Journal of Nuts 16(1) (2025) 1-13

Journal of Nuts

Journal homepage: sanad.iau.ir/journal/ijnrs/

ORIGINAL ARTICLE

Effect of Dormex® Application on Flowering Time and Vegetative and Reproductive Characteristics of Akbari Pistachio Cultivar

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K E Y W O R D S

Chilling requirements;
Hydrogen cyanimide;
Pistacia vera;
Volk oil

A B S T R A C T

In recent years, global warming and unusually warm winters have caused many problems for pistachio production in Iran. The effects of the Dormex® application were studied in a randomized complete block design with three treatments and three replications on the Akbari commercial pistachio cultivar in Kerman province. Treatments included Dormex® 4%, Volk oil 4%, and control, all applied in mid-February. Flowering time, gum secretion, current branch length, and qualitative and quantitative yield characteristics were measured. Results showed that Volk oil and Dormex® spring treatments caused early flowering in the Akbari cultivar. The Dormex® 4% treatment significantly prolonged flowering time (8 days earlier than Volk oil 4%, and 12 days earlier than the control). Based on the results, Volk oil treatment increased gum secretion from buds and perennial shoots compared to the Dormex® treatment and control. The results of yield indices showed that the weight of clusters in the Dormex® 4% treatment was 2.3 times higher than the control and about 21% more than the Volk oil 4% treatment, showing a significant difference with the control. Dormex® and Volk oil treatments increased split nut percentage by 66 % and 88%, respectively, but there was no significant difference between them. Based on the results, the blank nut percentage in the Dormex® 4% treatment significantly increased compared to the control and Volk oil treatment by 33% and 41%, respectively. The length of current season growth in the Dormex® 4% treatment significantly increased by 68% compared to the control. The Volk oil 4% treatment had no significant effect on the length of current season growth compared to the control.

Introduction

Agriculture is a vital part of the non-oil economy in Iran. Pistachios have a particular importance in agricultural production and cultivated areas. In recent decades, global warming and climate change have caused many problems in the farming sector (Vahdati *et al.*, 2019). The most important consequences of these climate changes in pistachio orchards are increasing temperatures, decreasing rainfall, and changes in distribution. Pistachio (*Pistacia vera* L.) is a semi-tropical and deciduous plant with a chilling requirement that must be fulfilled during winter and is cultivated in arid and semi-arid areas (Eslami *et al.*, 2019; Norozi *et al.*, 2019; Alipour, 2018). In additon, with suitable conditions, the natural growth and flowering of the buds could occur in spring (Romberger, 1963; Sharifkhah *et al.*, 2020). The suitable temperature range for chilling requirements is between 0 and 7°C, and the chilling unit needs for

***Corresponding author**: Email address: atajabadi@yahoo.com
Received: 29 July 2023; Received in revised form: 2 March 2024; Accepted: 1 June 2024
DOI: 10.60680/jon.2024.1240

optimal growth vary from 600 to 1400 depending on the cultivar type (Arora *et al*., 2003; Javanshah *et al*., 2005; Aslamarz *et al.*, 2009). Among the commercial pistachio cultivars of Iran, Kaleh-ghouchi has the least chilling requirement at around 600 hours, while the Akbari cultivar has the highest chilling requirement at 1200 hours, and Ahmad-Aghaei and Ohadi cultivars fall between them at 750 and 900 hours, respectively (Javanshah *et al*., 2005). During the last decade, unusually warm winters in Kerman province, especially in the Rafsanjan region, have caused problems for pistachio cultivars with high chilling requirements, such as Akbari. After insufficient chilling requirements of pistachio cultivars, adverse effects occur, such as a reduction in internode growth, number of leaflets, leaf area, pollen production, an increase in abnormal leaf percentage, bud drop, delay in flowering, and leafing times, low fruit set even in heavy years, and ultimately a reduction in yield (Javanshah *et al*., 2005; Javanshah and Ismailzadeh, 2004; Elloumi *et al*., 2013). Hydrogen cyanamide (formulated as Dormex®) is a plant growth regulator used to advance budburst and flowering, and has been widely used on various deciduous crops (Ghrab and Mimoun, 2014; Fayek *et al*., 2008). There are many reports of its positive effects, including increasing the percentage of bud stagnation in pistachios, grapes, peaches, nectarines, kiwis, cherries, apples, and pears (Bound and Jones, 2004; Godini *et al*., 2008; Clements *et al*., 2010; Ghrab & Mimoun, 2014). Ghrab & Mimoun (2014) tested only one application time and reported that a very early application 45 days before the expected bud break resulted in a significant advancement of bud break in pistachio trees. Müller (2008) found that Dormex® 4% had a positive effect on bud break in pistachio trees. Rahemi & Asghari (2004) showed that Dormex® 2% and 4% significantly increased the percentage of bud break compared to the control, with no difference between treatments; however, the 4% treatment had a slightly higher bud break percentage. The same result reported by Veloso *et al*. (2003) on kiwis. Based on their results, both Dormex® 4% and 6% had a significant increase in bud break, but Dormex® 6% only resulted in a slight increase in bud break compared to the 4% treatment.

