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Full Length Research Paper

Effect of water stress on essential oil yield and storage capability of *Matricaria chamomilla* L.

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To evaluate the effect of water stress on yield and storage capability (essential oil percentage, yield and harvest index during five years storage period) of *Matricaria chamomilla* L., a field experiment was conducted in 2005. Water stress treatments of irrigation after 30, 60, 90 and 120 mm evaporation from pan class A, were arranged as randomized complete block design with six replications. The harvested material was stored for five years and essential oil was extracted yearly. Results of the first year of experiment indicated that the highest (1347 kg/ha and 10084 g/ha) and lowest (952.2 kg/ha and 6672 g/ha) yields of dried flower and essential oil were obtained from irrigation after 60 and 120 mm evaporation from pan, respectively. Results from stored plant material (5-year data of storage) showed a significant effect during storage on essential oil percentage, yield of essential oil, harvest index of essential oil, reduction of essential oil percentage and yield. Means comparisons from the 5-year data indicated that the highest percentage and yield of essential oil (0.715% and 8442 g/ha) was observed at first year. After that, there was a 27% reduction trend in essential oil percentage to the 5-year storage result. So, the lowest essential oil content and yield (0.194% and 2335 g/ha) was obtained from flowers at the fifth year of storage.

Key words: *Chamomilla recutita*, dried flower yield, excess water, storage quality, water deficit.

INTRODUCTION

According to WHO, 80% of the world's population is currently dependent on plants for health care (Akerele et al., 2009). Plants provide a rich source of secondary metabolites that have medicinal and aromatic properties (Gomez-Galera et al., 2007). The world market for herbal medicine, including herb based products and raw materials, is growing at an annual rate of 5 to 15%. This indicates the likelihood of growing demand for plant-derived drugs in the coming years (Kumar and Gupta, 2008). Over harvesting and shrinkage of plant habitats from increasing urbanization have caused a significant decline in the volume of raw materials produced (Franke and Schilcher, 2005). Chamomile (*Matricaria chamomilla* L.), Family: Asteraceae, commonly known as German chamomile is a native of Europe and is cultivated extensively in Hungary, Germany, Russia and Yugoslavia (Singh, 1982).

M. chamomilla is an important medicinal plant that has multi-therapeutic, cosmetic, and nutritional values; established through years of traditional and scientific application (Schilcher, 1987). German chamomile essential oil obtained from flower heads, either by steam distillation or by solvent extraction, varies from 0.24 to 1.9% in fresh plant tissue. Distillation using the 4-h European pharmacopeia method typically yields 0.33 ml/100 g of tissue, whereas distillation, using the 2-h German Pharmacopeia method (DAB7) typically yields 0.29 ml/100 g of tissue (Mechler and Ruckdeschel, 1980). About 120 chemical constituents have been identified in chamomile as secondary metabolites, including 28 terpenoids, 36 flavonoids, and 52 additional compounds with potential pharmacological activity. Though (–)-α-bisabolol and chamazulene have proven to have the most bioactive inflammatory activity, the

en-indicycloethers have an antispasmolytic effect (Salamon, 1992).

The highest essential oil and prochamazulene concentrations are obtained after drying plant material in shade at 22 to 25°C (Schmidt et al., 1991). Slight losses (up to 7%) of prochamazulene, with no reduction in plant essential oil content, occur with drying in a stationary bulk dryer with active ventilation at 40 to 45°C. In another study, the greatest loss of active compounds in the essential oil generally resulted from storage of plant material at −6 to 25°C and 55 to 95% humidity (Walenciak and Korzeniowski, 1983). It seems that the essential oil content and constituents of essential oil had been changed by storage time. In this regard, climate factors may affect on quality and quantity of essential oil of medicinal plants. However, these factors can affect the maintenance of oil content of stored herbal raw material tissues in during storage period (Franke and Schilcher, 2005).

