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POTENTIAL, CULTIVATION AND QUALITY OF SOME CRAMBE SP. IN SOUTHERN TURKEY

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ABSTRACT. Crambe sp. is an oilseed crop from the Brassicae family and native to the Mediterranean region. It can be converted into a number of industrial and energy uses. Crambe oil is used in introducing in stain, primers, plastic and solid wax, cosmetic and engine portions in the form of nylon-13.13 exclude carburetor as eco-friendly. All these properties make it interesting. This study is the first report on yield, cultivation procedure, and quality characteristics of Crambe sp. cultivated in Turkey. Native Crambe seeds, collected from eight different locations in Turkey, were cultivated under Cukurova conditions in Mediterranean region. Two Crambe species, Crambe orientalis and Crambe tataria, determined at the locations were studied, and some morphological characteristics and oil compositions were sown from cultivars and native forms. Fatty acid composition of seeds was examined with GC and GC/MS. In native populations, high seed oil contents were obtained from C. tataria (Ankara -Faculty of Science and Letters) and *C. tataria* (Kahramanmaraş-Elbistan), as 45.62 and 45.50%, respectively. The highest erucic acid content (49.0 %) was found in *C. tataria* (Ankara-Bilkent). In Çukurova conditions, despite cultivated all collected species, just *C. orientalis* was bloom among this species, and so that seed yield (472.77 kg/ha) oil rate (% 27.43) and erucic acid (41.0 %) could determine just this species.

Keywords: bioenergy; bioplastic; *Crambe* sp.; erucic acid; fatty acid.

INTRODUCTION

Bio-renewables, such as plantderived oils, are a sustainable means of providing the essential products needed by society. In this context, plant oils are already major agricultural commodities with around 20% by value used for non-food applications. Two plant-derived fatty

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acids, erucic and lauric acid have been competing with petroleum alternatives for many years. Historically, cost has been the major bottleneck limiting the development of new plant derived oils. But, in the context of the escalating cost of crude oil and also the increasing concerns about both finite supply and security of supply, there is an emerging strategic need to additional renewable develop products from plant oils (Stymne and Dyer, 2006). Crambe is one of the most promising "new" plants. Crambe oil produced from pressing Crambe *Crambe*'s unsaturated seed fats erucic acid content is 55-60% and industry claim high rate of erucic acid. Crambe oil has very high capacity about removing warmth and high quality as well as some advantage about commercialization and storage. The research work still to do on breeding and chemistry is immense and requires support (Comlekcioglu et al., 2008; Razavi Nejad-Ebrahimi, and 2009). Furthermore, as a "new" plant it will have to challenge "old" plants which have been improved and supported for decades and petrol products with their powerful lobby. However, its strength comes from the will to preserve biodiversity and to limit pollution, i.e. the will to preserve the future. Surpluses in agriculture and pollution linked to the petroleum industry urge farmers and industrialists to look for new uses for "old" plants or directly for "new" plants, such as Crambe (Tittonel, 1994). A very important fuel's feature is the calorific value. The higher the calorific value, the higher is the yield of the fuel. The Crambe biodiesel shows the highest calorific value ($\Delta H= 40564 \text{ J} \text{ g}^{-1}$), influenced by the high amount of long chain ethyl ester originated from behenic acid (C22:0) that composes 57.2% of Crambe oil. The rapeseed, sovbean and Jatropha curcas biodiesel that exhibit approximately the same amount of long chain ethyl ester showed calorific values near to $\Delta H = 39450 \text{ J g}^{-1}$ (Oliveira et al., 2013). Moreover, the results show that the longer the carbon chain of the ethyl ester the higher is calorific value. Biodiesel from Crambe oil shows larger amounts of ethyl esters with long chain and shows the highest calorific value. Most of all energy consumed in the world comes from fossil fuels (oil, coal and natural gas). However, these sources are limited and will be exhausted in the near future (Anawar et al., 2010; Demirbas, 2005). Biodiesel is a fuel that can replace diesel and it is made from renewable sources, such as vegetable oils and animal fats. This fuel is biodegradable and non-toxic and has profile pollutant emissions, low compared to petroleum diesel. The use of biodiesel will allow the development of agriculture, economy and environment (Demirbas, 2008; Demirbas and Demirbas, 2011).