At the end of dormancy, which is a period of suspended growth and metabolic activity, a portion of the starch reserves stored in the plant is hydrolyzed (broken down) into soluble sugars (Gholizadeh *et al.*, 2017). This process is mediated by starch-degrading enzymes that become active in response to chilling temperatures (Klotke *et al*., 2004). Dormex® triggers the hydrolysis of starch reserves into soluble sugars to enter the pentose phosphate cycle, providing the necessary energy to break the stagnation.

Dormex works by altering herbal mechanisms, including the expression of genes related to day length perception, increasing specific stresses caused by free radicals such as hydrogen peroxide and superoxide, and adjusting biochemical compounds related to sleep and wakefulness mechanisms. It also affects the assessment of biochemical conditions. It increases the use of plant hormones like abscisic acid, ethylene, jasmonic acid, and cytokinin, as well as nitrogen and amino acids to accelerate and regulate the flowering process (Cooke and Weih, 2005; Yamane *et al*., 2011; Sudawan *et al*., 2016; Ionescu *et al*., 2017).

To initiate bud break, the activity of respiratory enzymes is crucial. However, free radicals can hinder enzyme activity. Dormex® has been shown to increase glutathione regeneration in flower buds of fruit trees like apples and peaches, eliminating free radicals and promoting the breakdown of stagnation (Asghari, 2011). Glutathione also impacts polysome activity, increasing enzyme synthesis necessary for breaking stagnation. Other effects of Dormex® include increasing ion leakage in plums, involvement in sulfur compounds in kiwi, and enhancing cytokinins containing zeatin, calcium, and magnesium in apple juice. By utilizing (applying or exposing the plant to) ethylene, the concentration or availability of the cyanamide ion can be increased. This, in turn, can accelerate the process of breaking the plant's dormancy or stagnation (Doizier *et al*., 1990; Fmetto, 1997; Powell, 2000).

Given the internal effects on plants, Dormex® appears to be a viable alternative to Volk oil when buds emerge from dormancy amid temperature fluctuations (Tohbe *et al*., 1999; Godini *et al*., 2008). With global warming and climate change impacting pistachio cultivation in Iran, the excessive use of mineral oil (Volk) to meet chilling requirements has both positive and negative effects. Negative effects include increased dieback and gum secretion from tree trunks in some years.

Considering the economic significance and sustainability of pistachio production in Iran, it is crucial to pay attention to climatic conditions for cultivation. Due to insufficient cold for chilling requirements of certain pistachio cultivars, like the Akbari cultivar, many orchards have suffered significant yield losses. Research on alternative compounds to mineral oil (Volk) with improved efficiency in pistachio orchards is essential. This study aims to investigate the impact of Dormex® on bud break and yield of pistachio trees.

Materials and Methods

This experiment was conducted in a completely randomized block design (CRBD) with three replications in mid-February on bearing pistachio trees of the Akbari cultivar in Kerman province. The selected orchard of Akbari cultivar was located in the Bayaz area of Anar city in Kerman province. The applied treatments included: 1) Dormex® 4%, 2) mineral oil (Volk) 4%, and 3) control (water spraying). After allocating the treatments to experimental units randomly, each treatment was sprayed on three rows of 50 meters (rows as repetitions) in mid-February. The total chilling hours accumulated by mid-February was 675 hours.