Water, as an important effective factor on plant growth and secondary metabolite production, increases levels of all of the compounds analyzed, particularly some of the phenolic compounds (Rostami-Ahmadvandi et al., 2011; Kahrizi et al., 2012). In flooded soil, the microorganisms reacted vigorously to a deficiency of lifesaving oxygen, but potentially inhibitory concentrations of carbon dioxide, hydrogen sulfide, methane, ethylene, manganese, iron, sulfate, and many organic substances may accumulate abnormally in large concentrations and lead to plant damage as a result of a reduction in oxygen (Rowe and Beardsell, 1973). Large quantities of medicinal plants are wasted during operations such as logging, chipping during semi-processing, powdering of leaves and tender stems during drying, there can be a loss of small particles during bundling and transportation and a certain percentage during washing. Collection and semi-processing at an inappropriate season might also lead to considerable waste. Documentation of the ideal season for harvest and storage is necessary to protect the active plant properties and to preserve their optimum therapeutic value (Akerele et al., 2009; Franke and Schilcher, 2005).

Due to defective storage, the raw drugs are often prone to attack by insects, fungi and bacteria. There is visible remarkable deterioration during storage (Krishnamurthy, 1993; Tajuddin et al., 1996). But there were few studies on reduction of essential oil quality and quantity in long time storage period in health condition. And there were no report on the effect of varying water availability (irrigation regimes) on the maintenance potential of chamomile essential oil percentage during long storage period. Based on current knowledge, information on the response of *M. chamomilla*, quantity and quality of herbal raw material tissue, to different irrigation regimes and storage periods is scarce. Therefore, the main objective of this study was to evaluate the effects of different irrigation regimes on yield of essential oil, and however on storage capability of dried flower (changes in percentage, yield and harvest index of essential oil) during long term (5 year) storage.

MATERIALS AND METHODS

This research was conducted at the experimental field of the Department of Agronomy and Plant Breeding, Faculty of Agriculture, Urmia University (latitude 37.53° N, 45.08° E, and 1320 m above sea level), Urmia, Iran in 2005. The soil texture of the site was clay-loam (28% silt, 33% clay, 40% sand) with 22.5% field capacity, 1.54 g cm^{-3} soil density, 1.98% organic mater, pH 7.6.

The research consisted of two experiments; firstly, a field experiment was conducted and secondly, tests on the harvested material after 1 to 5 years storage. In the first experiment, the treatments were irrigation after 30, 60, 90 and 120 mm evaporation from pan class A with six replications. *M. chamomilla* L. c.v. Bodegold, a tetraploide variety seeds were planted on 1st May 2005. Plant growth was continuously monitored for the duration of the experiment by the mechanical control of weeds. The harvested crop consisted of freshly gathered typical flower heads, with approximately 10% of flowers containing fragments of small flower stalks, which were up to 30 mm long. Flower heads were picked when they were fully developed and dried in a shady place. Flowers were hand harvested at the medium stage of development and dried at 25°C for 72 h (Bottcher et al., 2001).

In the second experiment, harvested dried flowers were stored at 25°C for 5 years. The air-dried parts of chamomile (15 g of the dry sample) were hydro-distilled in a Clevenger-type apparatus in 1000 ml round bottomed flask with 600 ml deionized water for 4 h (Salamon, 1992). The essential oil was extracted each year for with 5 years.

Statistical analysis

Statistical evaluation was performed using SAS software package, version 9.1 (SAS Institute, 2004). The effects of the irrigation regimes were analyzed with an analysis of variance test. The results of statistical analysis are expressed by F-values; asterisks indicate p-values: $p^* \leq 0.05$ and $p^{**} \leq 0.01$. The comparison of means was carried out with Student-Neuman Keul's test.

RESULTS

Field experiment (first year)

Results of data analysis of variance, from the field experiment showed significant effect of irrigation on dried flower yield ($P \leq 0.05$), biological yield ($P \leq 0.01$) and essential oil yield ($P \leq 0.01$) (Table 1).

