

Use of Plant Bioregulators to Stimulate Embryo Growth and Loosen Fruit to Increase Split Nut Yield of Pistachio

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Abstract

Nut size and split nut yield are key economic factors in commercial pistachio (*Pistacia vera* 'Kerman') production. Nut size tends to decrease as trees age and can be significantly reduced during the on-crop year. Whereas plant bioregulators (PBRs) are powerful tools for solving production problems in the field, research developing PBR strategies for the California pistachio industry has been limited. Gibberellic acid (GA₃), 3,5,6-trichloropyridyloxyacetic acid (3,5,6-TPA) and 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU) were tested for their capacity to stimulate embryo growth and increase nut size. Abscisic acid (ABA), methyljasmonic acid (MJA), 2-chloroethylphosphonic acid (CEPA) and 2,3,5-tri-iodobenzoic acid (TIBA) were tested for their capacity to loosen mature fruit and increase the number of fruit (nuts) harvested. Branch studies testing multiple application times, rates, and combinations of PBRs were undertaken with the goal of increasing split nut (dry weight) yield. Application of GA₃ (170 mg L⁻¹) during late bloom-early fruit set (20 May), CPPU (10 mg L⁻¹) at the initiation of the period of exponential embryo growth (initiation of nut fill, INF) (10 June), and the combination of GA₃ and CPPU 30 days after INF numerically, but not statistically, increased split nut (dry weight) yield per branch. TIBA (125 mg L⁻¹) applied 4 days before harvest was the most effective fruit-loosening treatment. MJA (10 mg L⁻¹) applied 14 days before harvest was nearly as effective as TIBA. Both treatments reduced adherence of fruit tissues to the seed (nut), thereby reducing shell staining. Neither treatment caused significant leaf drop. This is the first research testing these PBR strategies on pistachio. Our results identify PBR concentrations and application times that numerically increase yield parameters by large percentages, as well as some that cause significant negative effects, making our data valuable to PBR researchers in all pistachio-producing countries.

INTRODUCTION

Nut size and the total yield of split nuts on a dry weight basis are key economic factors in commercial pistachio (*Pistacia vera* 'Kerman') production. Nut size tends to decrease as trees age and nut size can be significantly reduced during a heavy on-crop year in an alternate bearing orchard. Use of plant bioregulators (PBRs) to increase nut size, especially in an on-crop year, might contribute significantly to keeping California pistachio growers competitive in the international market. Harvest efficiency is also an important economic factor in pistachio production that might be improved through the use of PBRs. Loosening fruit to increase the number of trees that can be harvested per unit time and to eliminate the need to go back through the orchard to harvest fruit that remain on the tree after the first harvest would be of great economic benefit to pistachio growers and contribute to sustaining the California pistachio industry.

Whereas PBRs are powerful tools for solving production and quality problems in an established orchard, use of PBRs on nut crops has been limited. The auxin 2,4-dichlorophenoxyacetic acid (2,4-D) was used to reduce alternate bearing of pistachio with minimal success, but 2,4-D enhanced nut maturity (Gawad and Ferguson, 1987). In

contrast, application of 6-benzyladenine (6-BA) combined with low-biuret urea to on-crop pistachio trees increased retention of floral buds and increased the kilograms of split nuts (dry weight) per tree the following off-crop year (Lovatt et al., 2005). Gibberellic acid (GA_3) enhanced all yield parameters of peanut (Bishnoi and Krishnamoorthy, 1995). Use of GA_3 to reduce fruit drop during fruit set of almond delayed maturation. Additionally, it was determined that large almonds had higher endogenous indole-3-acetic acid (IAA) concentrations than small almonds (Koukourikou-Petridou, 2003). Ethephon (2-chloroethylphosphonic acid [CEPA]), an ethylene-releasing agent, is used to promote uniform nut abscission of macadamia (Nagao and Hirae, 1992) and as a harvest aid for walnut (<http://ucipm.ucdavis.edu/PMG/r881900711.html>).

