

## Research Article

## Possible role of nitric oxide in budbreak in pistachio (*Pistaciavera* L.): A novel method

Zahra Pakkish\*, Soheila Mohammadrezakhani and Hadi Asghari

Department of Horticultural Sciences, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran

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### Abstract

In this study, one product derived from sodium nitroprusside (SNP) was used as a nitric oxide (NO) donor and evaluated as a stimulant bud break agent of Ahmad-Aghaei pistachio. Trees were treated by SNP 0, 0.30, 0.60, 0.90 and 1.20 mM in two stages (4 and 8 weeks before normal budbreak (February 1 and January 1, respectively)) with a factorial experiment in a randomized complete block design with four replications. Results showed that SNP treatment hastened the bud break date and increased the yield. SNP decreased the misshapen nuts and empty and non-splitting shells compared with the control treatment. In both ON and OFF years, the effect of SNP was greatly dependent on both times of application and concentrations used. Results showed that exogenous application of this compound, especially during the second application, increased splitting shell nut, number of nuts per cluster, number of nuts per ounce, leaf area, shoot length, and shoot diameter in both ON and OFF years. No limitations were encountered. The results of this study might apply to pistachio and temperate fruit-growing regions with a mild winter. Because in mild winter, pistachio trees' chilling requirements are not resolved, and this substance helps with this deficiency.

**Keywords:** Full bloom, Chilling requirement, Growth, Yield

### Introduction

Pistachio (*Pistaciavera* L.) is an important crop grown in arid and semi-arid regions (Eslami *et al.*, 2019). Pistachio, as an essential economic product, has a special place among Iranian agricultural products and constitutes a significant part of the country's non-oil exports. "Pistachio trees," like other fruit trees in temperate regions, need a cold period in their annual growth cycle, after which the natural flowering of the buds occurs with suitable conditions for growth. Climate plays a vital role in the successful production and sale of nuts products in world trade (Bhatti *et al.*, 2006).

Dormancy in plants has been described as a state in which visible growth is temporarily suspended (Samish and Lavee, 1962), a phase in plant development allowing it to survive under winter conditions (Saure, 1985) and a state in which deciduous plants are without leaf or are lacking visible growth (Nazoor *et al.*, 2008).

Crane and Takeda (1979) reported that, pollen production by much male inflorescence was inferior, and neither floral nor vegetative buds of the pollinator had begun to expand when the 'Kerman variety' was in full bloom, i.e., about the mid-April. These pistachio trees produced inadequately developed leaflets and were left with a reduced number of leaflets following

inadequate winter chilling in California (Crane and Takeda, 1979).

The molecular mechanisms of dormancy are unknown. Still, since this phenomenon coincides with the reduction of cell division, therefore, dormancy control should be related to the mechanisms that regulate the cell cycle that are involved with hormonal signals. It is stated that SNP (sodium nitroprusside) is a releasing compound of nitric oxide in plants. Evidence indicates that NO is involved in several cellular processes, such as growth, development, metabolism, respiration, death, maturity and response to biotic and abiotic stresses (Zhao *et al.*, 2004).

Researchers rarely use NO gas and usually release compounds that produce NO after passing through the membrane inside the cell. One of the most common NO releasers is sodium nitroprusside (SNP), which is relatively inexpensive and available and releases NO at intracellular pH (Neile *et al.*, 2003). Nitric oxide, as a highly active and reactive molecule effective in dormancy breaking, can interact with a large group of different molecules (Giba *et al.*, 2007).

Nitric oxide is one of the molecules that have recently received much attention from plant researchers (Fan *et al.*, 2012). Nitric oxide is a gaseous and diffuse radical produced endogenously in plants. Nitric not only

\*Corresponding Author, Email: smohammadrezakhani@yahoo.com

travels in hydrophilic regions of the cell, such as the cytoplasm, but also diffuses freely through the lipid phase of membranes (Arasimowicz-Jelonek *et al.*, 2009). There are many reports of various roles of nitric oxides, such as induction of seed germination, regulation of metabolism in aging, and response to biotic and abiotic stresses (Kopyra and Gwozdz, 2004). Examination of the sources showed that there was no report of the effect of SNP on bud break in pistachio trees and other trees. This study is the first report of the Different concentrations of SNP on dormancy breaking and bud growth in pistachio trees. Therefore, we aimed to compare the effects of different concentrations of sodium nitroprusside (SNP), a nitric oxide donor, on dormancy breaking and bud growth in pistachio trees in subtropical regions of Iran.