Means comparison indicated that the highest yield of dried flower (1347 kg/ha) was obtained from irrigation after 60 mm evaporation from pan class A. This greatest yield had no significant difference with yield of flower obtained from irrigation after 30 and 90 mm evaporation. But, the lowest yield of dried flower (952.2 kg/ha) was obtained from plants irrigated on 120 mm evaporation from pan. Regression function of dried flower yield along with irrigation was a binomial function with $R^2 = 0.9596$ (Figure 1A).

Table 1. Analysis of variance of yield and harvest index of *Matricaria recutita* L. affected by irrigation.

Source of variation	df	Dried flower yield	Biological yield	Harvest index of dried flower	Essential oil percentage	Essential oil yield	Harvest index of essential oil
Replication	5	47141.9^{ns}	123626.0^{ns}	0.005^{ns}	0.002^{ns}	0.008^{ns}	0.007^{ns}
Irrigation	3	172697.3*	2618468.3**	0.020^{ns}	0.006^{ns}	0.035**	0.029^{ns}
Error	15	43266.7	394476	0.014	0.005	0.006	0.014
Coefficient of Variation (%)		17.65	21.78	7.21	9.67	1.98	4.80

df, degree of freedom; ns, * and ** ; non-significant, significant at P ≤ 5% and P ≤ 1%.

