic parameter of the plant population ecology is the number of individuals within population (Lebreton *et al.* 1992). Population size is defined as the number of individuals present in a subjectively designated geographic range. During the study, overall population size and density were assessed according to the quadrant sampling plot method. Quadrants or transects were used to randomly sample a portion of the habitat.

So, quadrants are small plots, of uniform shape and size, placed in randomly selected sites for sampling purposes. By counting the number of individuals within each sampling plot, we can see how the density and abundance of individuals changes from one part of the habitat to another. The word "quadrat" implies a rectangular shape, like a "quad" bounded by four campus buildings. Quadrants or transects were used to randomly sample a portion of the habitat: plots used to measure the density, abundance and size of population. It has been determined quadrate size 50 cm x 50 cm in respect with to Maxted *et al.* guidelines.

Quadrates are chosen randomly by using a random number generator or a random number table to select coordinates. So, a sample is taken randomly in order to have an equal chance for the sampling area of being sampled in the study plot. The purpose for collecting the samples randomly is to avoid biasing the data during the study. Data become biased when individuals of some species are sampled more frequently, or less frequently, than expected at random. Such biases can cause the population size to be either over estimated or underestimated, and can lead to erroneous estimates of population size.

Each time a sample is taken randomly in order to have an equal chance for the sampling area of being sampled in the study area. To achieve this, it has been placed a tape measure along two sides of the area being studied and found random coordinates. Each plot length has been divided into the sampling interval. The length of one side of the quadrate forms the sampling interval. For instances, 10 x 10 plot and 50 x 50 cm quadrate, the intervals will be 0, 0.5, 1, 1.5, 2…..20. The interval numbers on the piece of paper, we have put in the box and draw out to identify the appropriate position along the tape measure for per plot. To cover one m square we need four 50 x 50 cm square quadrates therefore, its quantity waves to cover 2% of the total area of being sampled along with different plot (Table 1).

Table 1: Quadtate Random Sampling Coordinates

Regions	Populations	Study area, m^2	Quadrats / plot	SI[a]/plot	Species
Tavush	Getahovit	87	7	24	*Melissa officinalis* L.
Tavush	Ayrum	75	6	30	*Melissa officinalis* L.
Aragatsotn	Orgov	136	11	45	*Melissa officinalis* L.
Kotayk	Garni	165	13	66	*Melissa officinalis* L.
Vayots Dzor	Jermuk	163	13	54	*Melissa officinalis* L.
Syunik	Artsvanik	169	14	56	*Melissa officinalis* L.
Syunik	Srashen	189	15	54	*Melissa officinalis* L.
Syunik	Shikahogh	45	4	18	*Melissa officinalis* L.
Syunik	Kapan	56	5	16	*Melissa officinalis* L.
Syunik	Tsav	162	13	36	*Melissa officinalis* L.
Syunik	Karchevank	102	8	20	*Melissa officinalis* L.

[a] Sampling Intervals

Along with quadrant measurements it has been applied the mathematical formula of finding the average number of individuals for per plot:

$$N = (A/a) * n , \qquad \textbf{(1)}$$

Where, N is the estimated total population size, A is the total study area, a is the area of the quadrate and n is the number of organisms per quadrate. *M. officinalis* L. populations growth and habitat characteristics at each location were recorded. Plant height and number of stems for plants within each population were calculated by averaging measurements taken for plants that cover 2% of the total plot area in respect with randomly selected quadrants sampling within each plot.

3. Results and Discussions.

The study of population ecology under this research is covered how populations of the plant change over the time and space and interact with their environment. The significant characteristics that might reveal this specific interaction and population behavior have been posed. Such as, population size (the number of individuals in the population) density (how many individuals are in a particular area), grow (how the size of the population is changing over time) etc.

The study of about population growth in the certain habitat environment might supply better predictions about future changes in population sizes and growth rates. It is essential for the development of future conservation strategy of wild medicinal plant species in the country.