In this study, criteria such as flowering time, the opening time of 5% of the flowers as the beginning of flowering, the opening time of 50% of flowers as full flower, and the opening time of 100% of the flowers as the end of flowering were set. The number of days from the beginning to the end of flowering was considered as the length of the flowering period. The percentage of reproductive buds turned into flowers and fruits, and the time of leafing were measured.

At the time of harvesting from each replication, 20 clusters were randomly selected from different parts of the tree. The total weight of the cluster, axis, and weight of nuts of the cluster were measured. The average number of pistachios in clusters was calculated. After peeling and drying, the dry weight of pistachio was measured with a scale, and the average dry weight of pistachio per cluster was calculated.

To determine the percentage of split nuts, non-split nuts with whole kernels, half kernels, and blanks in each cluster, the number of these pistachios in the cluster was counted. The split nut percentage and blank nut percentage were calculated as a percentage of the total number of pistachios in the cluster. The number of split nuts in one ounce (28.3 grams) was counted in five replications for each treatment. The length and diameter of the current year’s branches and the number of flower buds were measured

The outflow of vascular sap from the branches and the main trunk, and the dieback of the trees for the treatments were evaluated by observation. Statistical analysis of data was done using Mstatc software, and mean comparison was done using Duncan's multi-range test at the 5% level.

Results

Flowering time

The results of flowering time in different treatments showed that Volk oil and Dormex® treatments caused early flowering of the Akbari pistachio cultivar. Spraying Dormex® at a 4% solution significantly advanced the flowering time. In general, the beginning of flowering in this treatment was 8 days earlier than the Volk oil at 4% treatment and 12 days earlier than the control (Fig. 1). The state of flowering is given in Table 1.

Fig. 1. Comparison of the flowering time of Akbari pistachio cultivar with Dormex® t 4%, (left) Volk oil 4% (middle) treatments and control (right).

Table 1. The effect of Volk oil and Dormex® treatments on the flowering time on Akbari pistachio cultivar.

Treatment	Bud swelling	Begin of flowering	Full flowering	End of flowering
Control	Apr.2	Apr.7	Apr.10	Apr.15
Volk oil 4%	Mar. 28	Apr.3	Apr.8	Apr.12
Dormex® 4%	Mar. 21	Mar. 26	Mar. 29	Apr.1

Gum secretion from branches

Observational studies indicated that the use of Volk oil increased gum secretion from buds, branches, and perennial branches, with the highest secretion observed in the Volk oil at 4% treatment. The control and Dormex® at 4% treatment had the lowest gum secretion.

Fresh cluster weight

In terms of fresh cluster weight, the highest weight was observed in the Dormex® at 4% treatment, showing a significant difference compared to the Volk oil at 4% treatment and the control (Fig. 2).

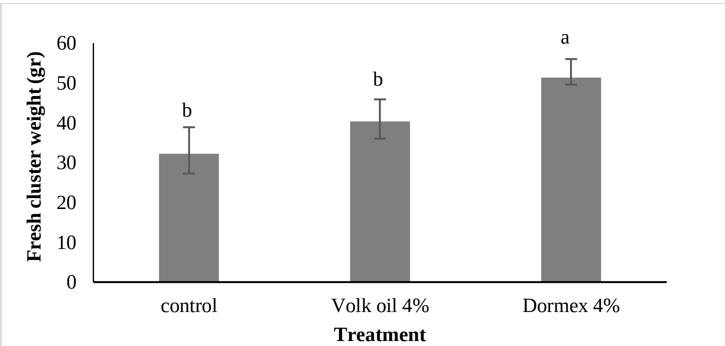

Fig. 2. The weight of pistachio cluster (g) in control, Volk oil and Dormex® treatments
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

Weight of dry pistachio in cluster

The investigation into the weight of dry pistachios in the cluster revealed that the Dormex® at 4% treatment had the highest weight, significantly differing from the control. There was no significant difference between the Volk oil at 4% and Dormex® at 4% treatments in this regard (Fig.3).