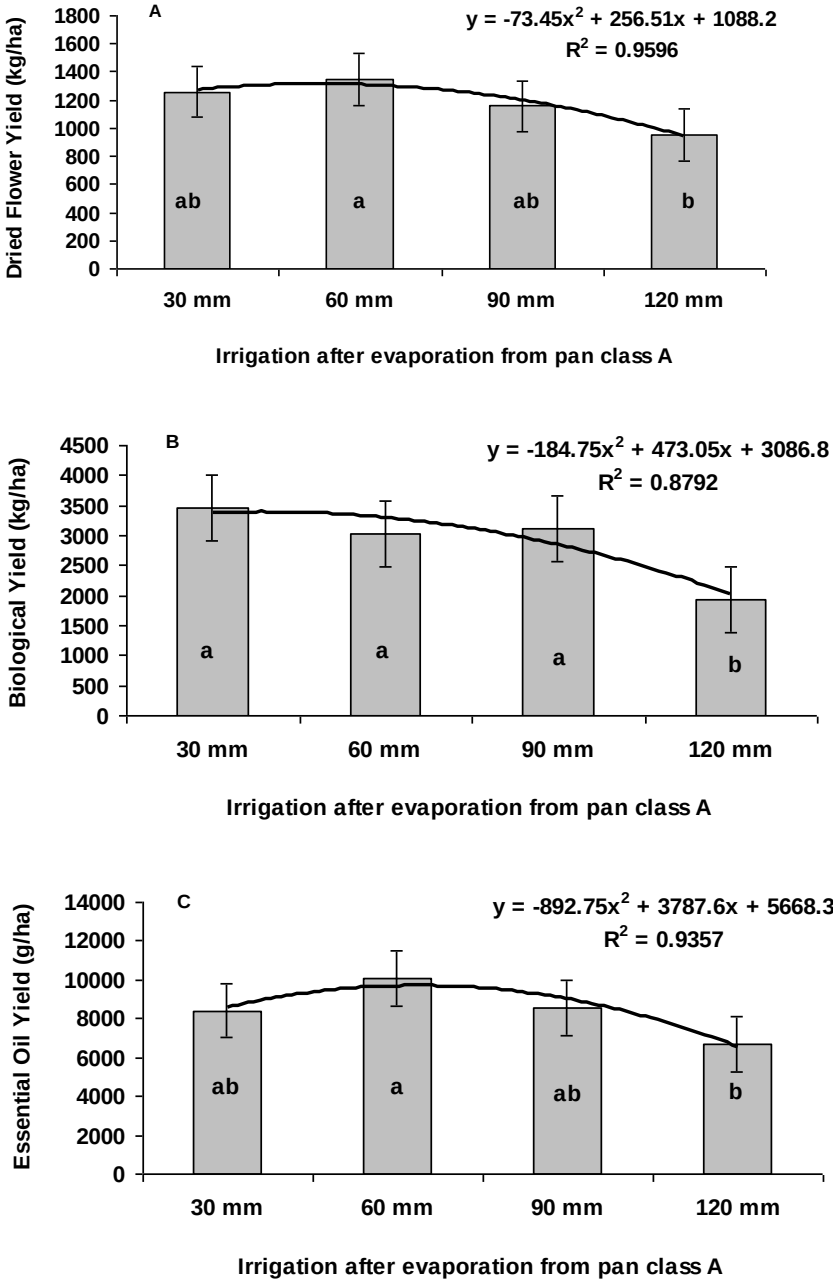


Figure 1. Means comparison of dried flower yield (A), biological yield (B) and essential oil yield (C) of German chamomile under irrigation regimes at first year. The same letters showed non significant differences.

Table 2. Analysis of variance of yield and harvest index of *Matricaria recutita* L. affected by irrigation and storage time.

Source of variation	df	Essential oil percentage	Essential oil yield	Harvest index of essential oil	Reduction of essential oil percentage	Reduction of essential oil yield
Replication	5	0.0004^{ns}	0.068*	0.046^{ns}	0.042^{ns}	0.04^{ns}
Storage	4	0.077**	1.176**	1.155**	13.57**	13.6**
Irrigation	3	0.002^{ns}	0.256**	0.083*	0.055^{ns}	0.06^{ns}
Storage × Irrigation	12	0.001^{ns}	0.019^{ns}	0.018^{ns}	0.063^{ns}	0.06^{ns}
Error	95	0.001	0.026	0.030	0.043	0.04
Coefficient of variation (%)		21.22	4.42	7.93	15.68	15.7

df, degree of freedom; ns, * and ** ; non-significant, significant at $P \leq 5\%$ and $P \leq 1\%$.

The minimum biological yield of German chamomile (1934 kg/ha) belonged to the most strength stress, irrigation after 120 mm evaporation from pan class A. But, the other three irrigation regimes (irrigation after 30, 60 and 90 mm evaporation) produced the maximum biomass (3464 kg/ha). Trends in biomass changes along with irrigation showed binomial regression with R^2 = 0.8792 (Figure 1B).

Similarity of changes in essential oil yield and dried flower yield indicated that the highest yield of essential oil (10084 g/ha) was obtained from irrigation after 60 mm evaporation that had any significant differences with yield of irrigation after 30 and 90 mm evaporation. But, the most strength stress, irrigation after 120 mm evaporation caused the lowest yield of essential oil (6672 g/ha). A binomial function between irrigation and yield of essential oil was made apparent by the means comparison (R^2 = 0.9357) (Figure 1C). The similarity between yield of dried flower and essential oil showed that dried flower yield had a correlation with oil yield.

Storage (five years)

Data analysis of variance of plant material (dried flower obtained from field experiment) stored for five years showed significant effect of storage on essential oil percentage, yield of essential oil, harvest index of essential oil, reduction of essential oil percentage and yield (Table 2).

Means comparison of data from 5-year storage indicated that the highest percentage of essential oil (0.715%) was observed at first year (harvesting year). After that, despite non-significant difference for second and third year's essential oil contents, there was a reduction trend in essential oil percentage along with that of 5 years' storage. So, the lowest essential oil content (0.194%) was obtained from flowers at the fifth year of storage. A polynomial equation of degree 3 ($y = -0.0286x^3 + 0.2685x^2 - 0.855x + 1.3238$; R^2 = 0.9809), showed the reduction as results of an analysis of variance (Figure 2A).