In three separate experiments, we screened the efficacy of PBRs to increase nut size and increase harvest efficiency. Gibberellic acid (GA_3), 3,5,6-trichloropyridyloxyacetic acid (3,5,6-TPA) and 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU) were tested for their capacity to stimulate embryo growth and increase nut size. Abscisic acid (ABA), methyljasmonic acid (MJA), 2-chloroethylphosphonic acid (CEPA) and 2,3,5-triiodobenzoic acid (TIBA) were tested for their capacity to loosen mature fruit and increase the number of fruit harvested versus the proportion left on the tree after mechanical shaking. Since success in bringing about a desired outcome from the foliar application of any PBR is dependent on both concentration and time of application, branch studies were undertaken to test multiple application times, rates and combinations of PBRs with the overall research goal of increasing split nut (dry weight) yield per branch of 'Kerman' pistachio.

MATERIALS AND METHODS

The research was conducted in a commercial orchard of 19-year-old 'Kerman' pistachio scions on Pioneer Gold I (*Pistacia integerrima*) rootstock at the University of California Kearney Research and Education Center in Parlier, Calif. (36.63 °N, 119.47 °W). Data trees were selected for uniform health, size, vigor and crop load. Treatments were in addition to standard cultural practices. The orchard was monitored annually to determine spring bud break, full bloom, fruit set, embryo development and fruit growth and maturation for the purpose of timing treatment applications to key stages of tree phenology. Calendar dates are given below in parentheses for convenience.

To stimulate embryo growth and increase nut size the following PBRs were applied during late bloom-early fruit set (20 May), at the initiation of exponential embryo growth (initiation of nut fill, INF) (10 June), 20 days after INF (30 June), and 30 days after INF (10 July): gibberellic acid (GA_3) (85 and 170 mg L⁻¹); 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU, forchlorfenuron) (10 and 20 mg L⁻¹); 3,5,6-trichloropyridyloxyacetic acid (3,5,6-TPA) (10 mg L⁻¹); GA_3 (170 mg L⁻¹) combined with CPPU (20 mg L⁻¹); and GA_3 (170 mg L⁻¹) combined with CPPU (20 mg L⁻¹) and 3,5,6-TPA (10 mg L⁻¹). The following year, GA_3 (85 mg L⁻¹), CPPU (10 mg L⁻¹) and GA_3 (85 mg L⁻¹) combined with CPPU (10 mg L⁻¹) were applied at the initiation of exponential embryo growth (initiation of nut fill, INF) (5 July), 15 days after INF (20 July), 30 days after INF (7 August), 45 days after INF (21 August) and on both 5 July and 7 August.

To increase harvest efficiency the following PBRs were applied 14, 11 and 4 days before commercial harvest to loosen the fruit: (2-chloroethylphosphonic acid [CEPA]) (100, 500 and 1,000 mg L⁻¹), methyljasmonic acid (MJA) (10 and 50 mg L⁻¹), abscisic acid (ABA) (250 and 500 mg L⁻¹), 2,3,5-triiodobenzoic acid (TIBA) (125 and 250 mg L⁻¹); and ABA (250 mg L⁻¹) combined with TIBA (250 mg L⁻¹) applied 11 days before harvest.

Each PBR treatment (concentration x application time) was applied to a branch on one of two trees comprising a block in a randomized complete block design. Each treatment was replicated eight times, with the control replicated 16 times (i.e., one control branch per tree in order to correct for tree to tree variation within a block). PBRs were applied in sufficient volume to provide good canopy coverage using a 2758 KPa handgun sprayer and protective barriers to prevent treatment sprays from drifting onto neighboring

branches. At each application time the number of fruit (nuts) per branch was determined.

At the time of commercial harvest, we determined the final fruit count and nut size (including fresh and dry weight of the exocarp and fresh and dry weight of the seed [nut]), the proportion of split nuts versus non-split nuts, and the proportion of aborted and blank nuts, on a dry weight basis. The effects of the PBR treatments on exocarp removal and staining of the seed coat (nut shell) were evaluated.

