Agronomy Research **19**(2), 648–658, 2021
https://doi.org/10.15159/AR.21.091

Analysis of the *Foeniculum vulgare* Mill. collection by the complex of features in the conditions of the Crimea foothills

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Received: February 24th, 2021; Accepted: May 8th, 2021; Published: Accepted: May 19th, 2021

Abstract. *Foeniculum vulgare* Mill. is a valuable essential oil plant, which raw materials and derived products, and, above all, essential oil, are widely used in the perfume and cosmetics, liquor, paint and varnish industries, in the food industry and medicine. The source material for cultivated plants selection, including *F. vulgare*, is the collections of the gene pool. The objective of this study was a comparative study of *F. vulgare* samples collection by the complex of features to clarify the possibility of identifying sources of economically valuable characteristics for creating promising breeding material. The study of the *F. vulgare* collection supported by the Research Institute of Agriculture of Crimea, which includes 75 samples rom 28 countries, was conducted in 2017–2019 at an experimental base located in the Crimea Foothills (Krymskaya Roza village, Belogorsky district). The collection samples were analyzed according to morphobiological parameters and productivity indicators. The work was guided by generally accepted methods, including those developed for essential oil plants. Statistical processing of the obtained data was carried out. The wide variability of the collection is shown, according to individual indicators (variation coefficients - from 8.3 to 54.4%). In this way, the mass fraction of essential oil (one of the most important indicators) varies within a wide range in the collection - from 1.09 to 3.86% (of absolutely dry mass) in whole plants and from 4.16 to 8.53% in fruits. The composition of the essential oil depends on the raw material. The anethole content reaches 80% in fruit oil, and the content of fenchone and terpene compounds is increased during the plant processing. The results of the collection analysis are basic, allowing preliminary sampling with high productivity indicators for inclusion in breeding studies.

Key words: collection, essential oil, *Foeniculum vulgare*, productivity parameters, variability.

INTRODUCRION

For the essential oils production in the world, about 300 species of cultivated and wild-growing essential-oil-bearing plants are used (Pashtetsky et al., 2018). Essential oils and products derived from essential-oil raw materials are used in the perfumery and cosmetics, pharmaceutical, liquor, paint, and varnish industries, in medicine and the food industry (Voitkevich, 1999; Tkachenko, 2011; Pashtetsky & Nevkritaya, 2018). One of the well-known essential oil crops is *Foeniculum vulgare* Mill. (Pashtetsky et al., 2018).

The herbaceous raw materials and fruits of *F. vulgare*, as well as the products of their processing, are widely used as a medicine and as a spice (Mehra et al., 2021). Valuable qualities are given to them by the essential oil and other valuable biologically active compounds that accumulate in them (Voitkevich, 1999; Timasheva & Gorbunova, 2012). The essential oil content in the *F. vulgare* fruits varies from 3.5% to 10.0% (Voitkevich, 1999). It contains more than 20 components, the main of which are anethol (60–80%), which gives a sweet taste and fenchon (up to 20%), which causes the bitter taste of the fruits. Methylchavicol is in significant amounts in the composition of the essential oil (3–15%) (Voitkevich, 1999; Hussein et al., 2016). The ratio of components that determines the quality of the essential oil, including its antimicrobial and antioxidant activity, varies depending on the type of raw material – whole plants or fruits. The phase of plant development and, accordingly, the state of fruit maturity is of great importance (Anwar et al., 2009).

In medicine, the fruits and essential oil of *F. vulgare* are used as a carminative, diuretic, lactic, aromatic stimulating and strengthening gastric remedy (Choi & Hwang, 2004; Rather et al., 2016; Akhbari et al., 2019). *F. vulgare* essential oil is a strong antiseptic. In terms of its bactericidal action, it is 13 times higher than phenol (Voitkevich, 1999). The antiamnestic, antidepressant, and anxiolytic effects of fennel have been proven (Perveen et al., 2017; Abbas et al., 2020). There is information that *F. vulgar*e essential oil not only exhibits antibacterial activity, in particular, against gram-negative strains of *Pseudomonas aeruginosa, Escherichia coli*, and *Shigella dysenteriae*, but also has high antitumor activity against human breast cancer (MDA-Mb) and cervical epithelial carcinoma (Hela) cell lines (Akhbari et al., 2019).

