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Comparison of Different *Pistacia* spp. in Terms of Pollination Biology in the Yunt Mountains of Manisa Province in Turkey

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Abstract: In this research conducted between 1999 and 2001 years, to use different *Pistacia* spp. (*P. vera*, *P. atlantica* and *P. terebinthus*) as source of pollen for pistachio types (Topan, Söbü, Çatlayan Kırmızı, Alyanak) in the Manisa-Yunt Mountain area of Turkey was investigated. The average percentages of pollen viability in different *Pistacia* spp. of three years ranged between 57.51 and 85.00% by IKI and TTC tests. *P. atlantica* had the highest pollen viability percentage in both two viability tests. In germination tests of three years, medium type of *P. terebinthus*, *P. vera* and *P. atlantica* pollen were active in viability in 20% sucrose medium caused higher germination than other media. The usage of different *Pistacia* spp. pollens resulted with higher fruit set than natural pollination in three years of this investigation. In general, the best fruit set was obtained in *P. vera* used pollinizers for pistachio types.

Key words: *Pistacia* spp., pistachio, pollination biology, fruit set

INTRODUCTION

Pollination and fertilization is of greater importance than the other fruits in relation to fruit set in nuts which seed is consumed like pistachio. As it is known, pollination in *Pistacia* spp. is mediated by wind. All *Pistacia* species are dioecious, male and female flowers are located on separate trees. The blooming period of male and female flowers do not overlap. Male flowers generally bloom earlier than females (Protandry). In modern pistachio nut growing, approximately one male tree is needed for 8-11 females (Crane and Iwakiri, 1980; Ak, 1998; Isfendiyaroglu *et al*., 2001; Kafkas *et al*., 2001). In case of insufficient pollination, blank nut forms in which only pericarps develop. Since the yield is reduced, this creates an economic problem. Therefore, ensuring good pollination is crucial to a good harvest in pistachio culture (Kaşka, 1995).

The pistachio is a native crop to Western Asia and Asia minor, where still found growing wild in Turkey, Syria, Iran, Iraq, India, Lebanon, Palestine, Southern Europe, Asia and Africa (Emeksiz and Şengül, 2001). Although the main region for pistachio is Southeast Anatolian region, some microclimates are also located in Turkey. One of these areas is the Yunt Mountains in Manisa province of Ege region in Turkey. The pistachio production of the Ege Region has long been suffering from an insufficient pollination with not enough male trees in the orchards. Since the orchards in the Yunt Mountain area are established by top working the naturally grown *Pistacia atlantica* and *Pistacia terebinthus* rootstocks, they are not in proper orchard design. These orchards are pollinated by the male *P. atlantica* and *P. terebinthus* trees scattered on the neighbouring fields. Therefore, pollination varies from year to year and from orchard to orchard, leading high production of blank nuts (Çağlar and Kaşka, 1995; Isfendiyaroglu *et al*., 2001).

Although, the available male *P. vera* can be used as pollinator for female pistachio trees in the Yunt Mountain, *P. vera* pollinators usually bloom earlier than female cultivars. Therefore, they are not considered as good pollinizers in this area and other pistachio areas of the country as well. It has been reported that in some countries such as Syria Tunisia, Iran, artificial pollination gives good result when natural pollination is not sufficient (Çağlar and Kaşka, 1995). It was demonstrated artificial pollination could be as much effective as natural pollination (Ayfer and Kuru, 1990). However, it is necessary to know germination ability and viability of pollen to apply this technique successfully. For this purpose, different viability and germination tests are carried out *in vitro* conditions (Therios *et al*., 1985; Ak, 1992; Atlı *et al*., 1995).

Any investigation on pollinizer selection has not been done in this area up to now. Consequently, this investigation was planned to determine and compare the usage of different *Pistacia* spp. which are available in large amounts in that area, source of pollen for pistachio cultivars.