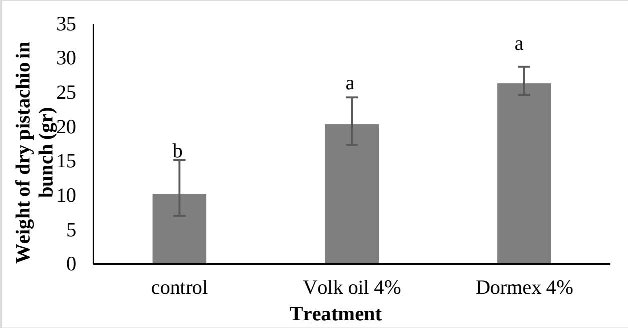

Fig. 3. The weight of dry pistachio in the cluster(g) in control, Volk oil and Dormex® spraied treatments
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

Number of nuts in cluster

The mean comparison of the number of nuts in the cluster showed that the Dormex® at 4% treatment had the highest number of nuts, significantly differing from the control. There was no significant difference between the Volk oil at 4% and Dormex® at 4% treatments in this aspect (Fig. 4).

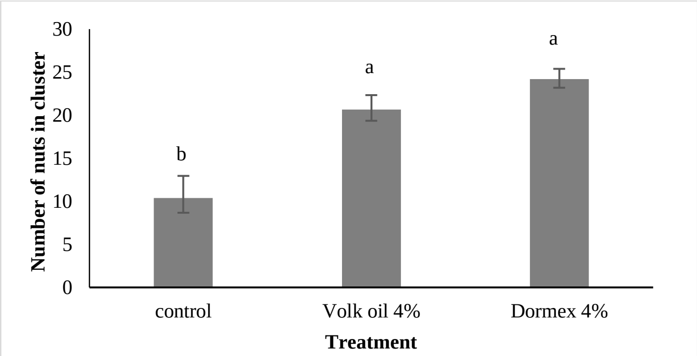

Fig. 4. The number of nuts in cluster in control, Volk oil and Dormex® spraied treatments
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

The weight of split nuts in cluster

When comparing the weight of split nuts in different treatments, the Volk oil at 4% treatment had the highest amount, significantly differing from the control. However, there was no significant difference between the Volk oil and Dormex® treatments in this trait (Fig. 5).

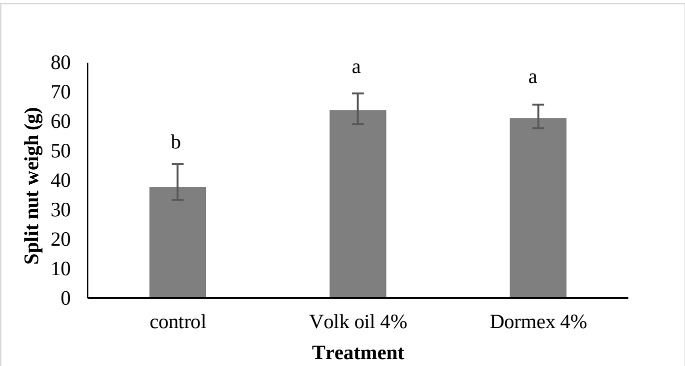

Fig 5. The weight of split nut (g) in control, Volk oil and Dormex® spraied treatments
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

Split nut percentage

The split nut percentage significantly increased in the Akbari cultivar with the Volk oil at 4% and Dormex® at 4% treatments compared to the control. There was no significant difference between the Volk oil and Dormex® treatments, with the highest percentage of split nuts, 88%, observed in the Volk oil at 4% treatment (Fig. 6).

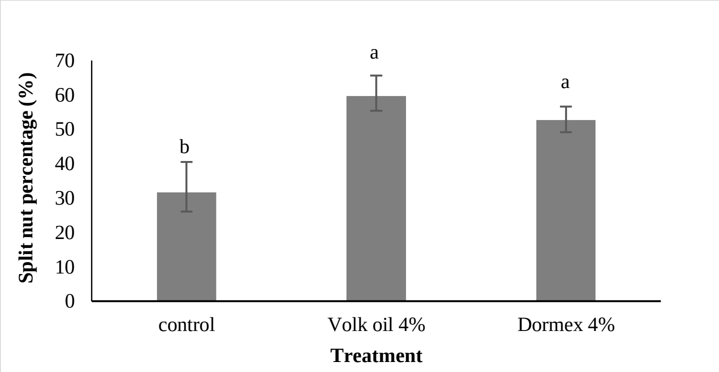

Fig 6. The percentage of split nut in control, Volk oil and Dormex® spraied treatments
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

Non-split nut percentage

The results of the percentage of non-split nuts (with whole kernels) in the studied treatments showed that the highest percentage belonged to the control group, which had a significant difference compared to the Volk oil 4% and Dormex® 4% treatments. There was no significant difference between the Volk oil and Dormex® treatments in terms of this trait (Fig. 7).