The possibility and prospects of using *F. vulgare* essential oil in the development of environmentally safe insecticides designed to protect plants from aphids have been experimentally demonstrated (Pavela, 2018).

The *F. vulgare* fruits are used in cooking as a seasoning for cheese, meat.

In the 80–90s of the twentieth century, *F. vulgare*, as an essential oil crop, was grown in many European countries and in the United States. The production of its essential oil in France alone reached 500 tons per year (Nazarenko & Afonin, 2008). Now it has significantly decreased due to competition with synthetic anethol. Currently, *F. vulgare* is cultivated in many countries: in the northern and southern regions of Europe, including Ukraine, Moldova, and Russia; in Asia (India, China, Japan), North and South America, in the oases of Africa (Pashtetsky et al., 2018).

F. vulgare is a perennial herbaceous plant of the Apiaceae family, reaching a height of 80–250 cm, with a hollow, branched, annually dying stem. The shoots are repeatedly branched and end, like the main stem, in inflorescences. The leaves are strongly divided. The flowers are small yellow in color. The inflorescence is a compound umbel, consisting of 10–25 simple umbels. The fruit is an ovoid-oblong, greenish-brown cremocarp, splitting into two half-fruits (Pashtetsky et al., 2018).

In Russia, *F. vulgare* is most often grown as an annual plant. In regions characterized by a warm long period and mild winters, including in the Crimea, *F. vulgare* can be cultivated as a perennial crop - up to 5–7 years (Nazarenko & Afonin, 2008). In comparison with the first year, in the following years of the growing season, the development of plants occurs 10–15 days earlier, and a higher yield is formed.

*F. vulgare*is a highly profitable crop, but, as for other agricultural crops, it is periodically necessary to carry out variety changes, including new promising varieties in production. The main direction of breeding is the development of effective varieties with high adaptive capacity, increased fruit yield and high content of high-quality essential oil.

The source of promising genotypes and donors of valuable traits of cultivated plants are the gene pool collections (Tkachenko, 2019).Evaluation and selection of collection material, represented by forms and samples of various origins, are important at the initial stages of the selection process (Saxena et al., 2016; Krivda et al., 2020; Nevkrytaya et al., 2020). At the same time, it is important to consider the variability of indicators depending on the regional soil and climatic conditions, meteorological conditions during plant growing season and agricultural techniques used in fennel cultivation (Shafeiee et al., 2019; Akbari-Kharaji et al., 2020).

The assessment of differences between genetically similar genotypes in breeding studies is very effective using molecular markers (Choudhary et al., 2018). Thus, Iranian scientists assessed 10 fennel genotypes from different regions for economically valuable traits. Twelve PCR primers were used to analyze genotypic diversity. Special attention was paid to the dependence of the main indicators on water stress. Obtained results made it possible to identify the most promising genotype for cultivation (Poudineh et al., 2018a, Poudineh et al., 2018b).

The study of the collection material of *F. vulgare* and the creation of varieties based on it has been carried out in the Crimea for decades (Pashtetsky et al., 2018). The State Register of Selection Achievements Authorized for Use for Production Purposes of the Russian Federation includes two varieties of the Research Institute of Agriculture of Crimea (RIAC) - Mertsishor and OxamitKryma (State Register of Selection Achievements Authorized for Use for Production Purposes, 2020). Currently, breeding studies of *F. vulgare* are continuing, using both traditional and modern biotechnological techniques for creating valuable source material (Zolotilova et al., 2017). Much attention is paid to the replenishment and study of the *F. vulgare* collection, as a source not only of donors of high productivity, but also of samples that differ in the component composition of the essential oil (Saxena et al., 2016; Zolotilova et al., 2019).

