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STUDIES ON THE COMPARISON OF POLLEN MORPHOLOGY AND VIABILITY OF FOUR NATURALLY DISTRIBUTED AND COMMERCIAL VARIETIES OF *ANEMONE CORONARIA* L.

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Abstract

The present study presents a comparison of the pollen morphology and viability of naturally distributed four varieties of *Anemone coronaria* L. These are *A. coronaria* var. *coccinea* (Jord.) Burn, *A. coronaria* var. *rosea* (Hanry) Batt, *A. coronaria* var. *cyanea, A. coronaria* var. *alba* Goaty & Pens) and its commercial cultivars. The four varieties were collected from areas near the road side along the Kırkağaç-Soma highway in the State of Manisa. The commercial cultivars were obtained from the commercial flower growers in the Urla region of İzmir. Pollen viability levels decreased in all commercial cultivars of *A. coronaria*. The highest reduction in pollen viability was recorded in *A. coronaria* pink cultivars of de Caen group. The general pollen type is prolate spheroidal in all pure forms, but there are some pollen morphological features which were not observed in the natural ones, although encountered in all commercial cultivars. On the other hand, various non-viable pollen types like wrinkled pollens, with abnormally shaped pollens or pollinia were found in the commercial cultivars. It was concluded that pesticides used to produce more flowers with rapid growth are the majör cause for his reduction. Another reason could be the use of tetraploid F_1 hybrids of *A. coronaria* cultivars of de Caen group as commercial samples.

Key words: Pollen morphology, *Anemone coronaria*, Ranunculaceae, Commercial cultivars.

Introduction

Ranunculaceae includes seven genera in the tribe Anemoninae (subfam. Ranunculoideae). The *Anemone* L. genus and *Anemone coronaria* species has been cited in some earlier valuable studies (Boissier, 1867). Monographs of the genus *Anemone* have been published by Tamura (1968, 1991). However, the data presented has been considerably reviewed, as a result the distinguishing characters at each taxonomic level have been changed and extended (Ziman *et al.,* 2008). According to Ziman *et al.* (2008, 2011) the genus *Anemone* sensu stricto includes 15 subgenera, 23 sections, 4 subsections, 23 series and 118 species throughout the world. The worldwide distribution and considerable diversity in the morphology of this genus has lead to undertake studies on its chromosomes and genome (Heimburger, 1959; Darlington & Wylie, 1961; Hagemann *et al.*, 1993). On the other hand, pollen morphology of Iranian species of *Anemone* was studied (Baladehi *et al.*, 2013). This genus has 7 recorded species in Turkey (Davis, 1965). Some anatomical and morphological investigations have been carried out on *Anemone coronaria* in Turkey (Candan, 2001; Candan & Şık, 2006a,b; Candan, 2013).

A. coronaria known locally as 'Tulip of Manisa' is represented by four varieties in Turkey, based on the tepal colours (Davis, 1965). These are *A. coronaria* var. *coccinea* (Jord.) Burn (with scarlet flowers), *A. coronaria* var. *rosea* (Hanry) Batt (with pink flowers), *A. coronaria* var. *cyanea* (with violet-blue flowers), *A. coronaria* var. *alba* Goaty & Pens (with white flowers). *A. coronaria* is a multifunctional species, recently many cultivars have been largely selected for cut flower production. These cultivars are classified into the two major groups namely: 'De Caen' and 'St. Brigid'. The plants used in this study belong to the 'de Caen' group which are characterized by their long stem and single flower (Laura & Allavena, 2007).

Although no culture studies on this species have not been done in Turkey but, diploid, triploid and tetraploid hybrid cultivars of the species have been developed in other countries. The hybrid *A. coronaria* has bigger and more beautiful flowers than the natural one, former is thus much more prefered. The major hybrid productions of the *A. coronaria* are found in Holland, France, England, Italy, the USA and Israel. The cultivated rhizomes are imported from these countries to produce cut flowers in Turkey.

No study available on the comparison of pollen morphology and viability of four varieties of *A.coronaria* distributed naturally in Turkey (*A. coronaria* var. *coccinea A. coronaria* var. *rosea, A. coronaria* var. *cyanea, A. coronaria* var. *alba*) and its commercial cultivars. Therefore, the main objective of this study has been to compare the pollen morphology and viability of these four varieties distributed in naturally with its commercial cultivars.