The biodegradable nature of *Crambe* oil is an advantage over mineral oils. As a lubricant it has a higher heat removable coefficient that mineral oil at temperatures of 700°C, whereas the better mineral oils reach a

maximum of 550-600°C, a potentially valuable characteristic for the refrigeration industry. It is a stable oil containing 3% of a polyphenol oxidant and was stable for 240 hrs at 180°C in evaluation with 'Rancimat' equipment. This together with the fact that it did not develop sandy oxidation or polymerization products make it suitable for chain saw lubricants. especially as it is biodegradable (Lazzeri et al., 1994).

RIRDC have recognized the potential of Crambe as a bio renewable and resource commissioned comprehensive а review of current and future potential of the crop (Knights, 2002). One of the selling points of the erucic-acidproducts oil is their enhanced biodegradability, compared to their petroleum-based counter parts (Dolack. 1996). Crambe was commercialized in the 1990s, through the efforts of a dedicated team of agribusiness people formers. and scientists to develop a reliable supply of erucic domestic acid (Carlson et al., 1997). Erucic acid can be converted into erucamide, which is then used as a slip agent in plastics. *Crambe* oil can then be converted into a number of industrial and energy The erucamide uses. market is growing at a rate of between 4% and 10% per year (Anonymous, 2012). Its interest and limits can be outlined by comparison with rape which is the main "old" oil brassica, already grown in the EU, and which is also a potential source of erucic acid. Rape is grown on a very large area of about 27 million hectares in the EU (Tittonel, 1994). In contrast, Crambe can be cultivated on the same areas as any cultivar of rape since they are not cross pollinated (Tittonel, 1994) Crambe has representation in the Mac-aronesian. Euro-Siberian, Mediterranean. Sindico-Saharan, Irano-Turkish and Sudan-Zambezian (Ethiopia and Tanzania) regions (Leppik and White, 1975). Many species registered Crambe are naturally found in Turkey. It has industrial uses due to its high erucic acid content, but there is no study on the cultivation of *Crambe* and its erucic acid content found in Turkey. until 2002 years. Despite its name, it probably originated from the Turko-Iranian area of South West Asia. Crambe is not a well-known species in Turkey, although it is present in the country's flora (Seyis et al., 2013). In this research, Crambe seeds, collected from different regions in Turkey, were identified and then to determine adaptation characteristics were cultivated under Cukurova conditions, in Mediterranean region.

MATERIALS AND METHODS

Plant material

Crambe sp. was collected from nine different locations from Central, East and Southeast Anatolian Region of Turkey. Collected specimens and collecting area were given in *Table 1* and *Fig. 1*. Plants were identified, according to Flora of Turkey and East Aegean Islands (Davis, 1982). Because the plants were collected from the various regions with different climates and soil conditions at different times, collecting the all of the data related

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to the characters to be investigated could not be possible. So necessary statistical comparisons for certain characters were only performed on the locations in which those data were collected. As seen in *Table 1*, two different *Crambe* species *C. orientalis* and *C. tataria* were identified at all locations and these species were cultivated under Çukurova conditions.



(Red circles show collection locations, yellow circle - experiment region). Figure 1 - Collection locations of *Crambe* sp. in Turkey Map

Table 1 - Collection a	areas of	Crambe s	p. in Turkey
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Crambe sp.	Collection area	Collection date	Altitude (m)	Latitude Longitude (N) (E)	Properties of habitat
Crambe tataria	Ankara- City Center (Faculty of Science and Letters, Dept. of Biology)	27.06.2000	875	40° 01.8' 32° 19.2'	Sliybum sp., Verbascum sp.,
Crambe tataria	Kahramanmaraş City-Elbistan	1.07.2000	1200	38° 34.8' 37° 55.8'	Stony place, rocky mountain, forage grasses
Crambe orientalis	Tortum-Erzurum City highway	19.08.2001	1900	40° 19.2' 41° 36.0'	Sliybum sp., Verbascum sp.
Crambe orientalis	Susuz-Kars City highway	19.08.2001	1750	40° 46.8' 43° 07.8'	Stony place, rocky mountain
Crambe orientalis	Şanlıurfa- Diyarbakır City highway	5.07.2001	540-600	37º 34.8' 38º 58.8'	<i>Sliybum</i> sp., <i>Verbascum</i> sp., <i>Isatis</i> sp., Stony and rocky place, (Step)
Crambe tataria	Ankara City- Bilkent	10- 22.08.2001 29.06.2002	875- 1225	39° 57.8' 32° 51.0'	Centaurea sp., Scabiosa sp., Isatis sp., Anthemis sp.,
Crambe orientalis	Kahramanmaraş City-Ahır Mountain	1.07.2000 10- 22.08.2001	650-700	37° 34.8' 36° 55.8'	<i>Centaurea</i> sp., Stony and rocky place,
Crambe tataria	Ankara City- Ayas	15.07.2002	875- 1225	40° 01.8' 32° 19.2'	<i>Centaurea</i> sp., <i>Scabiosa</i> sp