The objective of this study was a comparative study of *F. vulgare* samples collection by the complex of features in order to clarify the possibility of identifying sources of economically valuable characteristics for creating promising breeding material.

MATERIALS AND METHODS

In 2017–2019, in order to clarify the nature of the intraspecific diversity of the *F. vulgare* collection in the Crimea Foothills conditions and to determine its breeding value, a comprehensive assessment of samples by morphobiological and economically valuable characteristics was carried out. The collection includes 75 samples of various ecological and geographical origin (from 28 countries of the world), including varieties of domestic and foreign selection (Table 1).

The collection includes 16 RIAC samples, including the varieties Mertsishor and Oxamit Kryma, hybrids and regenerants obtained in culture *in vitro*.

Table 1. Samples of the *F. vulgare* collection

Region	Number ofsamples	Region	Number ofsamples	Region	Number ofsamples
Russia	19	USA	3	Israel	1
Italy	4	Germany	2	Morocco	1
India	4	Poland	2	Belgium	1
Canada	4	CzechRepublic	2	Hungary	1
Afghanistan	4	Switzerland	2	ArabRepublicofYemen	1
Argentina	3	Syria	2	Iran	1
Azerbaijan	3	Sweden	1	Pakistan	1
Bulgaria	3	Uzbekistan	1	Tunisia	1
France	3	Algeria	1		
Ukraine	3	China	1		
Total	75				

The study was conducted at the experimental base of the department of essential-oil-bearing and medicinal crops of RIAC in the village of KrymskayaRoza (Belogorsky district), located in the eastern part of the Crimea Foothill zone. This region belongs to one of the five agro-climatic regions - the upper foothill (Table 2) (Savchuk, 2006).

Table 2. Soil and climatic conditions of the study region

Indicator	Crimea Eastern Foothill
Type of climate	moderate continental
Annual sum of effective temperatures, °C	3,200–3,400
Annual precipitation, mm	450–500
Average annual evaporation from the surface, mm	460–484
Hydrothermal coefficient of humidification (G.T. Selyaninova)	0.92
Total annual solar radiation, kcal/cm^2	112–128
Average daily temperature of the warmest month, °C	+22.3 (July)
Average daily temperature of the coldest month, °C	-0.8 (January)
Duration of the period with an average daily temperature of > 0 °C, days	292
Soil type	southern carbonate chernozems
Granulometric composition of soils	Heavy loam
Acidity, pH	8.0

The collection nursery was founded in 2016. The samples were placed on plots with a length of 1 m with row spacing of 0.6 m. The area of the accounting plot is 0.6 m^2. The samples were analyzed in two replicates, which is acceptable when studying the collection.Sample placement randomized.The assessment was carried out in 2017–2019 on plants of 2–4 years of age.

The analysis of the collection samples was performed according to the developed methods (Beydeman, 1974; Arinshteyn, 1977). The following indicators were analyzed:

– phenological - period from the beginning of growing season to flowering, from flowering to maturation, total duration of vegetation period;

– morphobiological - winter hardiness, plant height, height of the lower umbrella attachment, number of shoots of the first order, weight of 1,000 fruits;

– productivity indicators - harvest from the plot, collection of essential oil;

– biochemical - content of essential oil in whole plants and in fruits.

Statistical processing of the obtained data was performed using the Microsoft Office Excel 2010 software package (Dospekhov, 2012).

RESULTS

The years of research (2017–2019) differed significantly by the hydrothermal conditions of the period of active vegetation of plants (Figs 1, 2).

The wettest period was the spring-summer period of 2017, and the hottest and driest period was 2018 (the exception was July, but precipitation during flowering did not favor pollination and fruit formation). Compared to the previous year, 2019 was generally more favorable. At the same time, the temperature regime in June was higher, and in July - significantly lower than in previous years. In addition, heavy precipitation fell in July. The peculiarities of weather conditions in the years of the research led to differences in the nature of the morphobiological parameters and productivity indicators of the studied samples of the *F. vulgare* collection.