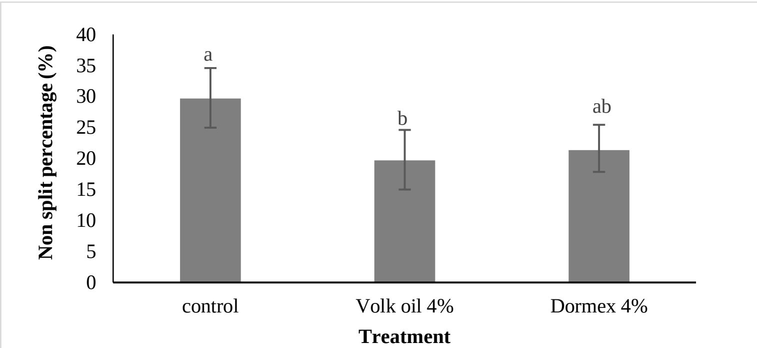

Fig. 7. The percentage of non-split nuts in the control, Volk oil and Dormex® spray treatments
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

Blank nut percentage

The results of the percentage of blank nuts showed a significant difference between the Volk oil and Dormex® treatments, as well as the control group, with the highest percentage of blank nuts observed in the Dormex® treatment (Fig. 8).

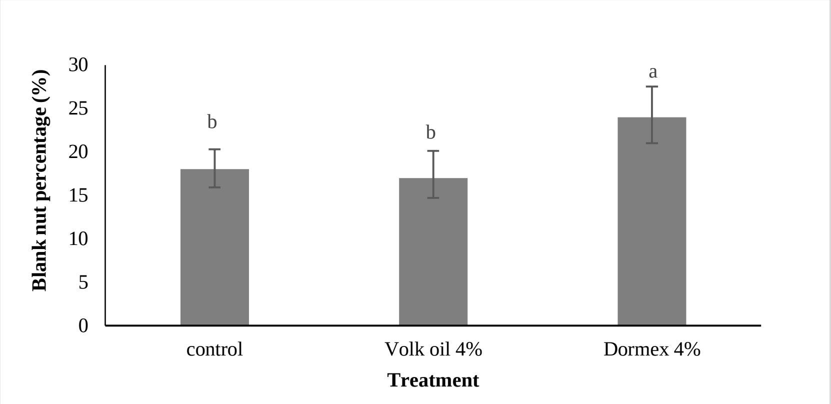

Fig 8. Blank nut percentage in control, Volk oil and Dormex® spraied treatments
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

The percentage of half kernel pistachios

The results of the percentage of pistachios with half kernels showed that there was no significant difference between the Volk oil and Dormex® treatments, as well as the control group, in terms of this trait (Table 2).

Ounce in split nut and mixed pistachio

The mean comparison showed that the ounce (number of nuts per 28.3 g) was not affected by the Volk oil 4% and Dormex® 4% treatments, and no significant difference was observed in terms of this trait compared to the control group (Table 2).

Table 2. The effect of Volk and Dormex® treatments on some quality traits of Akbari cultivar.

Treatment	Half kernal percentage	Ounce in mixed nut	Ounce in split nut
Control	8.33 a	29.2 a	23.2 a
Volk oil 4%	3.67 a	29.2 a	25 a
Dormex® 4%	4.33 a	29.93 a	24.67 a

Current season growth length

The length of current season growth in different treatments showed that the highest branch growth belonged to the Dormex® treatment, which had a significant difference compared to the control group but not compared to the mineral oil (Volk) treatment (Fig.9).

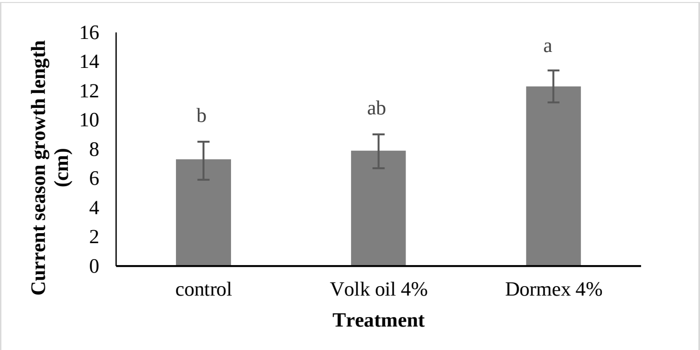

Fig. 9. The branch length in the control, Volk and Dormex® spray treatments
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

Current season growth diameter

The results of branch diameter showed that there was no significant difference in terms of branch diameter growth between different treatments (Fig.10).