Materials and Methods

The four varieties; *A. coronaria* var. *coccinea* (Jord.) Burn, *A. coronaria* var. *rosea* (Hanry) Batt, *A. coronaria* var. *cyanea*, *A. coronaria* var. *alba* Goaty & Pens; distributed naturally in Turkey were collected from areas alongside the road on the Kırkağaç-Soma highway of Manisa, in March 2011. The cultivars of the same species were obtained from the commercial flower growers in the Urla area of İzmir.

The cultivar group of *Anemone coronaria* L. from de Caen selections (scarlet, pink, violet-blue and white cultivars) was used. The shortening of the flowering period to just one year is enough to convince seed companies to invest in F_1 hybrid breeding, which carries with it both the important guarantee of uniformity and improved quality of the product (Laura & Allavena, 2007). In this study tetraploid F_1 hybrids were used as commercial forms. The 'Flora of Turkey' was used for identification of the plant specimens collected from their natural habitats (Davis, 1965).

Flower specimens from each groups were fixed in Carnoy solution, removed from Carnoy and then anthers taken from ripe floral buds with the help of a dissection needle were mounted on glycerine-gelatin-liquid safranine mixture (Wodehouse, 1965). A total of 1000 pollens from each groups were used for the determination of pollen shape. The investigations on the classification of pollens were followed according to Erdtman (1966). In addition, a total of 1000 pollens from each groups were used for the min-max measurement values of pollen equatorial length-width. These were made under a Leica ICC50 HD binocular light microscope by using a Leica Digital Camera.

To determine pollen viability, 1000 pollen grains from each group were counted under the light microscope, using TTC (a 2,3,5-triphenyl tetrazolium chloride) test (Norton, 1966). One drop of this solution was placed on slide, the pollens were spread by brush on the slide and a cover slip placed on it. Counting was made after the TTC application and it was divided into three groups based on staining density. Dark red stained pollens were referred to as viable, light red as semi-viable, and unstained as non-viable (Eti, 1991). Viable pollens in the natural group and non-viable pollens in the commercial group were photographed using a JEOL JSM-6060 Scanning Electron Microscope (SEM) (Nepi *et al.*, 1995; Giuseppe, 1999).

Results and Discussion

Ranunculaceae is a europalynous family. Pollen grains are (2)-3-(4) colpate, tricolporoidate, pantocolpate, pantoporate and pollen shapes ranges from oblate to spheroid (Erdtman, 1966). Our results revealed that the pollen type of all varieties is pantoporate(periporate) (Fig. 1a, Fig. 2 a, c and Fig. 3a, c). The pollen shape was prolate spheroidal.

The results of TTC test for pollen viability are presented in Table 1. As compared to the natural groups, the percentages of viable pollens is lower in the culture forms, whereas the percentages of semi-viable and non-viable pollens is higher in all commercial cultivars as compared to the natural groups. In contrast, various non-viable pollen types like wrinkled pollens ocur in commercial forms, with abnormally shaped pollens or pollinia of *A. coronaria* cultivars (Fig. 1b, Fig. 2b, d and Fig. 3b, d).

The species from the genus *Anemone* are cultivated in greenhouses for domestic consumption of cut flowers (Kamenetsky & Okubo, 2013). It is known that some diseases are easily caused under greenhouse conditions. The most common pathogens and diseases in the anemone cut flower production are *Pythium*, *Botrytis* and powdery mildew. Several fungicides are used fort his purpose. Several Aphid species and red spider species are considered to be the major pests of anemones. Some insecticides are also used against these pathogens. In the breeding of *A. coronaria* cultivars, for flowering purposes tubers are vernalised before planting (Ohkawa, 1987). One of the processes after vernalising is disinfection with fungicides (Laura & Allavena, 2007).