The eco-geographic data gathered from the field survey were organized into an eco-geographic conspectus, defined by Maxted *et al.* (1995) as a formal summary of the available geographic and ecological information of the habitats (Table 4.2). A summary of the ecogeographic conspectus is given below for each of the eight populations under the study. Also, it has been given the description of location including administrative unit and nearest settlement, growth stage and soil characteristics etc.

Investigations at smaller spatial scales on the ecological characteristics and the habitat types of wild *M. officinalis* L. populations (e.g., microhabitat, feeding patterns), especially comparisons among population should give conclusive results on the roles of competitive interactions and historical factors in shaping species/populations distributions and growth [6].

Summarized in table 2 indicate that *M. officinalis* L. displays an amazing ability in extremely different data to adapt to ecological conditions present in the various regions of Armenia.

Table 2: Habitat Characteristics of *M. officinalis* L. Populations

Populations		Location, m (above sea level)	Landscape type	Soil type	Soil pH	Humus Concentration, %	Annual mean precipitation, mm	Average month t^0 in summer
Regions	NS[a]							
Tavush	Getahovit	905	Forest	Mountain forest redbroun	5.0-6.0	5.0-7.0	570-750	17-20
Tavush	Ayrum▲	495	Dry mountainous steppes	Mountain forest brown	6.0-7.0	4.0-4.3	470	22-24
Aragatsotn	Orgov	1610	Mountain steep	Meadow blackearth	7.0-7.3	3.0-6.0	470-530	17-20
Kotayk	Garni	1310	Mountain steep	Mountain blackearth	6.9-8.1	2.0- 4.0	430	20-23
Vayots Dzor	Jermuk▲	2104	Forest	Forest gray soil	4.8-5.5	13-17	900-1000	10-15
Sjunik	Kapan	1486	Forest	Mountain forest broun soils	4.2-5.1	5.0-7.0	600-750	20-22
Sjunik	Artsvanik▲	1530	Forest	Mountain forest brown soils	4.3-5.5	7.0-9.0	700-900	18-20
Sjunik	Shikahogh	957	Dry steppe	Mountain meadow steepe soils	5.1-6.2	5.0-7.0	400-450	22-24
Sjunik	Srashen▲	1025	Meadow steppes	Mountain forest brown soils	4.2-5.0	8.0-9.0	750-900	17-20
Sjunik	Tsav▲	1130	Meadow steppes	Mountain forest broun soils	4.5-5.5	8.0-9.0	800-950	18-20
Sjunik	Karchevank	857	Dry steppe	Semi desert automorph solonetzs	4.7-5.9	6.0-8.0	600-700	24-26

a- Nearest Settlement

Exploration and the study of the populations' habitat types and other physical environmental factors that have been conducted during the research would be the most decisive key elements on the population grow intensity, density, distribution, abundance, sizes and their changes over the study period.

Populations were observed by growing at altitudes ranging from nearly 500 to 2100 m above sea level, from moist temperate forest regions (i.e., near Jermuk, with 900-1000 mm annual precipitation) untill dry mountain steppe habitats (i.e., near Shikahogh (south region), Garni (central region) with only 400-450 mm annual precipitation) (Table 2).

Plants tolerated soils with pH ranging between 4.0 (acidic) and 8.1 (alkaline), with a preferred range between 4.0 and 5.5. In fact, with this criterion mostly south regions to varying degree are preferable for wild *M. officinalis* L. species to grow. The locations of the populations demonstrate that this species can tolerate soils with various concentrations of humus and can even grow in nutritionally poor soil, as observed for the populations located near Ayrum and Garni. Plants in the latter populations, however, exhibited low growth characterstics (e.g., density and plant height) as compared to those observed in other populations,and the overall size of these populations decreased over the period of the study (Table 3; Fig. 2).