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MATERIALS AND METHODS

This investigation was carried out during 1999 and 2001 in pistachio and *Pistacia* spp. trees of the Yunt Mountain area of Ege region in Turkey and in the laboratories of the Department of Horticulture, Faculty of Agriculture, University of Ege, Turkey.

Materials: *P. atlantica*, *P. terebinthus*, male and female types of *P. vera* placed in the Yunt Mountain of Manisa province were used as plant material for this investigation. Female pistachio types were named locally. Topan (White-I), Söbü (White-II), Çatlayan Kırmızı (Red-I) and Alyanak (Red-II) were used as female pistachio types in this study.

Methods

Phenological observations: Male *Pistacia* species and female pistachio types were observed to determine flowering dates during three years (Kuru, 1984).

Pollen sources: Pollination were performed with the pollen collected male trees *P. vera*, *P. atlantica* and types of *P. terebinthus*.

Pollen collection: The branches carrying staminate clusters were collected from male *Pistacia* spp. trees mentioned above, just prior to pollen shedding time and pollens were put into glass vials. They were kept in a deep freezer at -18°C until used (Ak, 1992).

Pollen evaluation: All the pollens which were used for this study were evaluated by the following tests:

- Viability tests were done with 2, 3, 5 Triphenyl Tetrazolium Chloride (TTC) and Iodine in Potassium Iodide (IKI) methods (Eti, 1991).
- Germination tests were done with Agar-Pethri method *in vitro* conditions. As germination media, 10, 15 and 20% concentrations of sucrose were tested. All pollen germination tests were performed in 20-25°C for 4-5 h.

The viability and germination tests were arranged four replications.

Controlled pollination: In the selected two female trees belong to each types, 5 branches on each at the 4 directions and 3 clusters on each branch were isolated by using bags. In addition to bagged female flower clusters, some clusters marked for open pollination. The pollen were not mixed with any carrier and they were sprayed by means of a hand duster (Ak, 2001).

In the hybridization combinations (from obtained controlled pollination which different *Pistacia* spp. were used as pollinizers for female pistachios) and open pollination, fruit set ratios were determined.

Statistical analysis: All the data obtained from this study were analysed statistically by using SPSS statistical programme.

RESULTS AND DISCUSSION

Phenological observations: Phenological observations were carried out on male and female trees in the Yunt Mountain area during three years (1999, 2000 and 2001). The average dates determined in relation to beginning of flowering, full flowering and end of flowering in male and female trees are shown in Table 1.

According to the data, the flowering period of male trees did not fully coincide with the flowering of female trees and male trees flowered before females in three years. These differences occurred in flowering period of male and female trees were in parallel with the results of other researchers (Ayfer, 1959; Vargas *et al*., 1995).

Pollen viability tests: The pollen viability percentages of different *Pistacia* spp. used as pollinizer were determined by using TTC and IKI staining tests in this study.

The average percentages of pollen viability in *P. vera*, *P. atlantica* and *P. terebinthus* (early, medium and late types) of three years (1999, 2000 and 2001) by IKI viability test were shown in Fig. 1. Pollen viability percentages differed significantly among *Pistacia* species ($p<0.05$). As it was seen in Fig. 1, *P. atlantica* ranked the first row (85.00%) and this species was followed by *P. terebinthus* (early) (84.94%). *P. terebinthus* (late type) possessed the lowest value (61.46%). The percentage of viability was determined as 68.61% in *P. vera* (Fig. 1).

The results on pollen viability obtained by TTC test were similar to IKI test (Fig. 2). Statistical differences were determined among the species ($p<0.05$).The highest viability was obtained in *P. atlantica* (71.73%).Two types of *P. terebinthus* (medium and early) ranked the 2nd and 3rd row, respectively. Among the types, late type of *P. terebinthus* had the lowest pollen viability percentage in both IKI and TTC test. The lowest viability was found as 57.51% in *P. vera* (Fig. 2).