### Materials and methods

**Orchard management:** This research was conducted in 2019 and 2020 on mature 'Ahmad-Aghaei pistachio trees, in a commercial orchard in Orzoeieh, southwest of the Kerman province of Iran. This region generally receives below 610 h of chilling below 7°C annually. Pistachio trees require 700-1000 h of temperature at 7°C during winter (Crane and Iwakiri, 1981). The 20-year-old trees were grafted on 'Badami-e-Zarand' rootstock at a spacing of 5×5 m<sup>2</sup> and drip irrigation system, soil fertilization and pest and weed control. Scheduling was followed during the experiment. Trees were sprayed with sodium nitroprusside (SNP) solution at 0, 0.30, 0.60, 0.90 and 1.20 mM at the dormant bud stage. These treatments were applied separately at two stages: 4 and 8 weeks before normal budbreak (February 1 and January 1, respectively); Trees treated all the plants twice; Some plants received it in February and the rest in January. This experiment was conducted in two consecutive years (2019 and 2020).

**Determination of chilling hours:** Temperature was recorded with a Watch Dog Leaf Wetness and Temperature logger (Spectrum Technologies, Inc.), with an accuracy of ±0.7°C for 60-min intervals in two consecutive years (2019 and 2020). According to the Utah model, effective chill units were 240 units, or 610 h below 7°C (Alonso *et al.*, 2005)."

**Measurements:** The number of dormant buds at the end of winter was recorded on each treated branch. Budbreak was defined as the date when the green tip-forth new shoot was visible. Budbreak (%) = (No. of breaking buds/No. of dormant buds)×100

After separating the nuts from each cluster, the number of nuts per ounce was calculated. Nuts without hulls were dried and weighed to calculate the number of nuts per ounce. The yield was calculated by weighing the total dried split nuts separated from each shoot. Leaf area from fully expanded leaves was measured by a digital leaf area meter (ADC, Hoddeston, UK). The length and diameter of current-year shoots were measured at harvest time. At harvest time, the number of nodes was recorded for both years of the experiment.

To calculate the number of branches formed, the number of branches formed in the current year was counted on the treated branches. To calculate the percentage of empty fruits, one hundred fruits were randomly selected, and then the number of empty fruits was checked.

To calculate the percentage of indehisced fruits: One hundred fruits were randomly selected, and then the number of indehisced fruits was checked.

To calculate the percentage of dehiscent fruits: One hundred fruits were randomly selected, and then the number of dehisced fruits was checked.

To calculate the percentage of misshapen fruits: One hundred fruits were randomly selected and then the number of misshapen fruits was checked.

**Statistical analysis:** The experimental design was a factorial randomized complete block with four replicates; The replicate of each treatment was applied to one tree with five shoots. The analyses of variance were performed using the procedure of SAS (SAS Institute Inc., Cary, NC, USA). Means were separated by Duncan's multiple range test (P <0.05).

### Results

The results of the average comparison show that in both ON and OFF years, the measured traits were significant at the statistical level of 1 and 5% (Tables 1-4).

Table 5 shows that the foliar application of SNP treatment in both ON and OFF years greatly influenced the bud break and shoot formation of Ahmad Aghaeie's pistachio. However, the addition of SNP in both ON and OFF years, especially in stage 2, increased the budbreak (90.26%) and shoot formation (63.26%). The results of foliar application of SNP on pistachios of Ahmad Aghaeie cultivar in two steps show that the highest bud break and node formation were observed in trees treated with SNP 0.90 mM and the lowest rate was observed in control trees in both ON and OFF years (Table 5).