Table 3: *M. officinalis* L. Populations' Sizes, Density and Grow Intensity in Different Habitats

Population		**Density, plant/m²**			**Plant height, cm**			**Stems quantity/plant**		
Region	**NS[a]**	**2007**	**2008**	**2009**	**2007**	**2008**	**2009**	**2007**	**2008**	**2009**
Tavush	Getahovit	1.30	0.95	0.89	76	70	65	1-3	1-3	1-2
	Ayrum▲	1.10	0.97	0.93	67	62	58	1-1	1-2	1-3
Aragatsotn	Orgov	0.92	0.95	0.99	64	67	69	1-3	2-3	2-3
Kotayk	Garni	0.87	0.83	0.79	63	56	48	1-2	1-1	1-1
Vajoc Dzor	Jermuk▲	2.77	3.05	3.57	120	124	127	3-6	3-7	4-7
Sjunik	Kapan	2.2 0	1.97	1.89	107	101	97	2-3	2-5	2-4
	Artsvanik▲	2.50	3.50	4.20	117	122	125	3-5	4-6	5-7
	Shikahogh	1.80	2.10	1.80	85	81	77	2-3	3-4	2-3
	Srashen▲	2.70	3.10	3.70	118	124	127	4-5	4-6	5-7
	Tsav▲	1.70	1.90	2.50	91	94	92	2-3	2-4	2- 3
	Karchevank	1.5 0	2.10	2.70	108	114	117	2-3	3-4	3-5

[a] - Nearest Settlement;▲ –indicates new population. The plant height and stems quantity were measured at the end of stem forming -phenological phase in respect with assessment of 2% size of each population.

The measurement of population's size, density and abundance can give us a comprehensive understanding of why some populations are endangered, as well as to draw analysis about the environmental and any other constraints that can bring populations grow future changes [6, 7]. Realized different multiple field observations based on the quadrate sampling plot method, over the populations have exposed habitat and growing characteristics of the plants in different populations, as well as assessed population size and its abundance. A summary of the measurement of populations' size and density, as well as plant grow intensity is given below for the plant populations.

According to the collected data, average plant height measured within some populations displayed a slight increase over the study period, e.g., ca. 9 cm in the Srashen and Karchevank, ca. 8 cm in the Artsvanik and Jrrmuk populations located in the south regions. Interestingly, these populations demonstrate a positive increase, e.g. ca 1-2 plant/m^2 within the average density and stem quantities during the study (Table 3).

We hypothesize that these favorable growth characteristics may be attributed, at least in part, to the soil's higher humus concentration and acidic environment as well as relatively higher annual precipitation measured in these habitats as compared to other regions in central and northern Armenia [6].

The observation of population interaction with its environment, especially meaningful when considering the potential impacts of climate change and other changes in environmental factors (how will populations respond to changing temperatures? To drought? Will one population prosper after other declines?) [6-8].

A negative trend for the average plant height was observed e.g., ca. 15 cm in the Garni from the central region; ca. 11cm and 9 cm appropriately in the Getahovit and Ayrum populations from the northern regions. Also, the average number of stems of per plant ca. 1-2 and the population's density was slightly decreased ca.0.40 plant/m^2 in these populations over the study period (Table 3).

Populations located near the Getahovit and Ayrum in the northern Armenia, occupied forest landscape habitats. However, unlike populations from the south regions, the law grows capacity of these populations and reduction in sizes and density of them could be connected with less humus concentration and alkaline pH environment in the soils (Table 2). Soil characteristics, however, could represent only one of many possible factors that could have contributed to the reduction in plant population size and abundance over the study period [6, 7].

In fact, population located near Garni from the central region in Armenia, occupied mountain steep landscape with mountain black-earth soils, with low humus concentration, with a highest alkaline soil pH among the other populations have the smallest population density, plant height and stem quantity and exhibits negative trend in populations overall size and density during the study (Table 3).

No change in average stem number and plant height was observed over the study period for plants examined in Tsav population, from Syunik region (south). On the other hand, a slight increase ca. 0.8 plant/m^{2}over the population's density was observed during the study period.

A negative trend for the average plant height was observed e.g., ca. 10 cm in the Kapan and ca. 8 cm in the Shikahogh populations from Syunik region (south). Also, a slight decrease ca. 0.31 plant/m^2 was observed in the Kapan population's density during the study period, where in Shikahogh population this criterion fluctuates up and down maintaining average 1.8 plant/m^2 density over the study period. These populations have exposed the smallest average plants height among the other populations from south regions of Armenia (Table 3). In fact, these habitats are characterized with dry steppe landscape, relatively lower annual precipitation and contain poor soil with higher pH environment to compare with the other populations from south regions (Table 2).