Field trials

of С. Seeds orientalis and C. tataria, belonging to different locations were refrigerated until the sowing times, to break dormancy. Seed were sown in seedbeds, in greenhouse, in winter on 28 Nov. 2000. First emergence was seen on 7 Dec. 2000. Small seedlings transplanted with soil loaded in nylon bag on 20 Jan. 2001. Young seedlings grown in greenhouse were transplanted to open field, spaced 25 cm in rows on 21 April 2001. Plants were weeded and irrigated when necessary. Only C. orientalis cultivars, collected from Kahramanmaras, were flowered in second year, among the all cultivars. Thus, only C. orientalis (Kahramanmaras) fruits were harvested in physiological mature, when 50% of the seeds have turned brown on 29 May 2002. Field trials were arranged in the complete randomized block design with three replications in Çukurova conditions.

Lipid extraction

Crambe seeds were dried overnight at 50°C and ground into powder in a Moulinex coffee grinder. The oils were extracted by the Soxhlet method (Yaniv *et al.*, 1991). Samples of 5 g of ground dried seeds were used for each determination.

The oil from the seeds was extracted with n-hexane. The solvent was evaporated, and the lipid fraction residues were weighed. Three accessions were evaluated for each species and average values of these plants were recorded.

Methylation of Crambe oil

Two grams of normal KOH with methanol (2 g KOH / 100 ml MeOH) and 0.1 g *Crambe* oil were added in a 50 ml flask and boiled in a water bath with a condensator for 25 min. A quantity of 2 ml of Pentane was added on the mixture and transferred into a separatory funnel.

Then pentane was removed under nitrogen stream (Kalender, 2002).

GC and GC/MS analysis of methylated fatty acids

Gas chromatography (GC) analyses were conducted in Control Laboratory in Mersin/Turkey. Qualification of the oil was analyzed with a Hewlett-Packard GC-6890II series GC with flame ionization detector. SE-54 fused silica capillary column (600 m x 0.25 mm i.d.; 0.25 µm film thickness) and nitrogen was used as a carrier gas with flow rate of 1.0 mL/min. One µl of the oil was injected into the column. The GC oven temperature was kept at 100°C, for 5 min, and programmed to 200°C, at a rate of 2°C/min, and then kept at 200°C. The injector temperature was 200°C and the amount of injection was 1 µl.

Oil analysis was also repeated by GC/MS (Gas chromotography and Mass spectrofotometry) in the Plant Physiology Laboratory in Biology Dept. of Kahramanmaras Sutcu Imam University. Oualification of the oil was analyzed on an Agilent 5975C MS, coupled with an Agilent GC-6890II series. The GC was equipped with HP- 88 capillary column (100 m x 0.25 mm i.d., 0.20 um film thickness) and helium was used as carrier gas with flow rate of 1.0 mL/min. The GC oven temperature was programmed as follows: 170 (1 min), 220°C at an increase of 5°C/min, hold 10 min, at 220°C, and 230°C, at of 10°C/min, then kept at 230°C. The at 15 min. injector temperature was 250°C. The MS was operated in EI mode at 70 eV. Split ratio was 20:1. Mass range 35-400 m/z; scan speed (amu/s): 1000. The components of the oil were identified by mass spectra with those of pure authentic samples and wiley 7 n.1, Famdbwax. L, Famedb 23.L, libraries reference compounds.

All samples were repeated three times for GC/MS analysis.