Changes in essential oil yield, as for the results of oil content, showed a decreasing direction from the first to the fifth year of storage. We observed the minimum essential oil yield (2335 g/ha) in the fifth year of storage compared with the control (8442 g/ha). Polynomial equation between storage time (year) and oil yield was degree 3 ($y = -339.92x^3 + 3182x^2 - 10081x + 15596$; R^2 = 0.9746) (Figure 2B).

Harvest index of essential oil (HI; proportion of economic yield as the results for essential oil to total aerial dry matter), had a direction like essential oil yield because of consistency of biological yield for the duration of the experiment. So, the maximum (0.303%) and the minimum (0.083%) HI of essential oil from German chamomile was obtained at the first and fifth years of the experiment, respectively. This 27% reduction of essential oil was underlined with polynomial equation of degree 3 ($y = -0.0115x^3 + 0.1079x^2 - 0.3452x + 0.5491$; R^2 = 0.9785) (Figure 2C).

Reduction in essential oil content and yield compared to the control (first year of harvest) was significant and the highest reduction was recorded after storage for 5 years (73.23%). Polynomial equation of degree 3 ($y = 4.0905x^3 – 38.235x^2 + 120.91x – 85.91$; R^2 = 0.9818) showed this increasing trend (Figure 2D and E).

In the first year harvest, the correlation between biomass and dried flower yield was positive and significant ($P \leq 0.05$). But correlations of biomass yield versus harvest index of dried flower and harvest index of essential oil were negative and highly significant ($P \leq 0.01$). These relations indicate an ever-increasing trend in biomass, resulting in parallel changes to dried flower yields. However, higher biomass led to a decreasing harvest index ratio of both dried flower and essential oil because of the non-significant correlation between biomass and essential oil percentage. The non significant correlation of dried flower yield and harvest index of dried flower, like harvest index of essential oil, emphasizes the greater effect of biomass, because of its greater value in comparison with dried flower yield. The yield of essential oil showed significantly positive association with dried flower yield. The non-significant correlation between dried