Different biotic and abiotic factors could be postulated to affect the growth characteristics of the plants in different populations, but we can initially hypothesize that the poor soil quality and low annual precipitation rates measured at the populations contributed to the negative trend observed [6, 7].

The most fundamental demographic parameter of the plant population ecology is the number of individuals within population [6]. Population size is defined as the number of individuals present in a subjectively designated geographic range.

During field studies quadrate randomly sampling method has been identified as the key element for observations and assessment of populations' sizes. Through yearly measurements of population size and density, populations were identified that either expanded or diminished in size over the course of the five-year study (Figure 1). Studying population growth might reveal the causes that bring changes in population sizes and growth rates.

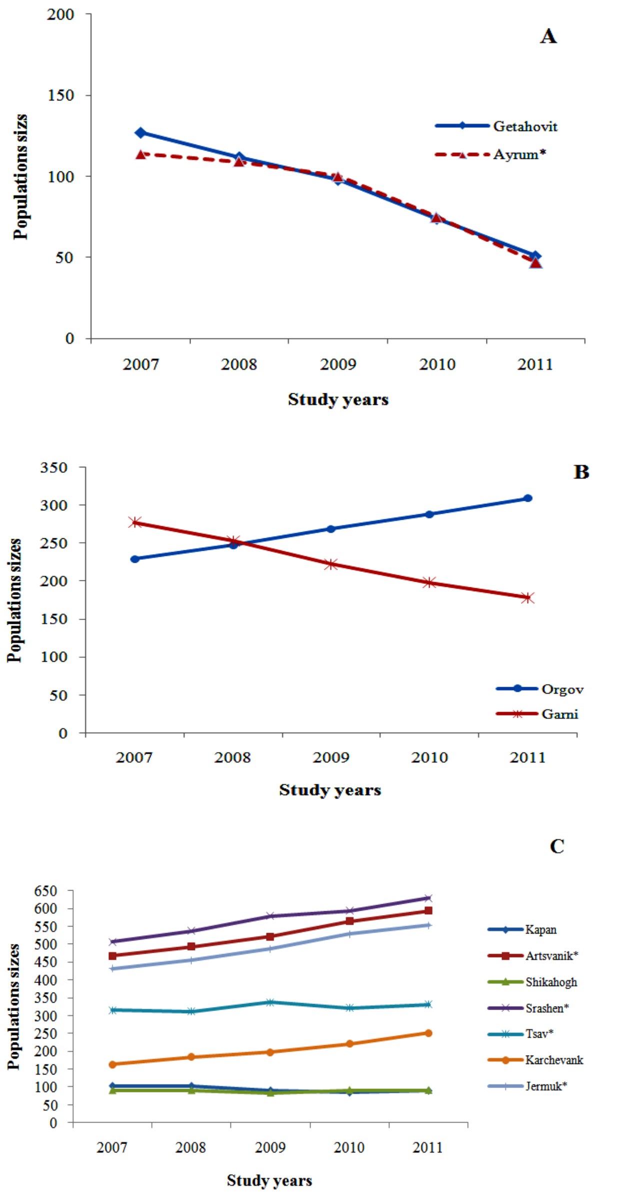

Fig 1: Populations Dynamics of *Melissa officinalis* L. across different habitats (A) in the North; (B) in the Central; (C) in the South Regions of Armenia, during the study period (in accordance with quadrate sampling plot method).

The dynamics of sizes in populations are exposed mostly with exponential growth pattern. That is the continuous increase or decrease in a population in which the rate of change is proportional to the number of individuals at any given time [9]. Populations demonstrate an increase in sizes and have mostly south location (Syunik and Vayots Dzor Regions, Fig 1., C).