Mean values of morphological and oil characters obtained from the locations were compared by means of an analysis of variance (ANOVA). Mean values were compared by the least significant difference (LSD), at the 5% probability level.

RESULTS AND DISCUSSION

Morphological characters and oil contents of *Crambe* sp.

Crambe species collecting from wild flora in this study were identified as Crambe orientalis and Crambe tataria. Both of Crambe species were found on the step regions of Turkey (Table 1 and Fig. 1). Morphological characteristics of C. orientalis were varied according to their geographic location. Fruit and seed weights of both collection C. tataria in 2000 and 2001 were higher than C. orientalis (Table 2). While the higher values in terms of fruit dimensions of Crambe sp. were determined from C. tataria (Ankara) and C. orientalis (Sanlıurfa-Divarbakır), the higher value seed dimension was determined from C. tataria (Ankara-Bilkent). Although the fruits and seed of C. orientalis (Tortum-Erzurum) and C. orientalis (Susuz-Kars) were smaller than other sp., theirs Crambe seeds fully developed in the periphery of fruit (Table 3). For this reason, seeds of C. orientalis (Tortum-Erzurum) and С. orientalis (Susuz-Kars) were broken during dehulling. Therefore sufficiently undamaged seed to obtain is very hard for germination. But, it may be advantage in extraction of direct fruit for oil. Thus, the higher fruit oil contents 26.80% and 18.85% were obtained C. orientalis (Tortum-Erzurum) and C. orientalis (Susuz-Kars). respectively (Table 3). However, the higher seed oil contents 45.62 and 45.50% were obtained from C. tataria (Ankara-Faculty of Science Letters) С. and and tataria (Kahramanmaras-Elbistan), respectively (Table 3).

A number of 1000 seed and fruit weights of cultivated *C. orientalis* (Kahramanmaraş) were higher than wild *C. orientalis* (Kahramanmaraş), but fruit+seed oil and seed oil rates were lowest (*Tables 2,3* and *4*).

Crambe seed-plus-hull The contains 26 to 38% oil, with 32% being about average (Earle et al., 1966). Dehulled Crambe seed has an oil content of 33 to 54% (McGregor et al., 1961). The oil content of the hulled Crambe seed was 30%, which equates to approximately 50% content of dehulled seed. The oil content of the hulled Crambe seed varied the most; this was probably related to the variation in thickness of the fruit coat (Francis and Campbell, 2003). C. abyssinica, having a significant amount of oil (32-36%) with a higherucic acid content (52-59%), along with its wide climatic and agronomic adaptation, is a promising alternative crop (Lazzeri et al., 1994; Mikolajczak et al., 1961).

	Collecting	time, 2000	Collecting time, 2001		
Crambe sp.	1000 seed weight (g)	1000 fruit weight (g)	1000 seed weight (g)	1000 fruit weight (g)	
<i>Crambe tataria</i> (Ankara- City Center)	17.50 a	22.30 a	17.80 a	22.50 a	
Crambe orientalis (K.maraş City-Ahır Mountain)	6.00 c	9.00 b	9.98 b	18.03 a	
<i>Crambe tataria</i> (K.maraş)	14.75 b	20.25 a	6.88 c	9.85 c	
Crambe orientalis (Susuz-Kars)	-	-	9.76 bc	15.65 b	
Crambe orientalis (Şanlıurfa-Diyarbakır)	-	_	9.95 b	14.46 b	
LSD (5 %)	2.61	2.62	2.72	2.23	

Table 2 -	1000 seed a	and 1000	fruit w	eight of	collection	some	native (<i>Crambe</i> sp.	. in
	2000-2001								

Table 3 - Dimensions of fruit and seeds of some native Crambe sp. (mm)