The results in Fig. 1 and 2 showed that the viability percentages of different *Pistacia* spp. pollen in IKI test were higher than TTC test. No investigation has been performed by using IKI test in *Pistacia* spp. up to now. Whereas, in TTC tests, Ak (1992) stated that the viability

Table 1: Phenological observations of male and female trees

Pollinizer (Male)	Beginning of flowering	Full flowering	End of flowering
P. vera	First week of April	Second week of April	Third week of April
P. atlantica	First week of April	Second week of April	Third week of April
P. terebinthus (E)	First week of April	Second week of April	Third week of April
P. terebinthus (M)	Second week of April	Third week of April	Last week of April
P. terebinthus (L)	Third week of April	Last week of April	First week of May
Type (Female)			
Topan	Third week of April	Last week of April	First week of May
Söbü	Third week of April	Last week of April	First week of May
Alyanak	Last week of April	First week of May	Second week of May
Çatlayan Kırmızı	Third week of April	Last week of April	First week of May

E: early, M: medium, L: late types of *P. terebinthus*

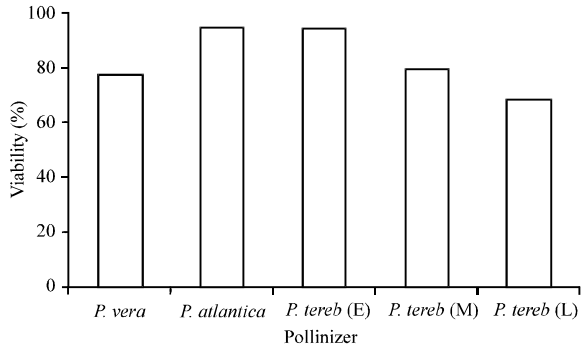

Fig.1: Average percentages of pollen viability in *Pistacia* spp. by IKI test

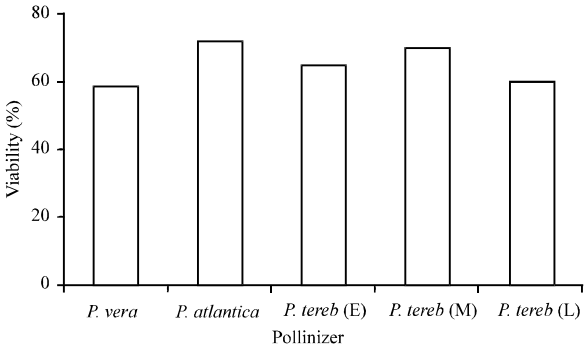

Fig. 2: Average percentages of pollen viability in *Pistacia* spp. by TTC test

percentages of *P. vera*, *P. atlantica* and *P. terebinthus* pollen were 94.57, 80.39, 82.78% in 1989 and were 90.30, 79.56, 74.67% in 1990 in Ceylanpınar State Farm (Southeast Anatolia), respectively. Additionaly, the viability percentage of pollen stored for a certain period dropped to 74.67% in *P. atlantica* and to 62.73% in *P. terebinthus*.

In the Yunt Mountain area, percentages of pollen viability in different *Pistacia* spp. were lower than those mentioned above. This could be due to different ecological conditions between two regions. As a matter of fact, the viability percentages were changed 22.36-49.20% in *P. vera*, 36.00-46.00% in a type of *P. terebinthus* and

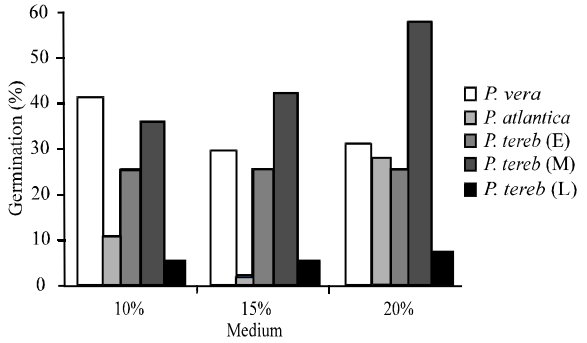

Fig. 3: The average pollen germination percentages of *Pistacia* spp. with different sucrose concentrations by agar-pethri method

3.33 -61.95% in another type of *P. terebinthus* according to the years at low altitudes of the Taurus Mountains of İçel province in a previous investigation (Çağlar and Kaşka, 1995). Besides, the pollinizer trees are markedly lack of several nutrients in pistachio orchards and the trees of *P. terebinthus* are generally grown as bushes in the Yunt Mountain area. However, due to the high pollen viabilities of these species in IKI test, their pollen could be considered as functional for fertilization.