The highest continues increase has been recorded in Artsvanik, Srashen and Jermuk populations that gained more than 120 individuals over the study period. According to logistic growth, we may contemplate these populations could be characterized with *intrinsic rate of increase* that are capable to achieve in maximum sizes until the environmental factors constrain to this type of grow (Fig. 2).

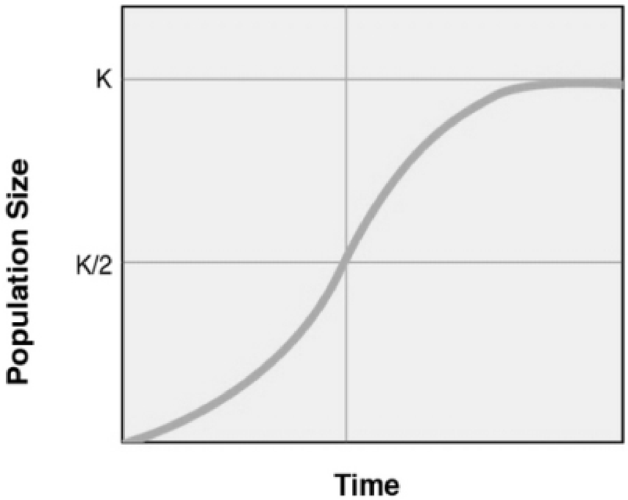

Fig 2: Logistic growths explicit the exponential grow in population until it approaches its carrying capacity (K).

Factors that enhance or limit population growth can be divided into two categories based on how each factor is affected by the number of individuals occupying a given area — or the population's **density**. As population size approaches the **carrying capacity** of the environment, the intensity of density-dependent factors increases. For example, competition for resources can eventually limit population size. Other factors, seasonal weather extremes, e.g. droughts, frosts, etc affect populations irrespective of their density, and can limit population growth simply by severely reducing the number of individuals in the population [8, 9].

In respect with logistic grow mechanism, high density of Jermuk, Artsvanik and Srashen populations does not afflict negatively on the exponential grow pattern, which might mean that they have not reached yet to their maximum grow capacity (Table 3). So, we might contemplate that the growth would continue in the future if we exclude other factors e.g. seasonal weather extremes, natural disasters etc. [7-9].

Size increase was recorded minor in Tsav population, only with ca. 23 more individuals over the study period. Among the other populations, Shikahogh population fluctuates up and down during the study period by maintaining comparatively constant size of 90 number of individuals. In compare with these populations, relatively higher increase was observed in Karchevank population with ca. 89 individuals. Kapan population has exposed a slight decrease with ca. 13 individuals over the study period from south regions of Armenia.

So, from seven populations located in the south part of the country, the increase is recorded in five populations and it is quite high in three of them, one population has minor decrease and the other is constant with its size over the study years. So, *Melissa officinalis* L. populations apparently is thriving in the south part of the country, in particular Srashen, Artsvanik and Jermuk by having located in the forest landscape along with forest gray and mountain forest brown soils which possess comparatively higher humus concentration and acid soil environment (Table 2).

On the other hand, populations with depleting sizes over the study period have been recorded mostly in Tavush Region from northern Armenia (Fig.1; A). The amount of decrease comprises with individuals of ca. 76 in Getahovit and ca. 67 in Ayrum populations over the study period. So, the most drastic change has occurred in Getahovit population. In fact, trend of reduction was especially higher during the 2010-2011 vegetative periods. It is almost two times higher in comparison with the previous years. Interestingly, the temperature was relatively higher in 2010 and 2011. So, we may contemplate that the rise in temperature could be one of the possible reasons to affect negatively on *M. officinalis* L. populations' grow pattern. This might increase their vulnerability towards the weather and climate change impacts. Further studies needed to reinforce this hypothesis.