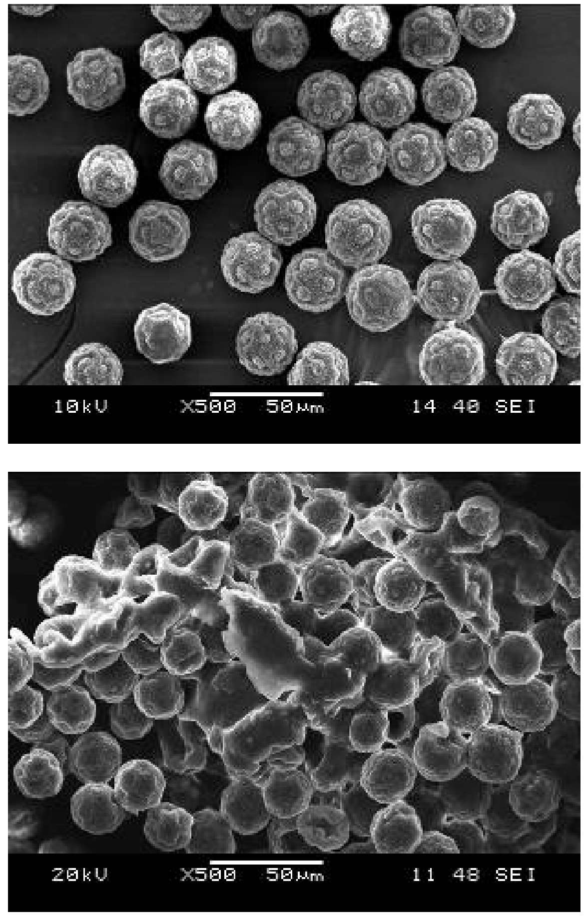

Fig. 1. SEM photographs **a.** General appearance of pollen in pure *Anemone coronaria* var. rosea (Hanry) Batt. **b.** Abnormally shaped pollens and some pollinia in commercial pink cultivars of de Caen group.

It is thought that the reason for the reduction in pollen viability belonging to commercial cultivars can be attributed to the use of these agricultural chemicals to produce many flowers and rapid growth. Different fungicide treatments have significant effects on the germination, the tube growth and pollen morphology. All these reduce the percentage of pollen germination, length of germ-tube elongation and morphological features of almond (*Prunus dulcis*) pollen (Zarrabi & Imani, 2011).

According to Öztürk & Candan (2010) the ACT-2; a K-vitamin group activator; treatment reduces the tomato (*Lycopersicon esculentum* Mill.) pollen viability. The germination and the tube length of olive pollen also got reduced when three insecticides (Dimethoate, Deltamethrine and Oleoparathion) were applied *In vitro* and *In vivo* to olive pollen culture mediums. The applications of the insecticide Oleoparathion are reported to have most severely reduced pollen germination, retarded germination, ruptured pollen tubes and damaged stigmatic surfaces *In vivo* (Mehri *et al.*, 2007). According to Öztürk (2008), the fungicide fosetly-Al causes non-viable pollens types, such as wrinkled pollens or pollens, with abnormal shapes. These were encountered in *L. esculentum* pollen. Moreover, pollen viability level decreases as the dosage increases.