In the fruit-loosening experiment, data branches were enclosed in paper bags to catch the fruit during commercial harvest with a mechanical shaker. After harvest, the number of fruit removed by mechanical harvesting and the number of fruit remaining on each branch were counted. The number of fruit that abscised after treatment but before harvest was calculated as follows: initial fruit number minus the sum of the number of fruit collected in the bags and the number of fruit remaining on the tree after harvest.

Statistical Analyses

The data were analyzed using the General Linear Model procedure of the SAS 9.2 statistical program (SAS Inst., Inc., Cary, N.C.). Analysis of variance was used to test for treatment effects on yield in grams per branch and nut quality parameters. To accommodate the non-parametric nature of data expressed as a percent, means were transformed by determining the square root of the arcsine. Means were separated using Fisher's protected LSD test at $P=0.05$.

RESULTS AND DISCUSSION

Effect of PBR Treatments on Yield and Nut Quality

No PBR treatment increased the yield of total fruit or split nuts per branch over that of the untreated control in the first year of the study. However, GA₃ (170 mg L⁻¹) combined with CPPU (20 mg L⁻¹) applied 30 days after INF increased the yield of fruit per branch 25% above that of the untreated control. GA₃ (170 mg L⁻¹) applied 20 days after INF increased yield of split nuts per branch 34% over that of the untreated control. No PBR significantly reduced the proportion of nuts with aborted embryos or the proportion of blank nuts, which was only 1% for the untreated control (Table 1).

Several PBR treatments had statistically significant negative effects on yield and nut quality parameters. Application of 3,5,6-TPA (10 mg L⁻¹) during the late bloom-early fruit set period (20 May) significantly reduced the fruit yield (g fresh wt/branch) by 43% compared to the untreated control ($P=0.0205$) and significantly below that of numerous other PBR treatments (Table 1). The yield of split nuts (dry wt) per branch was significantly reduced when 3,5,6-TPA (10 mg L⁻¹) was applied alone or in combination with GA₃ and CPPU at late bloom-early fruit set or at INF ($P=0.0028$). These treatments also significantly reduced the biomass of individual embryos ($P<0.0001$). The proportion of nuts with aborted embryos was significantly increased by the following treatments: 3,5,6-TPA (10 mg L⁻¹) at INF; GA₃ (170 mg L⁻¹) combined with CPPU (20 mg L⁻¹) and 3,5,6-TPA (10 mg L⁻¹) at INF, 20 days after INF, and 30 days after INF; and GA₃ (170 mg L⁻¹) combined with CPPU (20 mg L⁻¹) applied on all four treatment dates ($P<0.0001$). Six PBR treatments increased the number of blank nuts, but application of GA₃ (170 mg L⁻¹) combined with CPPU (20 mg L⁻¹) and 3,5,6-TPA (10 mg L⁻¹) at late bloom-early fruit set increased the proportion of blank nuts the most, from 1% for the untreated control to 21% ($P<0.0001$).

The more promising treatments were tested again the following year. Application of GA₃ (85 mg L⁻¹) combined with CPPU (10 mg L⁻¹) at 30 days after INF increased fruit yield (g fresh wt/branch) compared to all other treatments ($P=0.0776$), resulting in the greatest yield of split nuts per branch, 40% more than the untreated control but not statistically significant (Table 2). Whereas no PBR treatment reduced fruit yield, CPPU (10 mg L⁻¹) applied at INF and also 30 days after INF had a statistically significant negative effect on the yield of split nuts ($P=0.0078$). No PBR treatment had a positive effect on embryo biomass or the proportion of aborted embryos. Interestingly, there were

no blank nuts in any treatment. Two treatments increased the proportion of nuts with aborted embryos: GA₃ (85 mg L⁻¹) combined with CPPU (10 mg L⁻¹) applied at INF; and CPPU (10 mg L⁻¹) applied at INF and also 30 days after INF ($P=0.0148$).