The terms of passing the plants vegetation phases also differed in years. Spring regrowth of collection samples was observed in the period from March 3 to March 17, 2017, in 2018 - from February 26 to March 5, in 2019 - from February 24 to March 10. The difference between the samples at the beginning of the vegetation restoration was 14–16 days in 2017 and 2019, and 8 days in 2018.

The duration of the vegetation period from spring

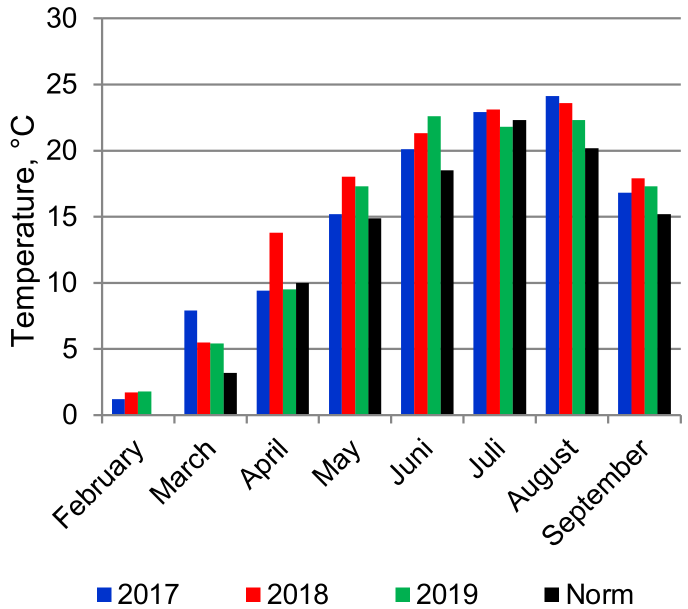

Figure 1. Average monthly air temperature during the period of active vegetation, 2017–2019.

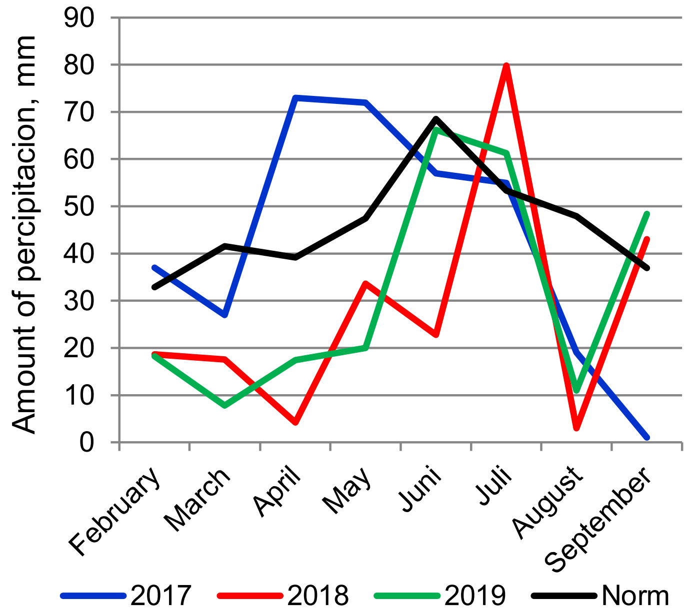

Figure 2. Average monthly precipitation during the period of active vegetation, 2017–2019.

regrowth to fruit maturation in F. vulgare is significantly longer compared to the related annual species of the Apiaceae family - *Coriandrum sativum* L., *Anisum vulgare*Gaertn. and *Anethum graviolens* L. When sown in the third decade of March, the fruit ripens in these crops in the third decade of July, and the mass ripening of fruits in *F. vulgare* is at least a month later. Accordingly, *F. vulgare* plants experience fluctuations in weather conditions for a longer time. Plants are particularly sensitive during flowering, fruit formation and ripening. Heavy precipitation during this period prevents normal fruit formation and accumulation of essential oil, and extremely high temperatures do not favor the development of fruits, reducing the time of their maturation, but stimulate the oil-forming process (Coban et al., 2018). The duration of the vegetation period in the studied samples of the collection for all years varied, on average, from 161 to 186 days. In 2017–2018, the difference between the samples was 17 and 16 days (161–178 and 164–180 days), and in 2019, due to the low temperature regime in June and heavy rains, this period was extended to 23 days (163–186 days) due to different genotype responses. According to the results of the analysis of three-year data, the samples were divided into three groups: early - up to 170 days - 5 samples (6.7%), mid - 171 to 175 days - 33 samples (44.0%), and late - more than 176 days -37 samples (49.3%). The varieties Mertsishor (174 days) and OxamitKryma (175 days) are included in the group of medium-ripened samples.