Pollen germination tests: The statistical analysis of the data obtained from germination tests showed that the differences among *Pistacia* spp. in terms of average germination percentages of three years ($p<0.05$) (Fig. 3).

In 10% sucrose medium, *P. vera* ranked first row with 37.87%. This species was followed by the medium type of *P. terebinthus* (34.92%) and early type of *P. terebinthus* (25.00%). Both of them were in the same statistical group. The lowest germination was obtained from the late type of *P. terebinthus* (5.22%) (Fig. 3). Similarly, *P. atlantica* gave less germination (1.90%) than other types in 15% sucrose medium. In the same medium, the highest germination was found in medium type of *P. terebinthus* (41.18%). *P. vera* (28.49%) and early type of *P. terebinthus* (22.46%) took place in the same group (Fig. 3). In 20% sucrose medium, the highest and lowest values were obtained from medium

type of *P. terebinthus* (56.37%) and late type of *P. terebinthus* (9.16%), respectively. *P. vera* (30.54%) and *P. atlantica* (27.17%) were located in the same statistical group (Fig. 3).

Similarly, the highest germination was seen in all investigated media with medium type of *P. terebinthus*. This species was followed by *P. vera* and *P. atlantica*. 20% sucrose medium caused higher germination than other media. According to the results of germination tests of three years, medium type of *P. terebinthus*, *P. vera* and *P. atlantica* pollen were active in viability. As a matter of fact, Dokuzouz (1957) reported that if pollen germination percentages were 30% or more, they had sufficient activity in fertilization biology.

The average germination percentages may be found to be lower than those in some previous Works (Crane *et al*., 1974; Ak, 1992, 1998). However, it was determined that the germination percentages were rather low in early types of *Pistacia* (Rashed *et al*., 1995).

In this investigation, the germination percentages of pollen were generally low but these values were higher than 30% in some media with some *Pistacia* spp. So, these pollen could be considered as functional for fertilization (Dokuzouz, 1957). Crane *et al*. (1974) and Ak (1992) also reported that pollen viability and germination percentage could be changed by species, types and years.

The fruit set: As a result of three years' statistical analysis on the average fruit set ratio in this study, it was found that there were statistical differences among pistachio types and different *Pistacia* spp. used as pollinizer and open pollination ($p<0.05$).

When *P. vera* pollen was used, the highest fruit set value was obtained as 22.15%. This species was followed by *P. atlantica* (18.51%). The lowest fruit set values were also obtained in open pollination (14.17%) and *P. terebinthus* (13.44%) (Fig. 4). In all evaluations on the ratio of fruit set in this study, *P. vera* was the best pollinizer for all pistachio types. In artificial pollination, it was necessary to use early type of *P. vera*. It has been reported that early type of *P. vera* pollen keep viability for a long time (Polito and Luza, 1988). Thus, usage of *P. vera* pollen in artificial pollination can increase the ratio of fruit set. Similar findings have also been reported by Crane and Iwakiri (1980) and Ak (1992).