Effect of PBR Treatments on Harvest Efficiency

The highest percentage of harvested fruit (98%) resulted from the application of TIBA (125 mg L⁻¹) 4 days before harvest (Table 3). Both CEPA (500 mg L⁻¹) applied 4 days before harvest and MJA (10 mg L⁻¹) applied 14 days before harvest achieved 94% harvested fruit. In contrast, application of the 5-fold higher concentration of MJA (50 mg L⁻¹) 14 days before harvest resulted in the greatest amount of fruit abscission (10%) during the period from application to just before harvest, moreover an additional 10% of the fruit remained on the tree after harvest. Three PBR treatments resulted in 11% and 12% less fruit harvested than the untreated control: CEPA (100 mg L⁻¹) applied 4 days before harvest; TIBA (250 mg L⁻¹) applied 11 days before harvest; and ABA (250 mg L⁻¹) combined with TIBA (250 mg L⁻¹) applied 11 days before harvest. These treatments left 27, 24 and 25% of the total fruit remaining on the trees after harvest, respectively.

MJA and TIBA caused advanced senescence of fruit tissues, which significantly reduced their adherence to the surface of the nut, facilitating removal and decreasing shell staining. Both are beneficial effects. No PBR treatment increased leaf abscission.

CONCLUSIONS

GA₃ combined with CPPU applied 30 days after the initiation of exponential embryo growth (initiation of nut fill, INF) was the most effective treatment in both years of the study. Use of the lower concentration of each PBR in year 2 improved the efficacy of this treatment. In year 2, application of GA₃ (85 mg L⁻¹) combined with CPPU (10 mg L⁻¹) 30 days after INF increased the yield of fruit (fresh wt) per branch compared to all other treatments ($P=0.0776$), resulting in 43% more split nuts (dry wt) per branch than the untreated control (not significant). TIBA (125 mg L⁻¹) applied 4 days before harvest increased the number of fruit harvested. Similar results were obtained with CEPA (500 mg L⁻¹) applied 4 days before harvest and MJA (10 mg L⁻¹) applied 14 days before harvest. The TIBA and MJA treatments increased the ease with which the nut was separated from the fruit and decreased the incidence of shell staining. Over the range of concentrations and application times tested, the proportion of harvested fruit obtained with MJA varied from only 82 to 94%, whereas TIBA, CEPA and ABA treatments produced percentages of harvested fruit that were 10% lower as well as higher than the control. Thus, MJA may be more reliable. The promising treatments reported herein now need to be tested as full canopy sprays in commercial pistachio orchards.

The results of this research identified PBR strategies (concentrations and application times) that show promise for increasing the yield of split nuts (dry weight basis) of 'Kerman' pistachio, as well as some that cause significant negative effects, making these data valuable to PBR researchers in pistachio-producing countries.

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Tables

Table 1. Effect of GA₃ (85 and 170 mg L⁻¹), CPPU (10 and 20 mg L⁻¹), 3,5,6-TPA (10 mg L⁻¹), GA₃ (170 mg L⁻¹) combined with CPPU (20 mg L⁻¹); and GA₃ (170 mg L⁻¹) combined with CPPU (20 mg L⁻¹) and 3,5,6-TPA (10 mg L⁻¹) applied during late bloom-early fruit set (20 May), the period of exponential embryo growth (initiation of nut fill, INF) (10 June), 20 days after INF (30 June) and 30 days after INF (10 July) on total fruit fresh weight and split nut dry weight per branch and nut quality of 'Kerman' pistachio (Year 1).