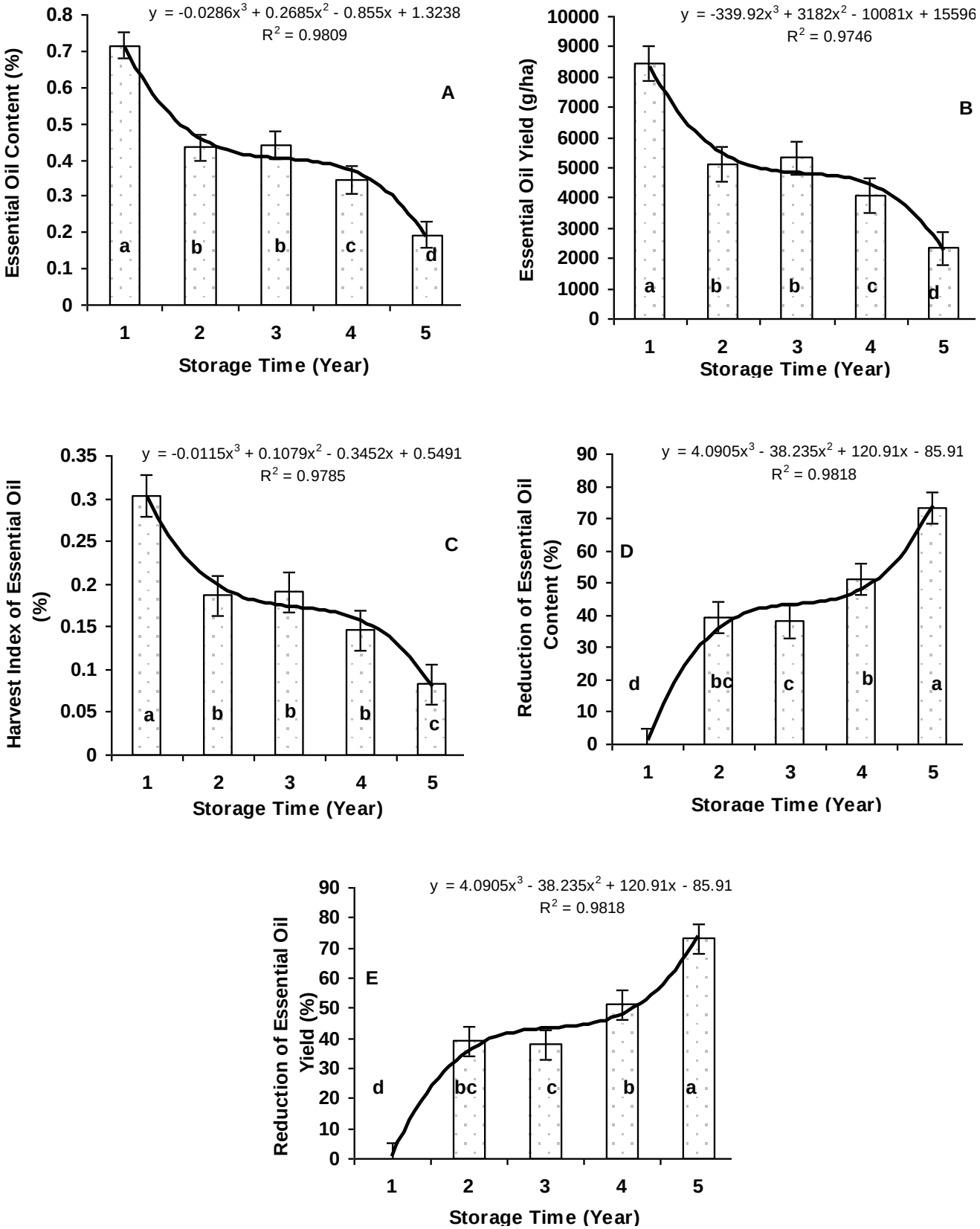


Figure 2. Means comparison of essential oil percentage (A), essential oil yield (B), harvest index of essential oil (C), reduction of essential oil percentage (D) and reduction of essential oil yield (E) of German chamomile during five storage year. The same letters showed non significant differences.

Table 3. Correlation coefficients among biomass yield, dried flower yield, harvest index of dried flower, essential oil percent, and essential oil yield at first year of field experiment.

Variable	Biomass yield	Dried flower yield	Harvest index of dried flower	Essential oil percent	Essential oil yield
Dried flower yield	0.431*				
Harvest index of dried flower	-0.702**	0.308^{ns}			
Essential oil percent	-0.090^{ns}	0.076^{ns}	0.058^{ns}		
Essential oil yield	0.331^{ns}	0.904**	0.302^{ns}	0.490*	
Harvest index of essential oil	-0.683**	0.315^{ns}	0.950**	0.327^{ns}	0.420*

ns, * and ** ; non-significant, significant at P ≤ 5% and P ≤ 1%.

Table 4. Correlation coefficients among biomass yield, dried flower yield, essential oil percent, essential oil yield, harvest index of essential oil, essential oil percent reduction, and essential oil yield reduction during five-year storage.

Variable	Biomass yield	Dried flower yield	Essential oil percent	Essential oil yield	HI of essential oil	Essential oil percent reduction
Dried flower yield	0.431**					
Essential oil percent	0.075^{ns}	0.082^{ns}				
Essential oil yield	0.221*	0.448**	0.909**			
HI of essential oil	-0.269**	0.214*	0.859**	0.849**		
Essential oil percent reduction	-0.094^{ns}	-0.062^{ns}	-0.976**	-0.874**	-0.832**	
Essential oil yield reduction	-0.094^{ns}	-0.062^{ns}	-0.976**	-0.874**	-0.832**	1.000**

ns, * and ** ; non-significant, significant at P ≤ 5% and P ≤ 1% .

flower yield and harvest index (harvest index of flower and essential oil) relates to the importance of biomass in comparison with flower yield (Table 3).