When Ahmad Aghaeie's pistachio trees were treated with SNP, the lowest amount of blank nuts (8.23%), in dehiscent shell (9.41%) and misshapen nuts (6.65%) in both stages are related to SNP 0.90 mM, and the highest is connected to control trees in both ON and OFF years. Comparison of the time of application of the treatment shows that in the first stage, the highest amount of blank nuts, indehisced shell, misshapen nuts compared to the second stage is observed. In addition, the alleviation effect of SNP 0.90 mM was detected best compared with other treatment in both ON and OFF years (Table 6).

Dehisced shell nut, no. of nuts per cluster, no. of nuts per ounce and yield by SNP treatment increased compared with control in both ON and OFF years. The lowest level of Dehisced shell nut in both stages is related to control and the highest is related to SNP 0.90 mM both ON and OFF years. A comparison of the application time of SNP shows that the maximum value of Dehisced shell nut in the second stage is related to SNP 0.90 mM treatment in both ON and OFF years.

**Table 1. Analysis of variance of nitric oxide on quality and quantity characteristics of pistachio (OFF)**

Variation Sources	Degree Free	Mean of Squares					
		Budbreak (%)	Shoot formation (%)	Blank nuts (%)	Indehisced shell (%)	Misshapen nuts (%)	Dehisced shell nut (%)
Block	3	0.02*	32.15*	21.04*	11253.0*	1854.25*	3215.02*
Treatment	4	0.06*	40.22*	29.18*	13258.01*	5214.23*	721.05*
Time	1	0.09*	29.11*	44.01*	2581.12*	1589.32*	328.07*
Treatment × Time	4	0.11*	33.40*	25.43*	1965.29*	1458.62*	35.18*
Error	14	0.012	1.31	1.09	2.28	10.22	31.02
cv	-	5.28	3.18	5.13	9.27	3.28	9.91

P = 5% significant probability level\*, P = 1% significant probability level\*\*, ns = not significant

**Table 2. Analysis of variance of nitric oxide on quality and quantity characteristics of pistachio (OFF)**

Variation Sources	Degree Free	Mean of Squares					
		No. of nuts per cluster	No. of nuts per gram	Yield per shoot (g)	Leaf area (cm <sup>2</sup> )	Shoot length (cm)	Shoot diameter (mm)
Block	3	0.01*	1.08*	178.12*	1.02*	6.38*	6.38*
Treatment	4	1.01*	1.01*	298.23*	1.04*	8.21*	7.41*
Time	1	0.150*	0.150*	364.21*	1.12*	19.25*	13.28*
Treatment × Time	4	0.08*	0.08*	65.28*	0.33*	0.35*	1.09*
Error	14	0.018	0.018	35.01	0.005	0.14	19.34
cv	-	5.39	5.39	5.98	6.37	11.28	8.05

P = 5% significant probability level\*, P = 1% significant probability level\*\*, ns = not significant

**Table 3. Analysis of variance of nitric oxide on quality and quantity characteristics of pistachio (ON)**

Variation Sources	Degree Free	Mean of Squares					
		Budbreak (%)	Shoot formation (%)	Blank nuts (%)	Indehisced shell (%)	Misshapen nuts (%)	Dehisced shell nut (%)
Block	3	0.01*	41.02*	23.14*	1258.12*	2154.01*	235.17*
Treatment	4	0.05*	51.02*	38.21*	14001*	7030.01*	602.74*
Time	1	0.15*	36.24*	57.01*	3574.02*	2871.1*	258.51*
Treatment × Time	4	0.01*	47.12*	27.39*	2048.01*	1850.11*	36.12*
Error	14	0.005	0.25	0.89	3.11	12.01	25.14
cv	-	4.11	3.18	4.02	1.40	7.62	11.31

P = 5% significant probability level\*, P = 1% significant probability level\*\*, ns = not significant

**Table 4. Analysis of variance of nitric oxide on quality and quantity characteristics of pistachio (ON)**

Variation Sources	Degree Free	Mean of Squares					
		No. of nuts per cluster	No. of nuts per gram	Yield per shoot (g)	Leaf area (cm <sup>2</sup> ) <sup>a</sup>	Shoot length (cm)	Shoot diameter (mm)
Block	3	1.05*	0.01*	125.08	1.28*	4.32*	5.29*
Treatment	4	1.06*	0.05*	247.89*	1.54*	7.12*	6.38*
Time	1	0.295*	0.02*	344.12*	0.99*	19.4*	11.44*
Treatment × Time	4	0.52*	0.01*	81.26*	0.27*	0.85*	1.24*
Error	14	0.005	0.005	27.01	0.001	0.04	11.01
cv	-	7.41	9.35	4.28	5.98	9.46	4.28