Crambe sp.	Fruit width	Fruit length	Seed width	Seed length	Fruit+ seed oil	Seed oil
<i>Crambe tataria</i> (Ankara- City Center)	3.05 a	3.38 a	1.86 b	2.02 ab	20.25 b	45.62 a
<i>Crambe orientalis</i> (K.maraş-Ahır Mountain)	2.75 ab	2.87 ab	1.94 b	2.06 ab	15.66 c	39.94 b
<i>Crambe orientalis</i> (Tortum-Erzurum)	1.85 c	1.89 c	1.18 b	1.26 c	26.80 a	40.62 b
Crambe orientalis (Susuz-Kars)	1.91 bc	2.16 bc	1.29 b	1.38 c	18.85 b	37.24 c
<i>Crambe tataria</i> (Ankara-Bilkent)	3.19 a	3.29 a	2.83 a	2.88 a	13.20 d	28.82 d
Crambe orientalis (Şanlıurfa-Diyarbakır)	3.11 a	3.58 a	1.84 b	1.95 ab	10.77 e	23.31 f
<i>Crambe tataria</i> (K.maraş-Elbistan)	-	-	-	-	20.20 b	45.50 a
<i>Crambe orientalis</i> cultivars (experiment in Çukurova)	2.77 ab	2.85 ab	2.00 ab	2.21 ab	12.30 de	27.43 e
LSD (5 %)	0.89	0.85	0.88	1.03	1.89	1.20

All collected *Crambe* species were germinated under the Çukurova conditions, in southern Turkey, but only *C. orientalis* (Kahramanmaraş) succeeded in reaching a full grown state while other *Crambe* sp. stands at rosette periods and were not flowered,

so that seed yield (472.77 kg/da) and oil rate (27.43%) could determine just this species (*Tables 3 and 4*). Lancaster (2012) stated one of which from two plant side by side of *C. cordifolia*, which is perennial has not flowered for 3 years. It was

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flowered in fourth year. Although there has been a lot of rain here in Worcestershire but with just enough sunshine and heat to encourage flower. As some *Crambe* sp., which in perennial hardy plants could not be seen flowering even in first and second year (Lowery, 1999). However, *C. abyssinica* is annual and flowering in first year. We could not found *C. abyssinica* in Turkey, although its origin is Mediterranean region. *C. abyssinica* Hochst. ex. R.E. Fries has a significant amount of oil (32-36%) with a high erucic acid content (52-59%), along with its wide climatic and agronomic adaptation (Vargas-Lopez, 1999).

 Table 4 - The some morphological properties of Crambe orientalis were grown at Çukurova conditions in 2002

Crambe orientalis (K. maraş)	Mean	Mean standard deviation	Confidence limit
Plant lenght (cm)	93.98	1.59	93.98±3.20
Number of main branches	1.82	0.14	1.82± 0.29
Number of secondary branches	25.59	0.61	25.59±1.23
Yield of fruit per plant (g)	72.35	21.939	72.35±43.88
Number of fruit per plant	3771.24	152.55	3771.24±306.92
1000 seed weight (g)	12.99	0.31	12.99± 0.62
1000 fruit weight (g)	19.52	0.21	19.52± 0.43
Length of spike pedicel (cm)	4.00	0.06	4.00± 0.13
Length of spike (cm)	22.92	0.35	22.92±0.70
First branch height (cm)	10.00	0.12	10.00±0.23
Yield of fruit (kg/da)	1165.26	173.21	1165.26±346.41
Yield of seed (kg/da)	472.77	122.44	472.77±245.86

Fatty acid composition

Fatty acid compositon of the Crambe species were analyzed by GC and GC/MS. Methylated oil compounds were identified with GC/MS, according standard to compounds and MS database and ratio of methylated components were calculated according to GC, coupled with FID detector.

According to GC/MS results, eighteen oil compounds were identified in the *C. tataria* and *C. orientalis* oil and main compounds at the all samples were erucic acid (30.33%), oleic acid (10.83%), linoleic acid (10.68%) and cis-11 eicosenoic acid (8.71%) were other important compounds in the all *Crambe* oil (*Table 5*). These results were expected for fatty acid profile of crambe oil (Lalas *et al.*, 2012).

According to GC results, fatty acid ratio of *Crambe* spp. collected from different location and cultivated in Çukurova region were given in *Table* 6. The highest erucic acid content (49.0%) was found in *C. tataria* (Ankara-Bilkent) (*Table* 6). The lowest level of erucic acid was obtained in location with the shortest and coolest growing season as Susuz-Kars (31.82%) and Tortum Erzurum (36.05%). Erucic acid contents of

C. orientalis were changed, depending influence of environment in on different location (Table 6). Erucic acid content was affected by Crambe species and different locations. Generally, variation in erucic acid content was lower than in other fatty acids, such as oleic, linoleic or linolenic which acid. were significantly influenced by temperature during the period of seed

development (Vollmann and Ruckenbauer, 1993).