In fact, it is not only the environmental or habitat and the plant biological factors that influenced on the plant population size and distributional abundance, it is also antropogenetic and other biotic factors that are different across the regions and habitats and might affect on populations growing condition. Some anthropogenic threats that were identified as part of the study included poor land management (erosion, overgrazing), increasing population pressure (impact of livestock overpopulation, improper human development), and excessive or inappropriate collection for the purposes of local sale/usage (due to lack of knowledge/training of collectors). Especially in the northern and central regions, it was observed that the wild harvesting of medicinal plant materials (e.g., *O. vulgare* L., *Hypericum perforatum* L., and *Melissa officinalis* L.) at inappropriate times for sale at Yerevan markets negatively impacted population sustainability. Records indicate that approximately 200 tons of wild edible and/or medicinal plants are sold in Yerevan markets each year [10]. Wild *M. officinalis* has been used by local inhabitants primarily in the form of an infusion (tea) as to treat disorders of the nervous and digestive systems.

A negative trend for the population size was observed in the Garni population (Kotayk Region) from the central Armenia, over the study period. The decrease was gradually by comprising ca. 100 individuals overall the study. In fact, *Powedery mildew* diseases were observed in this population (5, June 2009). The leaves observation at colba plots with room temperature during 3-4 days has been conducted to identify the disease at Gene Pool Laboratory of Plants Cultivation and Vegetable Growing Department of Armenian National Agrarian University. It is important to mention that *Powedery mildew* is not recorded in other populations across the country. The disease spread over 40% of the Garni population in 2009 and is remarkable, especially during the plant flowering phenological phase (June-July). In the next study years, the disease was spread by comprising 67% in 2011. Future studies could be conducted to reveal the causes of the origin of disease in this population that might have either the population' genetic, or other environmental reasons. Furthermore, one thing is certain that the dramatic decrease in sizes of this population obviously connected with this phenomenon as well.

However, the exponential increase ca. 20 individuals were observed in the Orgov population (Aragatsotn Region) in the central Armenia. The overall growth in the population is ca. 80 individuals over the study period.

In fact, populations may display distinctive behaviors based on their size. Depleting and small populations face a greater risk of extinction and are more susceptible to climate change impact [11]. Additionally, individuals in small population are more susceptible to random deaths e.g. fire, floods, and disease have a greater chance of suppressing the growth of population, such as depleting grow pattern in the Garni population caused by disease.

In addition, populations with depleting sizes have exposed comparatively lower density (Table 3). In this respect, we may contemplate that the density dependent factor does not limit on population growth [9]. However, these habitats located mostly in central and northern regions have relatively less carrying capacity, in other words, less favorable conditions for the plant to grow (Table 2). Thus, even lower density could be decisive along with unfavorable environmental and extreme weather factors. Also, we should not exclude a human negative impact which is more intense in the central and northern regions of Armenia [10]. So, populations located in central and north regions of Armenia may comparatively be more vulnerable under global climate change impact [6-12].

In addition, a significant concept is the grasp comprehension that none of this factor might separately causes the population size to alter. Only, the all components can rightly show their intricate and complex interactions among them and the correct picture of their functioning impact on the changes of the plant populations' sizes.

4. Conclusion

In conclusion, data indicated the significant dynamics of wild *M. officinalis* L. populations occurred during the period of 2007-2011 in Armenia. This study, conducted over a five years period, allowed the identification of particular factors that could be assessed either quantitatively (e.g., annual precipitation, soil pH, humus concentration) or qualitatively (e.g., interaction of the plant grow pattern and the environment, anthropogenic threats) that had either negative or positive effects on the survival and fitness of the located populations. The study of populations' ecology under this research is covered changes in populations' sizes and abundance over the time and space and their interaction with the environment. These investigations are necessary for predicting populations future conditions their flexibility and/or vulnerability in different habitats under global climate change impacts. The extensive phenotypic variability and adaptive ability displayed by individual plants within these populations suggests that *M. officinalis* in Armenia represents a rich genetic resource for the species as a whole. This research has provided a baseline dataset that can be used for the development of further *ex situ* and *in vitro* strategies to conserve unique genotypes, as well as assess the sustainability of wild populations with regard to the IUCN Red Book Criteria, of this important medicinal and culinary species in Armenia. The researchers recommend continuous monitoring of specific sites and the effects of the independent variables that influence populations' dynamics of wild *Melissa officinalis* L. and identifying threats to conservation.

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