P = 5% significant probability level\*, P = 1% significant probability level\*\*, ns = not significant

The effect of different concentrations of SNP 0.90 mM in the two stages of foliar application showed that the highest number of nuts per cluster was observed in trees treated with SNP 0.90 mM in the second stage. The lowest number of nuts per ounce was seen in the second stage and in fruits treated with SNP 0.90 mM and the highest in control fruits in the first stage in both ON and OFF years. The highest Yield per shoot (g) is in fruits treated with SNP 0.90 mM and in the second stage in both ON and OFF years (Table 7).

The highest leaf area, stem length and stem diameter were observed in trees treated with SNP in the second

stage of foliar spraying and the lowest in control trees in both on and off years (Table 8).

## Discussion

The higher the concentration and the more appropriate the application time, the greater the effect of the material. Results of the current study showed that the effects of Nitric oxide application in different concentrations and stages are significantly different from the control treatment. Treatment of nitric oxide increased the budbreak and also hastened the yield in pistachio trees. Sodium nitroprusside (SNP) releases

**Table 5. Effects of nitric oxide treatment on budbreak and shoot formation of ‘Ahmad Aghaeie’ pistachio in both ON and OFF years**

Treatment	Budbreak <sup>a</sup> (%)		Shoot formation (%)	
	Stage 1 <sup>b</sup>	Stage 2	Stage 1	Stage 2
On year				
Control <sup>c</sup>	60.21±0.15 <sup>f</sup>	60.01±0.17 <sup>f</sup>	31.11±0.11 <sup>f</sup>	31.18±0.14 <sup>f</sup>
SNP 0.30 mM	68.21±0.1b <sup>e</sup>	73.23±0.14 <sup>d</sup>	39.21±0.0b <sup>e</sup>	41.23±0.10 <sup>d</sup>
SNP 0.60 mM	73.21±0.15 <sup>d</sup>	80.25±0.10 <sup>c</sup>	42.21±0.13 <sup>d</sup>	52.25±0.12 <sup>c</sup>
SNP 0.90 mM	86.24±0.21 <sup>b</sup>	90.26±0.21 <sup>a</sup>	57.24±0.12 <sup>b</sup>	63.26±0.15 <sup>a</sup>
SNP 1.20 mM	78.11±0.21 <sup>c</sup>	85.42±0.24 <sup>b</sup>	47.11±0.01 <sup>c</sup>	56.42±0.21 <sup>b</sup>
Off year				
Control	48.01±0.01 <sup>f</sup>	49.23±0.01 <sup>f</sup>	22.13±0.01 <sup>f</sup>	23.21±0.02 <sup>f</sup>
SNP 0.30 mM	61.14±0.11 <sup>be</sup>	68.41±0.19 <sup>cd</sup>	30.18±0.11 <sup>be</sup>	31.08±0.31 <sup>ed</sup>
SNP 0.60 mM	66.11±0.01 <sup>d</sup>	73.18±0.11 <sup>c</sup>	34.17±0.02 <sup>d</sup>	41.33±0.22 <sup>c</sup>
SNP 0.90 mM	77.30±0.20 <sup>b</sup>	83.38±0.27 <sup>a</sup>	50.04±0.10 <sup>b</sup>	57.41±0.04 <sup>a</sup>
SNP 1.20 mM	69.12±0.10 <sup>c</sup>	78.21±0.16 <sup>b</sup>	38.16±0.01 <sup>c</sup>	48.64±0.17 <sup>b</sup>

<sup>a</sup> value are means ± SE. <sup>b</sup> Trees were sprayed at the dormant bud. These treatments were applied separately at two stages: (4 and 8 weeks before average bud break (February 1 and January 1, respectively)). <sup>c</sup> Colum means followed by the same letter are not significantly different ( $P < 0.05$ ) according to the Duncan's multiple range test.