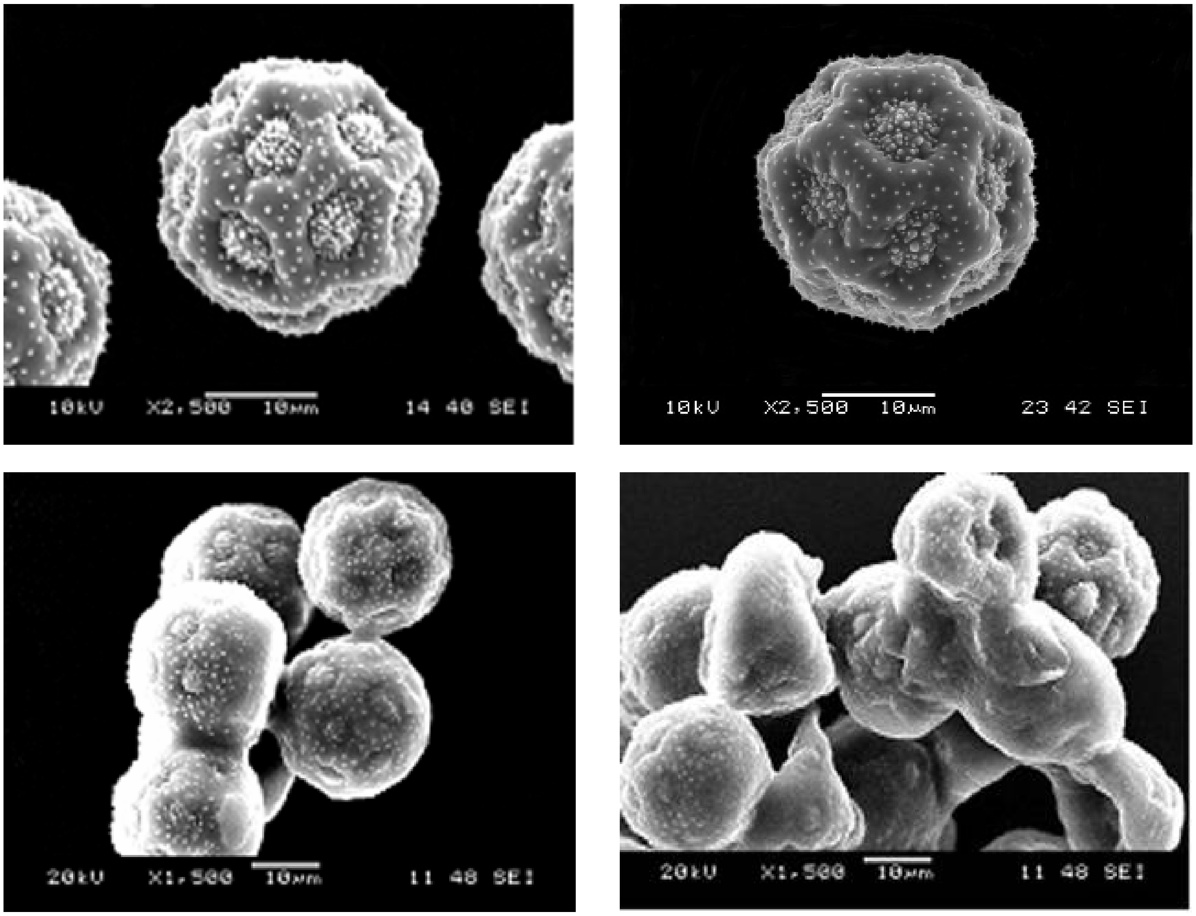

Fig. 2. SEM photographs **a.** General appearance of pollen in pure *A. coronaria* var. *coccinea* (Jord.) Burn. **b.** Pollinium in commercial *A. coronaria* scarlet cultivars of de Caen group **c.** General appearance of pollen in pure *Anemone coronaria* var. *rosea* (Hanry) Batt. **d.** Abnormally shaped pollens, wrinkled pollens and a pollinium in commercial *Anemone coronaria* pink cultivars of de Caen group.

Another reason for the reduction in pollen viability could be using hybrid forms of *A.coronaria* in the commercial cultivars to get big and beautiful flowers. Most modern *Anemone* cultivars have been derived either by selection from within old 'De Caen' types, or from crosses between 'De Caen' cultivars and selections from wild populations. 'Wicabri', 'Mona Lisa', 'Cristina', 'Jerusalem', 'Tetranemone', 'Mistral' and 'San Piran' are cultivated throughout Europe, USA and Israel and include a number of sub-cultivars distinguished by their flower colour. Cultivars bred for cut flower production are characterized by prostrate leaves and by long stemmed flowers (Laura & Allavena, 2007). The cultivar 'Tetranemone', bred from 'Wicabri' using this technology (Meynet, 1993), is characterised by greater flower stalk length and width, sepal thickness and flower size. Recently, Jacob *et al*., (1997) induced tetraploids in 'Mona Lisa' and compared 'Tetranemone', tetraploid 'Mona Lisa' and the 'Tetranemone' x tetraploid 'Mona Lisa' hybrid with their originating diploid cultivars. The hybrid maintained the tetraploid advantage, was as early flowering as 'Mona Lisa' and produced a comparable number of stems.

According to Bures *et al*. (2010), all hermaphrodite plants of the *Cirsium* hybrids have viable pollen, though generally at lower levels than those found in natural species. In the same study, it was found that the pollen viability of a hybrid generally decreases with increase in genetic distance. In another study, F_1 generation of hybrids created from *Erica ciliaris* and *Erica tetralix* had low pollen viability and high seed sterility (Gay, 1957). Using tetraploid F_1 hybrids of *A. coronaria* as commercial cultivars leads to decreased pollen viability levels.