The general characteristics of the main economically valuable indicators of collection samples are given in Table 3.

Table 3. Characteristics of the *F. vulgare* collection by complex of features, 2017–2019

Indicator value	Indicator									
	Winter hardiness, point	Plant Height, cm	Number of shoots 1 order, pcs.	Weight o f 1,000 fruits, G	Yield from the plot0 (0.6 m^2)		Mass fraction of essential oil, % of absolutely dry mass		Collection of essential oil, g from the plot (0.6 m^2)	
					Green raw materials, kg	Fruits, g	In green raw materials	In fruits	From green raw materials	From fruits
Average indicator in the collection	4.2 ± 0.1	149.7 ± 1.5	6.9 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	40.9 ± 2.7	2.32 ± 0.06	5.92 ± 0.11	18.4 ± 0.7	2.6 ± 0.2
Indicator range	1–5	115.4–179.1	5.4–8.4	2.0–5.4	1.6–6.7	5.2–108.1	1.09–3.86	4.16–8.53	6.2–34.3	0.4–6.3
Variation coefficient, (v),%	21.4	8.9	8.3	16.6	28.2	54.4	21.1	14.7	31.9	54.1

Since *F. vulgare* can be cultivated in a perennial culture, the winter hardiness indicator is of particular importance, as a guarantee of plants overwintering in certain climatic conditions. The winter hardiness of the samples was determined in the spring at the beginning of the growing season by the degree of damage to the plants on the accounting plots after overwintering. The highest winter hardiness (5 points) for three years of study showed 24 samples - 32.0%, the lowest winter hardiness (1–3 points) was noted in 14 samples - 18.7%, the average winter hardiness (4 points) have 37 samples or

49.3% of the number of studied samples. The varieties Mertsishor and OxamitKryma were characterized by the highest winter hardiness - 5 points. As the results of the evaluation show, it is possible to select forms in the collection that are promising for creating winter-hardy varieties.

The range of variability of the average height of the samples over three years is quite large - 63.7 cm (115.4–179.1 cm) with an average height of 149.7 ± 1.5 cm in the collection. The main number of samples (51 out of 75 or 68.0%) - medium-sized (131–160 cm). This group also included the variety Mertsishor (on average, 145.2 ± 5.9 cm). Significantly fewer samples (17 or 22.7%) were characterized as tall (more than 161 cm). This group includes the variety OxamitKryma (on average, 160.9 ± 3.8 cm). Undersized specimens with a height of up to 130 cm are least present in the collection (7 or 9.3%).

The number of shoots of the 1st order is an indirect indicator of the potential productivity of *F. vulgare*, since they form the main productive inflorescences. The average number of shoots per plant varied from 5.0 to 8.6 pieces in the samples of the collection. Varieties of Mertsishor and OxamitKryma - 7.2 ± 0.3 and 7.8 ± 0.3 pcs. A sample from Iran with an average number of shoots - 8.4 ± 0.1 pcs, is notable.

For mechanized harvesting, it is important to know the height of the cut of plants. The study showed that the height of the lower inflorescence attachment in the collection samples is, on average, 77.5 ± 0.1 cm. At the same time, the range of variability of this indicator is quite high - from 57 to 106 cm, on average, over three years. When sampling for analysis, the plants of each sample were cut considering this indicator.

F. vulgare essential oil is obtained both from whole plants (in the phase of the end of flowering - the beginning of fruit formation) and from mature fruits.