**Table 6. Effects of nitric oxide treatment on blank nuts, in dehiscent shell, and misshapen nuts of ‘Ahmad Aghaeie’ pistachio in both ON and OFF years**

Treatment	Blank nuts (%) <sup>a</sup>		Indehiscent shell (%)		Misshapen nuts (%)	
	Stage 1 <sup>b</sup>	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2
On year						
Control <sup>c</sup>	24.16±1.38 <sup>a</sup>	24.25±2.16 <sup>a</sup>	28.02±1.17 <sup>a</sup>	28.25±1.14 <sup>a</sup>	18.27±2.08 <sup>a</sup>	17.32±1.24 <sup>a</sup>
SNP 0.30 mM	18.61±1.71 <sup>b</sup>	16.25±1.01 <sup>c</sup>	22.19±1.31 <sup>b</sup>	18.32±1.81 <sup>c</sup>	15.01±1.32 <sup>b</sup>	13.11±2.21 <sup>c</sup>
SNP 0.60 mM	15.91±2.19 <sup>c</sup>	13.38±2.18 <sup>d</sup>	19.01±1.09 <sup>bc</sup>	15.23±1.08 <sup>d</sup>	14.07±1.12 <sup>bc</sup>	11.53±2.12 <sup>d</sup>
SNP 0.90 mM	11.20±2.51 <sup>e</sup>	8.23±2.03 <sup>f</sup>	13.54±1.71 <sup>e</sup>	9.41±1.27 <sup>f</sup>	9.22±2.01 <sup>e</sup>	6.65±1.13 <sup>f</sup>
SNP 1.20 mM	13.02±2.40 <sup>d</sup>	11.14±1.16 <sup>e</sup>	16.02±2.05 <sup>d</sup>	14.37±1.14 <sup>e</sup>	11.67±1.31 <sup>d</sup>	9.41±1.01 <sup>e</sup>
Off year						
Control <sup>c</sup>	19.31±1.2 <sup>a</sup>	18.47±1.87 <sup>a</sup>	23.17±0.34 <sup>a</sup>	24.31±1.08 <sup>a</sup>	15.24±1.01 <sup>a</sup>	15.04±1.55 <sup>a</sup>
SNP 0.30 mM	15.71±1.011 <sup>b</sup>	12.17±1.21 <sup>c</sup>	15.23±1.58 <sup>b</sup>	11.66±1.48 <sup>c</sup>	12.31±1.02 <sup>b</sup>	10.28±1.28 <sup>c</sup>
SNP 0.60 mM	11.87±1.01 <sup>c</sup>	9.47±1.41 <sup>d</sup>	12.38±2.01 <sup>bc</sup>	9.54±1.37 <sup>d</sup>	11.11±1.01 <sup>bc</sup>	7.48±1.10 <sup>d</sup>
SNP 0.90 mM	7.46±1.20 <sup>e</sup>	6.65±1.18 <sup>ef</sup>	7.69±1.25 <sup>e</sup>	4.39±1.64 <sup>f</sup>	7.19±1.01 <sup>d</sup>	6.20±1.19 <sup>e</sup>
SNP 1.20 mM	9.11±1.23 <sup>d</sup>	7.32±0.19 <sup>e</sup>	10.1±1.38 <sup>d</sup>	7.81±1.70 <sup>e</sup>	8.69±1.08 <sup>d</sup>	6.38±2.01 <sup>e</sup>

<sup>a</sup> Values are means ± SE. <sup>b</sup> Trees were sprayed at dormant bud. These treatments were applied separately at two stages: (4 and 8 weeks before normal budbreak (February 1 and January 1, respectively)). <sup>c</sup> Colum means followed by the same letter are not significantly different ( $P < 0.05$ ) according to the Duncan's multiple range test.