When the pistachio types compared based on the fruit set, the highest fruit set occurred in Topan type (22.30%). Söbü type (19.69%) ranked the second row. Çatlayan Kırmızı type exhibited the lowest fruit set

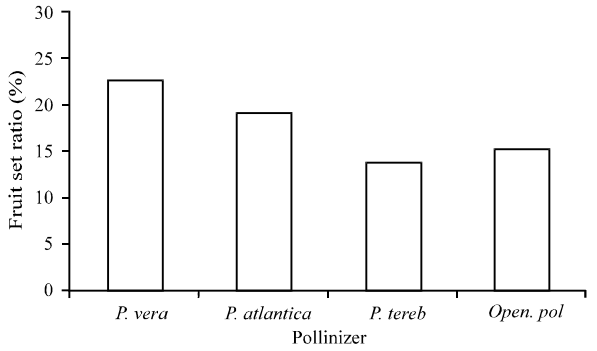

Fig. 4: Fruit set ratio in *Pistacia* spp.

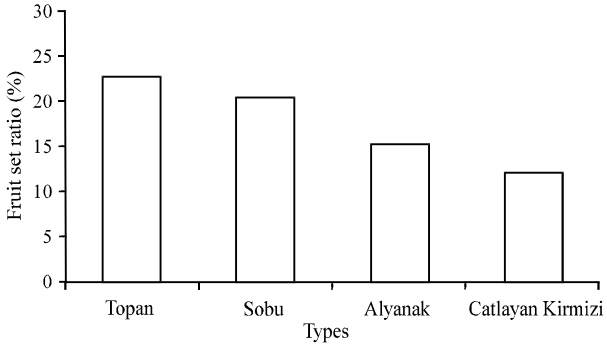

Fig. 5: Fruit set ratio in different pistachio types

(11.95%) (Fig. 5). The fruit set ratios of White types (Topan and Söbü) were higher than Red types (Alyanak and Çatlayan Kırmızı) in this study. Ak (1992) determined that ratios of fruit set in Kırmızı, Ohadi, Vahidi and Bilgen cultivars were 21.20, 19.00 and 17.00%, respectively in the artificial pollinated trees.

The results obtained from this experiment were paralled with the findings reported by Ak (1992). But, the values of fruit set were lower in Yunt Mountain area than the values in Ceylanpınar State Farm (Urfa). This situation may be due to the differences of ecological conditions in Yunt Mountain area from Ceylanpınar's ecological conditions.

The effect of type×pollinizer interaction on the average fruit set ratio was also found to be significant during three years of this study ($p<0.05$).

In hybridization combinations which different *Pistacia* spp. were used as pollinizers for pistachio types, the best fruit set was obtained in Topan×*P. vera* combination (28.00%). This combination was followed by Topan×*P. atlantica* (27.50%) and Söbü×*P. vera* (25.43%). The lowest fruit set occurred in Alyanak×*P. terebinthus* (11.50%) and open pollination of Çatlayan Kırmızı (10.20%) and (Fig. 6).

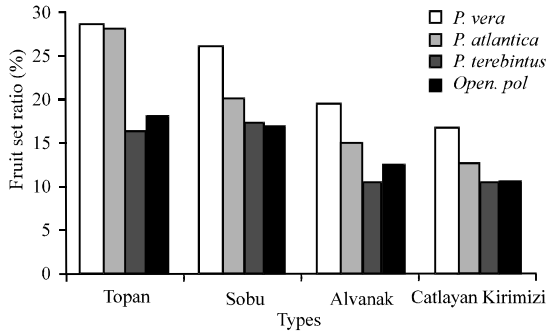

Fig. 6: Fruit set ratio in different hybridization combinations

The usage of different *Pistacia* species pollens resulted with higher fruit set than open pollination in three years of this study. Çağlar and Kaşka (1995) stated that ratios of fruit set were 12.47 and 6.06% by pollination with pure pistachio pollen and open pollination, respectively. In a previous research, the fruit sets in pollination with *P. vera*, *P. terebinthus* and open pollination were determined as 14.2, 13 and 9.1%, respectively (Ayfer, 1959).

Based on the results of this experiment, it is suggested that growers in Yunt Mountain area should plant the pollinizer trees overlapped the flowering period with female trees and the artificial pollination should be applied in their orchard. Especially *P. vera* and *P. atlantica* pollen should be used and their pollen should not be stored in this area. Artificial pollination should be performed at the beginning of blooming period.

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