In the 5-year storage, the correlation between biomass and dried flower yield (P ≤ 0.01), however between biomass and essential oil yield (P ≤ 0.05) was observed positive and significant. But the correlation of biomass yield to harvest index of essential oil was negative and highly significant (P ≤ 0.01). These relations indicate that the ever-increasing trend in biomass caused parallel changes to dried flower and essential oil yields, but led to decreasing trend for harvest index of essential oil. The correlation between dried flower and essential oil yield was also positive and significant (P ≤ 0.01). The significant (P ≤ 0.01) correlation of essential oil percentage versus essential oil yield and harvest index of essential oil was observed during five years of storage. The correlation of essential oil percentage, yield and harvest index to reduction of essential oil percentage and yield was negative and significant (P ≤ 0.01) (Table 4).

DISCUSSION

Based on the results of current study, there was a reduction in the yield of biomass, dried flower and essential oil in irrigation after 120 mm evaporation from pan, because of the strongest water deficit condition. In the 5-year storage, there was a sharp slope in reduction of essential oil content and yield at the second year of storage. And a significant reduction was occurred in the percentage and yield of essential oil at the forth and fifth year because of destruction in herbal raw material tissues. Quality of dried flower will be lost after storage for 6 months, because of respiration as a very stringent process in living cells of freshly harvested chamomile. It mediates the release of chemically bound energy through the breakdown of carbon components and the formation of carbon skeletons necessary for maintenance and synthetic reactions after harvest. A secondary problem of prolonged storage is the result of respiration, the release of energy as heat. Therefore, heat from respiration needs to be monitored and heat needs to be abducted from stacks of the stored product. This difference, which was statistically significant (P ≤ 0.01), was also maintained during postharvest storage. The complex relationship between storage temperature, postharvest storage time, and respiration course was reported by Franke and Schilcher (2005). Thus, the quantity of constituents, in general, turned out to have been relatively stable during the postharvest period, but there were some unfavorable reactions (Bottcher et al., 2001).

Natural samples of chamomile flowers after a postharvest storage period of 80 days contained a quantity of +46 ml essential oils/100 g dried herbal raw material at 10°C and of +40 ml/100 g dried herbal raw material at 20°C in comparison to the quantity at harvest date. However, after conditions of 30°C there was a

decline by 9.75 ml/100 g dried herbal raw material. Chamazulene was marked by small decreases, rising with higher temperatures at 10°C –1.95%; at 20°C -8.8%, and at 30°C –10.7% (Bottcher et al., 2001).

It was determined that the quality of the herb deteriorated with storage and its quality was affected (Singh et al., 1994). The pattern of accumulation of monoterpenes and the effect of storage on the herb prior to distillation has been reported in other research (Singh et al., 1994, 1996). There are several reports that the oil content increases with storage prior to distillation (Singh et al., 1994). These show that the safe limit of herb storage varied according to the species and storage conditions. But, there is no exactly report on the changes of essential oil content in longtime storage. Essential oil from the leaves of *Cymbopogon martinii* was tested for toxicity against *Fusarium oxysporum*. Fungitoxicity remained unchanged in temperature treatment after a long storage period (Akhila, 2010). Storage of the herb *Cymbopogon lexuosus* always caused a reduction in oil content except during the summer, when it was not affected by 3 days of storage under shade. Little variation in the geranial and neral contents of essential oils of *C. lexuosus* leaves was observed during storage for 15 days (Singh et al., 1994). Hay storage of *C. martinii* (during summer) either in the shade or in the open increased its essential oil content. A slight difference was observed in geraniol and geranyl acetate contents of the essential oils from leaves of *C. martinii* (Franke and Schilcher, 2005). The infrared spectrum of lemongrass oil proved that changes in samples during storage could be detected; especially changes that occurred in the carbonyl group (Foda et al., 1975). Finally, because of no references on changes in essential oil yield from long term storage of medicinal plants, that of German chamomile, quality traits of other medicinal plants is yet to be discussed.

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