**Table 7. Effects of nitric oxide treatment on dehiscent shell nut, No. of nuts per cluster, No. of nuts per ounce and yield of ‘Ahmad Aghaeie’ pistachio in both ON and OFF years**

Treatment	Dehiscent shell nut (%) <sup>a</sup>		No. of nuts per cluster		No. of nuts per ounce		Yield per shoot (g)	
	Stage 1 <sup>b</sup>	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2
On year								
Control <sup>c</sup>	71.98±1.18 <sup>f</sup>	71.75±0.18 <sup>f</sup>	12.19±1.08 <sup>f</sup>	12.27±0.16 <sup>f</sup>	28.38±0.23 <sup>a</sup>	28.25±2.10 <sup>a</sup>	63.87±0.19 <sup>f</sup>	65.24±1.14 <sup>f</sup>
SNP 0.30 mM	77.81±1.1b <sup>e</sup>	81.68±0.12 <sup>d</sup>	17.22±1.2b <sup>e</sup>	21.41±0.14 <sup>d</sup>	26.31±1.01 <sup>b</sup>	24.11±1.01 <sup>c</sup>	74.21±0.1b <sup>e</sup>	80.34±0.18 <sup>d</sup>
SNP 0.60 mM	80.91±2.16 <sup>d</sup>	84.77±0.11 <sup>c</sup>	20.91±1.06 <sup>d</sup>	24.71±1.11 <sup>c</sup>	23.67±1.23 <sup>c</sup>	21.01±2.03 <sup>d</sup>	80.01±0.18 <sup>d</sup>	85.17±1.11 <sup>c</sup>
SNP 0.90 mM	86.46±1.21 <sup>b</sup>	90.59±0.20 <sup>a</sup>	26.14±2.01 <sup>b</sup>	30.21±1.20 <sup>a</sup>	19.01±2.01 <sup>e</sup>	17.38±2.03 <sup>f</sup>	90.31±1.01 <sup>b</sup>	95.67±1.10 <sup>a</sup>
SNP 1.20 mM	83.98±0.43 <sup>c</sup>	85.63±0.14 <sup>b</sup>	23.68±0.21 <sup>c</sup>	27.01±0.1 <sup>b</sup>	21.12±1.28 <sup>d</sup>	19.15±1.19 <sup>e</sup>	84.53±1.27 <sup>c</sup>	91.03±0.08 <sup>b</sup>
Off year								
Control <sup>c</sup>	76.83±1.33 <sup>f</sup>	75.69±1.12 <sup>f</sup>	19.01±0.12 <sup>f</sup>	19.04±0.18 <sup>f</sup>	23.27±1.08 <sup>a</sup>	23.18±0.23 <sup>a</sup>	41.87±1.35 <sup>f</sup>	40.24±1.65 <sup>f</sup>
SNP 0.30 mM	84.77±1.01b <sup>e</sup>	88.34±1.02 <sup>d</sup>	24.40±0.01b <sup>c</sup>	28.24±0.02 <sup>d</sup>	21.08±1.16 <sup>b</sup>	19.34±1.71 <sup>c</sup>	57.21±1.20b <sup>e</sup>	60.34±2.01 <sup>d</sup>
SNP 0.60 mM	87.62±0.16 <sup>d</sup>	90.43±1.16 <sup>c</sup>	27.11±0.17 <sup>d</sup>	31.46±0.18 <sup>c</sup>	18.54±1.60 <sup>c</sup>	16.14±0.34 <sup>d</sup>	61.01±0.39 <sup>d</sup>	66.17±0.01 <sup>c</sup>
SNP 0.90 mM	92.31±1.81 <sup>b</sup>	95.61±0.84 <sup>a</sup>	34.21±0.21 <sup>b</sup>	37.65±0.61 <sup>a</sup>	14.23±1.31 <sup>e</sup>	12.08±1.07 <sup>f</sup>	71.31±0.42 <sup>b</sup>	74.67±1.01 <sup>a</sup>
SNP 1.20 mM	89.9±1.25 <sup>c</sup>	92.19±1.03 <sup>b</sup>	30.39±0.07 <sup>c</sup>	34.30±0.19 <sup>b</sup>	16.40±1.51 <sup>d</sup>	14.27±1.03 <sup>e</sup>	63.53±0.06 <sup>c</sup>	70.03±1.02 <sup>b</sup>

<sup>a</sup> Values are means ± SE. <sup>b</sup> Trees were sprayed at the dormant bud. These treatments were applied separately at two stages: (4 and 8 weeks before normal bud break (February 1 and January 1, respectively)). <sup>c</sup> Colum means followed by the same letter are not significantly different ( $P < 0.05$ ) according to the Duncan's multiple range test.