The yield of green mass, on average, in the collection was 3.5 ± 0.1 kg from the accounting plot (0.6 m^2). 74 samples were evaluated by this trait. One, with low winter hardiness, did not form a generative sphere in 2017-2018 and was not evaluated in 2019 due to the lack of seed reserve. Fluctuations in the yield of green mass in the collection ranged from 1.6 to 6.7 kg per plot (V = 28.2%). The largest number of samples refers to medium-yielding - 38 (51.4%). Their harvest was 3.1–4.5 kg from the plot. This group includes both RIAC varieties - Mertsishor and OxamitKryma, with an average yield of 4.2 kg per plot. In the group of low-yielding (less than 3 kg per plot) - 28 samples (37.8%). The most interesting is the high-yielding group, which included 8 (10.8%) samples with an average yield of more than 4.6 kg from the plot (4.6–6.7 kg). Samples of this group can potentially serve as donors of high yield.

During the period of full maturation, the harvest was recorded in 67 samples that formed the fruits. Eight samples did not form fruit due to poor overwintering and poor plant development. The range of variability, on average for 2017–2019, was in the range from 5.2 to 108.1 g per plot. According to this indicator, all samples are divided into three groups: high-yielding (over 60 g per plot) - 14 samples out of 67 (20.9%), medium-yielding (30–60 g per plot) - 31 samples (46.3%) and low-yielding (less than 30 g per plot) - 22 samples (32.8%). Mertsishor variety with an average yield of 66.5 ± 20.5 g per plot it is included in the group of high-yielding, and the OxamitKryma variety - 50.1 ± 2.4 g per plot - in the group of medium-yielding samples. The high variability of the collection by this feature (V = 54.4%) and the presence of samples with a high yield of fruits exceeding the varieties Mertsishor and OxamitKryma indicate the prospects of working with the collection in order to create valuable breeding material.

The weight of 1,000 fruits (m_{1000}) varied quite widely in the collection, on average, over three years, from 2.0 to 5.4 g. The bulk of the samples (47 out of 67–70.1%) had medium-sized fruits (m_{1000} from 3.1 to 4.0 g). 9 out of 67 samples (13.4%) were assigned to the small-fruited group (m_{1000} up to 3.0 g). Large fruits (m_{1000} more than 4.0 g) were formed by 11 samples (16.5%). OxamitKryma and Mertsishor varieties form a fruit of medium size - 3.8 ± 0.1 and 4.1 ± 1.0 g, respectively. The weight of the fruits depends on both genetic determination and hydrothermal conditions during their formation. The smallest fruits were formed in extremely dry and hot conditions in 2018. It should be noted that there are quite stable samples by this feature, which remained belonging to the same size group for all three years: 12 from the group of medium-sized, 4 from the group of large-sized, and 2 from the group of small-sized.

The most important indicator of essential oil plants is the content of essential oil in the raw material. The mass fraction of essential oil in the whole plants of the collection samples of *F. vulgare* ranged quite widely - from 1.09 to 3.86% (of the absolutely dry mass). The average figure in the collection for three years was 2.32 ± 0.06%. For breeding purposes, samples with an essential oil content of less than 2.0% are not of interest. In this regard, all samples (74) of the collection are divided into three groups: low-oil, with a mass fraction of essential oil up to 2.0% - 20 samples (27.0%); medium-oil (2.1–3.0%), the main group - 48 (64.9%) samples and high-oil (more than 3%) - 6 (8.1%) samples. The most valuable are samples from the high-oil group, as donors of this trait. Both varieties - Mertsishor (2.32 ± 0.34%) and OxamitKryma (2.72 ± 0.03%) were included in the middle group.