When the results on min-max values of length-width measurements of pollens in equatorial view are evaluated, it is generally seen that the min measurement values decrease, whereas the max measurements increase (Table 2). Several studies have been carried out on detrimental effects of pesticides on the length-width measurements of pollens in equatorial and polar view (Öztürk & Candan, 2009a; Öztürk, 2008). Another reason could be the hybrid nature of the *Anemone* varieties. According to Hossain *et al*. (1990), pollen of the amphidiploid interspecific hybrids between *B. oleracea* var. *capitata* and *B. campestris* var. *pekinensis,* and between *B. campestris* var. *chinensis* and *B. oleracea* var. *capitata,* are significantly longer and wider than those of their diploid parents, presumably due to the phenotypic expression of the hybrid genomes. In the same study it was reported that the exine ridges and pores of the amphidiploids are well-developed and significantly larger than those of their diploid parents.

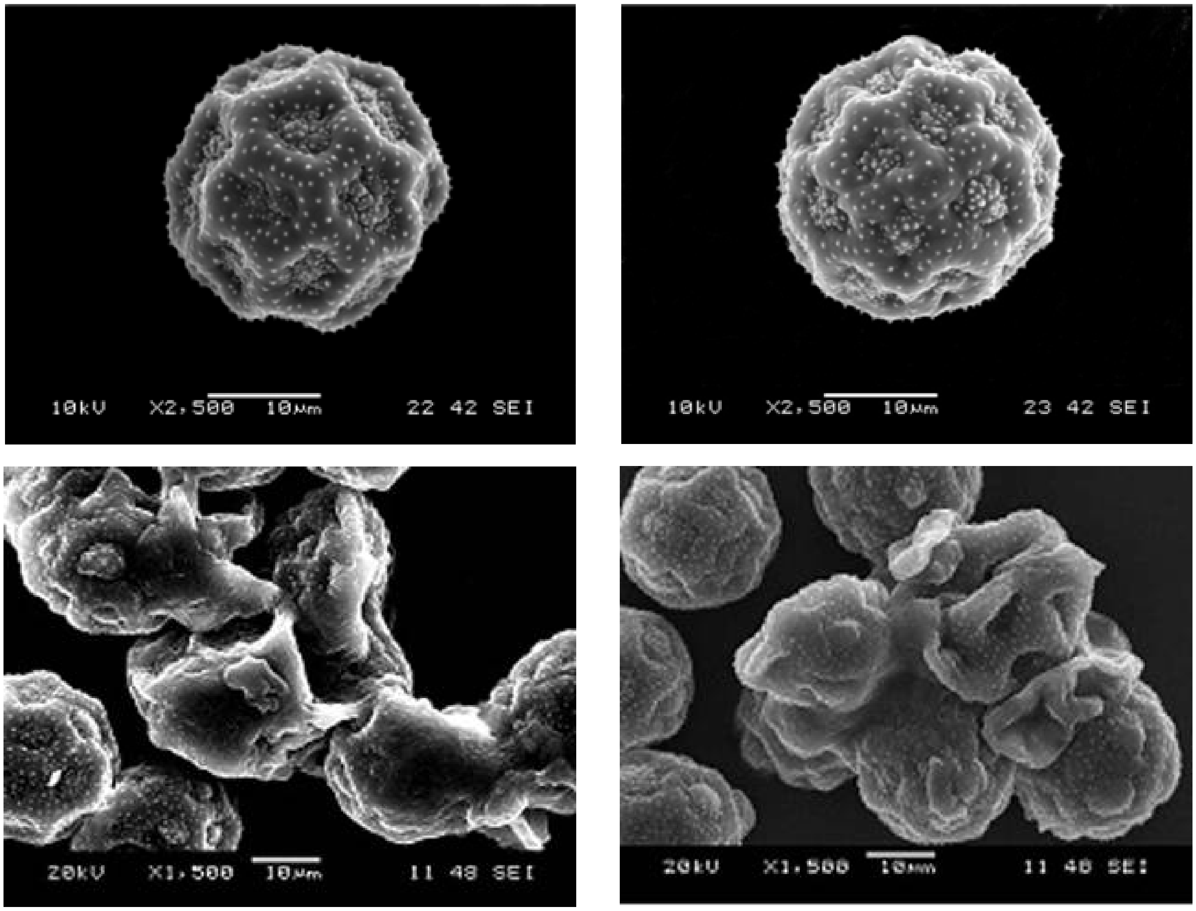

Fig. 3. **a.** General appearance of pollen in pure *Anemone coronaria* var. c*yanea*. **b.** Abnormally shaped pollens and a pollinium in commercial *Anemone coronaria* violet-blue cultivars of de Caen group. **c.** General appearance of pollen in pure *Anemone coronaria* var. *alba* Goaty & Pens. **d.** Abnormally shaped pollens, wrinkled pollens and a pollinium in commercial *Anemone coronaria* white cultivars of de Caen group.