nitric oxide (NO), a highly reactive gas and ubiquitous bioactive molecule that plays a central role in signal transduction in plant stress response (Arasimowicz and Wieczorek, 2007). The effects of NO on different types

of cells indicate that NO is a potent oxidant or an effective antioxidant (Qiao and Fan, 2008). SNP releases NO in a pH-dependent manner that promotes plant growth and development and retards senescence

**Table 8. Effects of nitric oxide treatment on leaf area, shoot length and shoot diameter of 'Ahmad Aghaeie' pistachio in both ON and OFF years**

Treatment	Leaf area (cm <sup>2</sup> ) <sup>a</sup>		Shoot length (cm)		Shoot diameter (mm)	
	Stage 1 <sup>b</sup>	Stage 2	Stage 1	Stage 2	Stage 1	Stage 2
On year						
Control <sup>c</sup>	70.04±1.10 <sup>f</sup>	69.54±1.03 <sup>f</sup>	10.71±0.01 <sup>f</sup>	10.69±1.16 <sup>f</sup>	5.62±1.01 <sup>f</sup>	5.61±0.04 <sup>f</sup>
SNP 0.30 mM	76.90±0.1b <sup>e</sup>	79.33±0.02 <sup>d</sup>	10.75±0.11b <sup>e</sup>	10.81±0.12 <sup>d</sup>	5.73±1.11b <sup>e</sup>	5.78±0.12 <sup>d</sup>
SNP 0.60 mM	80.91±2.16 <sup>d</sup>	86.97±0.11 <sup>b</sup>	10.80±0.03 <sup>d</sup>	10.83±0.15 <sup>c</sup>	5.80±0.00 <sup>d</sup>	5.85±1.08 <sup>c</sup>
SNP 0.90 mM	87.46±1.26 <sup>ab</sup>	89.37±1.03 <sup>a</sup>	10.85±0.1 <sup>b</sup>	10.91±1.04 <sup>a</sup>	5.87±0.1 <sup>b</sup>	6.32±0.10 <sup>a</sup>
SNP 1.20 mM	83.18±1.30 <sup>c</sup>	88.70±1.01 <sup>ab</sup>	10.82±0.21 <sup>c</sup>	10.86±0.07 <sup>b</sup>	5.84±0.01 <sup>c</sup>	5.89±0.01 <sup>b</sup>
Off year						
Control	71.94±1.13 <sup>f</sup>	72.05±0.19 <sup>f</sup>	11.69±0.12 <sup>f</sup>	11.67±1.00 <sup>f</sup>	5.71±1.01 <sup>c</sup>	5.69±0.04 <sup>c</sup>
SNP 0.30 mM	78.28±0.7 <sup>c</sup>	82.01±0.11 <sup>d</sup>	11.73±0.01b <sup>e</sup>	11.79±0.07 <sup>d</sup>	5.82±1.11b <sup>b</sup>	5.87±0.12 <sup>b</sup>
SNP 0.60 mM	84.01±1.21 <sup>d</sup>	88.85±0.11 <sup>b</sup>	11.77±0.03 <sup>d</sup>	11.80±0.03 <sup>c</sup>	5.89±0.00 <sup>d</sup>	5.94±1.08 <sup>b</sup>
SNP 0.90 mM	89.94±0.30 <sup>ab</sup>	93.01±0.16 <sup>a</sup>	11.82±0.01 <sup>b</sup>	12.68±0.09 <sup>a</sup>	5.96±0.1 <sup>b</sup>	6.24±0.10 <sup>a</sup>
SNP 1.20 mM	86.24±1.17 <sup>c</sup>	91.14±1.43 <sup>ab</sup>	11.79±0.01 <sup>c</sup>	11.83±0.11 <sup>b</sup>	5.95±0.01 <sup>b</sup>	6.01±0.01 <sup>a</sup>

<sup>a</sup> Values are means ± SE. <sup>b</sup> Trees were sprayed at the dormant bud. These treatments were applied separately at two stages: (4 and 8 weeks before normal bud break (February 1 and January 1, respectively)). <sup>c</sup> Column means followed by the same letter are not significantly different ( $P < 0.05$ ) according to the Duncan's multiple range test.