Seed oil content of *C. tataria* (Ankara-Fac. of Science and Letters), *Crambe tataria* (Kahramanmaraş-Elbistan), *C. orientalis* (Tortum-Erzurum), *C. orientalis* (Kahramanmaraş-Ahır Mountain 2000) and *C. orientalis* (Susuz-Kars) were higher than *C. abyssinica*, but theirs erucic acid contents were lower than *C. abyssinica* (Tables 3 and 5).

Table 5 - GC-MS resu	Its of Crambe orienta	lis and Crambe tataria seed	oil

Fatty acid	Retention time	Carbon number	% Ratio of <i>Crambe</i> <i>orientalis</i> (Kahramanmaraş Ahir mountain)	% Ratio of <i>Crambe tataria</i> (Ankara- Bilkent)
Miristic acid	10.92	14:0	0.10	0.09
Pentadecanoic acid	11.60	15:0	0.06	0.03
Palmitic acid	12.38	16:0	2.72	1.89
Palmitoleic acid	12.92	16:1	0.31	0.21
Heptadecanoic acid	13.014	17:0	-	0.24
Stearic acid	14.24	18:0	0.50	0.55
Oleic acid	14.99	18:1	10.83	16.16
Elaidic acid	15.06	18:1 trans9	1.64	2.18
Linoleic acid	16.06	18:2 (cis,cis 9,12)	10.68	11.21
Linolelaidic acid	16.29	18:2 trans- trans-9,12)	-	0.14
Eicosenoic acid	16.58	20:0	0.56	0.33
α-Linolenic acid	17.45	18:3	7.13	8.55
Cis-11 Eicosenoic acid	17.55	20:1	8.71	14.18
Eicosadienoic acid	18.87	20:2	0.88	0.71
Behenic acid	19.61	22:0	0.43	0.22
Erucic acid	20.96	22:1	30.33	26.10
Tricosanaote	21.45	23:0	0.09	-
Docosadienoate	22.50	22:2	0.54	-
Lignoseric acid	23.51	24:0	0.17	0.11
Nervonic acid	24.56	24:1	1.20	1.17
Total ratio			76.88	83.90
DT D L L				

RT=Retention time

Sample Name	C. or <i>ientalis</i> Çukurova cultivation (K. maraş origin)	C. <i>orientalis</i> K. maraş (Ahır mountain)	C. o <i>rientalis</i> Şanlıurfa- Diyarbakır	C. <i>orientalis</i> Susuz- Kars	C. <i>orientalis</i> Tortum- Erzurum	C. <i>tataria</i> Ankara- Bilkent
Palmitic	4.29	2.56	2.48	2.47	2.35	3.68
Palmitoleic	-	0.51	-	-	0.16	-
Stearic	-	-	0.47	0.5	0.56	0.58
Oleic	13.97	18.1	14.55	18.34	21.93	15.00
Linoleic	15.04	12.03	10.20	11.21	11.02	11.00
Linolenic	7.60	7.30	6.48	7.56	7.80	6.90
Eicosenoic	12.47	14.80	10.91	16.42	18.22	12.00
Erucic	41.00	44.32	43.32	31.82	36.05	49.00

Table 6 – GC-FID results of some Crambe sp. collecting from Turkey

The both among seed oil of *C. tataria* and *Crambe orientalis* were seen variation between location in Turkey, similarly findings of Francis and Campbell (2003). The source of the variation can be attributed to both genetics and environment in Crambe (Francis and Campbell, 2003). As Francis and Campbell (2003) stated, the highest level of erucic acid was obtained in location with the longest and coolest growing season, as Sanlıurfa-Divarbakır Ankara. and Kahramanmaras (Table 6).

CONCLUSION

In this study, two different *Crambe* sp., as *C. orientalis* and *C. tataria*, were found in Turkey flora. Actually all entries were cultivated under the warmer conditions comparing to their natural living conditions. *C. orientalis* from Kahramanmaraş, where the nearest

collection site to the experimental area, was flowered and seeded one. This implies that some *Crambe* species needs the certain cold period for flowering. Erucic acid rate (49.0%) in seed oil of *C. tataria* was the higher than *C. orientalis* (44.32%).

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