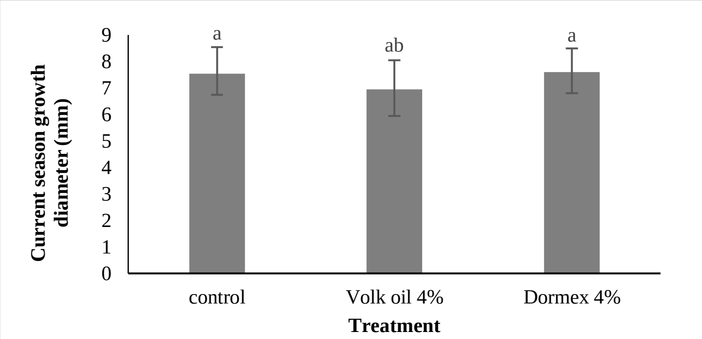

Fig. 10. The branch diameter in the control, Volk and Dormex® spray treatments
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

The number of flower buds

The number of flower buds on the branch of the current year in different treatments showed that there was no significant difference in the number of flower buds between different treatments. However, the highest number of flower buds was observed in the Dormex® treatment and control group (Fig. 11).

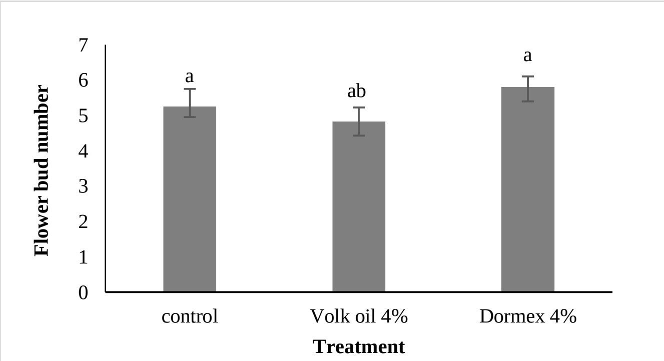

Fig. 11. The number of flower buds on the branch in the control, Volk oil and Dormex® spray treatments
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

Flower bud drop percentage

The flower bud drop percentage on the branch of the current year showed that there was no significant difference in terms of this trait between the Volk oil and Dormex® treatments, but these differences were significant compared to the control group (Fig. 12).

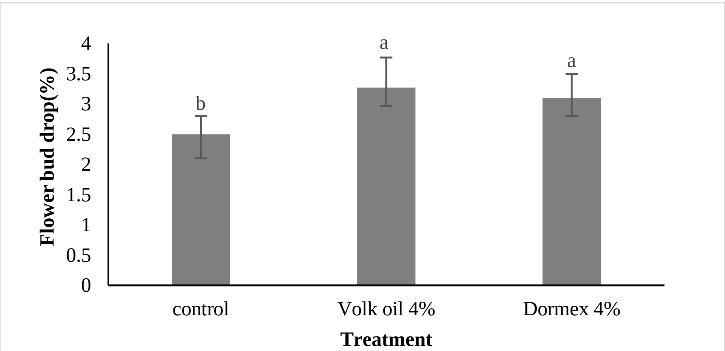

Fig 12. The flower buds drop percentage in control, Volk oil and Dormex® spraied treatments
(Means followed by same letter are not significantly different at 5% probability using Duncan's test)

Discussion

The lack of sufficient chilling hours in the Akbari pistachio cultivar resulted in a delay in flowering and a significant decrease in yield in the control group. In pistachio trees that did not receive the necessary cold, buds bloomed late and pollen production in most male inflorescences was greatly reduced. Additionally, many inflorescences may become sterile and drop off. Female flower buds also appeared weak and had low attachment to the branch. Even if they were pollinated with suitable pollen, they would drop, leading to a significant reduction in fruit set and yield (Javanshah and Ismailizadeh, 2004; Javanshah, 2005; Asghari, 2004).