(Kolberz *et al.*, 2008). NO (precursor of SNP) has been reported to influence several plant developmental events in which gibberellins (GAs) play crucial roles such as seed germination, hypocotyl elongation, acquisition of photomorphogenic traits, and primary root growth (Beligni and Lamattina, 2000).

NO has been described as acting upstream of GAs (Bethke *et al.*, 2006), regulating both biosynthesis and perception/transduction of GAs (Leon and Lozano-Juste, 2011). Nitric oxide, as an intracellular and extracellular messenger molecule, is known to be useful in regulating physiological and biochemical reactions of the plant (Neile *et al.*, 2003). The positive effect of sodium nitroprusside on increasing the germination of lettuce (Beligni and Lamattina, 2000) and Arabidopsis (Libourel *et al.*, 2006) has also been reported. In some grass species, the use of nitric oxide has been used to break of seed dormancy (Sarath *et al.*, 2006). Nitric oxide activates the enzymes degrading abscisic acid, which causes dormancy (Bethke *et al.*, 2006). The positive effect of sodium nitroprusside on increasing the germination of lettuce (Beligni and Lamattina, 2000) and Arabidopsis (Libourel *et al.*, 2006) has also been reported. In some grass species, the use of nitric oxide has been used to break seed dormancy (Sarath *et al.*, 2006). In fact, nitric oxide activates the enzymes that degrade abscisic acid, which causes dormancy (Bethke *et al.*, 2006). SNP decreased the percentage of malformed nuts, blanks, and indehiscent shells compared with the control treatment. Nitric oxide probably prevents pistachio misshapen nuts, blank, and indehiscent shells by increasing nutrient uptake, increasing the hormones auxin and gibberellin, and breaking the dormancy in the buds and pollination, and also avoiding environmental stresses by removing reactive oxygen species of pistachio trees. Nitric oxide regulates physiological processes such as germination, stomatal opening and closing, and photosynthesis and is effective in responding to biotic and abiotic stresses (Siddiqui *et al.*, 2011). SNP increases leaf nitrogen concentration, given that it increases nitrate uptake

(Manai *et al.*, 2012). NO produced by SNP has recently been considered a new member of the phytohormones (Leterrier *et al.*, 2012) and may scavenge other reactive intermediaries like reactive oxygen species (ROS) (Laspina *et al.*, 2005). NO produced by SNP has recently been considered a new member of the phytohormones (Leterrier *et al.*, 2012) and may scavenge other reactive intermediaries like reactive oxygen species (ROS) (Laspina *et al.*, 2005). This gas is known as a signaling and regulatory molecule in biological processes involved in regulating plant hormones such as cytokinin (Leshem and Kuiper, 1996), auxin (Forde and Lorenzo, 2001) and ethylene (Muday *et al.*, 1995). Dehisced shell nut, the number of nuts per cluster, the number of nuts per ounce and leaf area, shoot length, and shoot diameter by SNP treatment increased compared with control. The role of sodium nitroprusside on root and shoot elongation may be related to the effect of nitric oxide on the activity of xyloglucan-degrading enzymes. Xyloglucans bind to cellulose and other cell wall polysaccharides, and their breakdown leads to loosening of the wall, allowing the cell to increase in volume, resulting in longer organs (Qu *et al.*, 2009). NO, with its direct effect on cell wall components, could relax the cell wall and improve membrane fluidity, inducing cell enlargement and, therefore, stimulating plant growth. NO and SNP function signals in the auxin-induced signaling cascade leading to adventitious root development.

### Conclusion

Our study demonstrated that SNP application can increase budbreak percentage and hasten yield in pistachio trees in subtropical regions in Iran. We also showed that two-stage foliar application of SNP was more effective than one-stage application in both on and off years. We proposed that SNP acts as a dormancy breaking agent and as a growth promoter by increasing nutrient uptake and assimilation in pistachio trees. Our findings provide a novel and practical approach to improve pistachio production in warm climates.

**Conflict of interest**

The authors declare no conflict of interest.

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