The content of essential oil in mature fruits is much higher than in whole plants. This is primarily due to the fact that the massive stem, which occupies a significant share in the total mass of raw materials, practically does not contain glandular conceptacles in which essential oil accumulates, and, in fact, is a ballast. The essential oil content in the fruits of 65 samples was analyzed. (The remaining samples either did not form fruit or were not sufficient for analysis.) On average, over three years, the mass fraction of essential oil in the fruits of the studied samples was 5.92 ± 0.11% (of the absolutely dry mass) and was characterized by a fairly wide variability range - from 4.16 to 8.53%. Taking into account the value for breeding purposes, the samples are conditionally divided into three groups: low-oil (up to 5.5%) - 22 samples (33.8%), medium-oil (5.5–6.5%) - 29 samples (44.6%), and the most valuable for breeding - a group of high-oil (more than 6.5%) - 14 samples (21.5%). The Mertsishor variety (7.25 ± 1.46%) is included in the group of high oily specimens, and the OxamitKryma (6.30 ± 0.55%) variety is in the group of medium oily samples.

The main indicator of the productivity of essential oil crops varieties is the collection of essential oil. This is the resulting indicator, depending on the yield of raw materials and the content of essential oil in it.

The collection of essential oil during the processing of whole plants in the *F. vulgare* collection ranged, on average, from 6.2 to 34.3 g per plot. The average value in the collection is 18.3 ± 0.7 g per plot. Samples with the collection of essential oil of up to 15 g per plot, inclusive, formed a low-oil group (23 samples out of 74–31.1%). In 50.0% of the samples, the collection of essential oil was in the range of 15.1 to 25.0 g per plot. The high-oil group (from 25.1 g per plot) included 14 (18.9%) samples, including the varieties Mertsishor (25.1 ± 8.0 g per plot) and OxamitKryma (27.7 ± 10.1 g per plot).

The variability range of the collection indicator of essential oil from fruits in the collection was, on average, 0.4–6.3 g per plot, and the average indicator was 4.3 ± 1.0 g. The group with a low indicator (less than 1.5 g $plot^{-1}$) includes 16 samples - 24.6% of the number analyzed (65), the group with an average collection of essential oil (1.5–3.5 g $plot^{-1}$) - 33 samples (50.8%). The group with a high indicator (over 3.5 g $plot^{-1}$) consists of 16 samples (24.6%), including the Mertsishor and OxamitKryma varieties.

The collection of essential oil from whole plants is far superior to that of whole fruits.

The high variability of the samples in terms of essential oil collection (V = 31.9% when processing whole plants and 54.1% when processing fruits) indicates the prospects of working with the collection when creating varieties, providing a high collection of essential oil from a unit area.

The component composition of the essential oil depends on the processed raw materials. In this way, the content of anethole can reach 80% in essential oil from fruits, and when processing whole plants, the content of fenchone and terpene compounds is increased in essential oil. In this regard, the choice of raw materials for processing is determined by the subsequent use of essential oil.

In this way, the analysis of the *F. vulgare* collection showed its high variability in terms of the main productivity indexes, which is consistent with the conclusions of similar studies by a number of scientists, including the use of genetic markers (Maghsoudi Kelardashti et al., 2015; Hadli et al., 2021). Obtained results indicate the possibility of identifying sources of valuable traits in the collection in accordance with the breeding tasks.

The study was carried out on the basis of the RIAC collection of the gene pool of spicy-aromatic, essential oil and medicinal plants, registered in the Russian Federation as a unique scientific installation USI No. 507515 (http//www.ckp-rf.ru).

CONCLUSIONS

The analysis of the *F. vulgare* collection, which includes 75 samples of various origins (from 28 regions), has been carried out for a complex of morphobiological and economically valuable traits.

Wide variability of the collection samples for the studied parameters is shown (the variation coefficients are from 8.3 to 54.4%). The groups of samples that are promising for the creation of valuable source material in accordance with the selection tasks have been identified.

The results of the collection analysis are basic, for preliminary selection of samples with high indicators for inclusion in breeding studies.

The obtained characteristics of collection samples will be used to create a reference manual for researchers conducting breeding studies of *F. vulgare* and related species of the Apiaceae family - *A. vulgare* Gaertn. and *C. sativum* L., *A. graviolens* L., *Carum carvi* L., etc.

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