Spraying Dormex® at a 4% concentration significantly advanced the flowering time compared to the control and Volk oil at a 4% concentration. Cyanamide compounds, used in the forms of calcium cyanamide and hydrogen cyanamide (Dormex®), have shown beneficial effects on fruit trees like grapes, pistachios, kiwis, pecans, etc. (Doizier *et al*., 1990; Finetto, 1997; Powell, 1999; Asghari, 2011). These results were consistent with sources indicating that Dormex® was more effective in overcoming bud stagnation (Pin, 2012).

Dormex® inhibits the activity of the catalase enzyme, which plays a crucial role in plant metabolism by breaking down toxic substances resulting from the plant's cellular activities into hydrogen oxide. When catalase is active, hydrogen peroxide accumulates, allowing the cycle of glycolysis to proceed through the pentose phosphate pathway. This activation provides the necessary materials for sprouting and the conditions needed to overcome stagnation. Excessive enzyme activity is necessary to break stagnation, but free radicals in the cell can affect enzyme activity (Zhang *et al*., 2018).

Reports have shown that Dormex® increases the regeneration of glutathione, an antioxidant substance, in the flower buds of fruit trees in temperate regions such as apples and peaches. This process removes free radicals and helps overcome stagnation. Glutathione also affects the activity of polysomes, leading to increased synthesis of proteins and enzymes needed to break stagnation (Doizier *et al*., 1990; Finetto, 1997; Powell, 1999; Asghari, 2011).

Volk oil caused early flowering in the Akbari cultivar. Volk oil and compounds like di nitro ortho cresol, di nitro phenol, and di nitro cyclo hexyl phenyl have had positive effects on deciduous trees like apples and pistachios (Procopiou, 1973; Beede and Padillia, 1999; Kuden *et al*., 1995; Asghari, 2011).

Yield indicators showed that the pistachio cluster weight in the Dormex® 4% treatment was higher than in the control and Volk 4% treatment. Both Dormex® and Volk oil treatments increased the split nut percentage by 66% and 88%, respectively, compared to the control. This suggests that while chemical treatments may cause early overcoming of the stagnation period and more successful pollination of Akbari trees in the first season, the lack of cold requirement can still lead to disorder in the vegetative and reproductive structure, reduced pollen production, excessive bud drop, and ultimately decreased yield (Talaei *et al*., 2009).

Other research has shown that using Volk oil prevents oxygen from entering the bud by creating an oxygen-impermeable layer. Reports indicate that a lack of oxygen can cause stagnation in peach leaf buds and apple embryos to break, with the impact varying based on the amount of oxygen reduction and the duration of Volk oil stability. In response, the plant increases its metabolism to break down the oil, leading to early bud growth. Higher concentrations and longer durations of oil application result in more successful breaking of stagnation (Procopiou, 1973; Beede and Padillia, 1999; Kuden *et al*., 1995; Asghari, 2011).

The use of Volk oil increases gum secretion from buds, branches, and perennial branches. Volk oil, a petroleum-based product, can have negative side effects with prolonged use. Some orchards treated with high concentrations of Volk oil have experienced branch dieback, trunk color changes, and enlarged lenses.

The percentage of blank nuts significantly increased in the Dormex® 4% treatment compared to the control and Volk oil 4% treatment, by 33% and 41%, respectively. Early flowering in the Akbari cultivar due to Dormex® spraying led to an inconsistency in flowering times between male and female trees. This lack of synchronized pollen availability during flowering caused pollination and fertilization issues, resulting in an increase in blank nuts in Dormex®-treated trees. Additionally, the high yield from Dormex treatment and synchronized nutrition times led to some nuts receiving inadequate nutrition, resulting in embryo abortion.

In conclusion, spraying Dormex® 4% caused earlier flowering in the Akbari cultivar, with reduced gum secretion compared to other treatments. The effects of Dormex® on flowering in off-year pistachio trees were less pronounced compared to on-year trees.

Conclusions

Spraying Dormex® 4% caused earlier flowering in Akbari cultivar. Gum secretion was much less visible in Dormex® treatment. The effects of Dormex® on flowering in off year pistachio trees are less compared to on year trees.

Acknowledgements

We acknowledge the assistance and cooperation received from the Pistachio Research Center.

Conflict of Interest

The authors declare no conflicts of interest.

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