Table 1. Pollen viability level in the pure and the commercial groups.

Groups	Pollen viability percentage (%)		
	Dark red (viable)	**Light red (semi-viable)**	**Unstained (non-viable)**
A. cor. var. *coccinea* (Pure)	94	5	1
A. cor. scarlet cultivars of de Caen group	69	18	13
A. cor. var. *rosea* (Pure)	96	3	1
A. cor. pink cultivars of de Caen group	67	24	9
A. cor. var. c*yanea* (Pure)	97	2	1
A. cor. violet-blue cultivars of de caen group	74	13	13
A. cor. var. *alba* (Pure)	95	3	2
A. cor. white cultivars of de Caen group	72	18	10

Table 2. Min-Max values of length-width measurements of pollens in equatorial view (µm).

Groups	Equatorial view	
	Width (µm) Min-Max	**Length (µm) Min-Max**
A. cor. var. *coccinea* (Pure)	26.0-30.0	26.5-30.2
A. cor. scarlet cultivars of de Caen group	27.5-37.5	7.5-37.5
A. cor. var. *rosea* (Pure)	30-35	30-35
A. cor. pink cultivars of de Caen group	27.5-37.5	27.5-37.5
A. cor. var. c*yanea* (Pure)	35.0- 37.5	35.0- 37.5
A. cor. violet-blue cultivars of de caen group	25.0– 37.5	27.5– 40.0
A. cor. var. *alba* (Pure)	28.4-34.0	28.6-34.5
A. cor. white cultivars of de Caen group	25 – 32.5	25 – 35.0

Table 3. Pollen shape classification in the pure and the commercial groups in the equatorial view.

Groups	Pollen viability percentage (%)		
	Oblate spheroidal %	Prolate spheroidal %	Subprolate %
A. cor. var. *coccinea* (Pure)	-	100	-
A. cor. scarlet cultivars of de Caen group	33.33	66.66	-
A. cor. var. *rosea* (Pure)	-	100	-
A. cor. pink cultivars of de Caen group	-	93.33	6.66
A. cor. var. c*yanea* (Pure)	-	100	-
A. cor. violet-blue cultivars of de caen group	6.66	93.33	-
A. cor. var. *alba* (Pure)	-	100	-
A. cor. white cultivars of de Caen group	6.66	93.33	-

A comparison of the pollen shape classification in the pure and commercial groups reveals that only prolate spheroidal pollen grains are observed in all natural groups, whereas oblate prolate and subprolate are determined with prolate spheroidal in the commercial groups (Table 3). This could be due to the use of fungicides, which cause some changes in pollen shapes. According to Öztürk & Candan (2009b), the fungicide Agri Fos 400 (Mono and di-potassium phosphanate) causes changes in the morphological features of tomato pollens. Some pollen morphological features not observed in the control group were encountered in the pollens due to the application of Agri Fos 400 in equatorial view at 8 ml/l and in polar view at 4 ml/l. In another study, the fungicide Equation Pro (22.5% Famoxadone + 30% Cymoxanil) has caused morphological deformity in *L. esculentum* pollen (Öztürk, 2009). Applying fungicides to commercial rhizomes has given rise to morphological changes on pollens of *A.coronaria* cultivars in the study. We also found that various non-viable pollen types like wrinkled pollens, abnormally shaped pollens or pollinia are found in the commercial cultivars.

In conclusion, a homogeneity in the pollen dimensions was observed between the natural forms of varieties in this species, but there were noteworthy differences in the pollen dimensions, the pollen viabilities and the pollen shape changes between commercial cultivars. The cause of all these differences is thought that pesticides are used to get more flowers and rapid growth during the cultivation of commercial forms. Another reason could be use of tetraploid F_1 hybrid *Anemone* forms to produced more beautiful and big flowers than the pure ones. Because of this, the hybrids are much more preferred than the pure ones.

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