| Treatment | Concentration (mg L ⁻¹) | Application time | Total fruit fresh wt. (g/branch) | Split nut dry wt. (g/branch) | Embryo dry wt. (mg/nut) | Aborted embryo (%) | Blank nuts (%) |
|-------------------------------------|--|----------------------------|--|------------------------------------|-------------------------------|--------------------------|----------------------|
| GA ₃ | 85 | Late bloom-early fruit set | 102.23 abcd ^z | 6.21 abcd | 17.03 bcdef | 29 fghij | 1 fg |
| GA ₃ | 170 | Late bloom-early fruit set | 122.23 ab | 8.40 a | 20.56 abc | 19 j | 1 fg |
| CPPU | 10 | Late bloom-early fruit set | 94.13 bcd | 8.08 ab | 14.95 cdefg | 19 j | 2 fg |
| CPPU | 20 | Late bloom-early fruit set | 105.30 abcd | 4.01 abcdef | 18.26 bcd | 24 hij | 1 fg |
| 3,5,6-TPA | 10 | Late bloom-early fruit set | 59.76 e | 0.96 ef | 9.22 gh | 24 hij | 0 g |
| GA ₃ +CPPU | 170+20 | Late bloom-early fruit set | 105.83 abcd | 1.94 def | 18.52 abcd | 29 efghij | 2 fg |
| GA ₃ +CPPU +3,5,6-TPA | 170+10+10 | Late bloom-early fruit set | 81.95 de | 0.81 ef | 10.35 efgh | 31 defghij | 1 fg |
| GA ₃ | 85 | INF | 102.03 abcd | 6.18 abcd | 19.32 abcd | 26 hij | 1 fg |
| GA ₃ | 170 | INF | 106.63 abcd | 5.58 abcde | 17.96 bcd | 32 defghij | 3 efg |
| CPPU | 10 | INF | 118.80 abc | 8.58 a | 18.21 bcd | 22 ij | 7 cde |
| CPPU | 20 | INF | 107.64 abcd | 4.05 abcdef | 14.48 cdefg | 42 bcde | 3 efg |
| 3,5,6-TPA | 10 | INF | 87.77 cde | 0.21 f | 6.38 h | 48 bc | 2 fg |
| GA ₃ +CPPU | 170+20 | INF | 107.13 abcd | 4.28 abcdef | 18.00 bcd | 29 efghij | 0 g |
| GA ₃ +CPPU +3,5,6-TPA | 170+10+10 | INF | 94.38 bcd | 0.31 f | 5.91 h | 62 a | 1 fg |
| GA ₃ | 85 | 20 days after INF | 104.86 abcd | 8.29 a | 17.21 bcde | 28 hij | 1 fg |
| GA ₃ | 170 | 20 days after INF | 102.39 abcd | 8.95 a | 16.35 bcdefg | 27 hij | 2 fg |

Table 1. Continued.

| Treatment | Concentration (mg L ⁻¹) | Application time | Total fruit fresh wt. (g/branch) | Split nut dry wt. (g/branch) | Embryo dry wt. (mg/nut) | Aborted embryo (%) | Blank nuts (%) |
|-------------------------------------|--|----------------------|--|------------------------------------|-------------------------------|--------------------------|----------------------|
| CPPU | 10 | 20 days after INF | 81.09 de | 8.82 a | 12.22 defgh | 28 ghij | 6 def |
| CPPU | 20 | 20 days after INF | 94.16 bcd | 2.31 cdef | 16.89 bcdef | 41 bcdefg | 2 efg |
| 3,5,6-TPA | 10 | 20 days after INF | 110.96 abcd | 4.15 abcdef | 19.91 abc | 36 cdefgh | 4 efg |
| GA ₃ +CPPU | 170+20 | 20 days after INF | 92.40 bcd | 4.14 abcdef | 17.89 bcd | 25 hij | 2 fg |
| GA ₃ +CPPU +3,5,6-TPA | 170+10+10 | 20 days after INF | 80.15 de | 3.17 bcdef | 9.69 fgh | 49 b | 10 bcd |
| GA ₃ | 85 | 30 days after INF | 122.33 ab | 4.55 abcdef | 25.89 a | 25 hij | 14 b |
| GA ₃ | 170 | 30 days after INF | 95.11 bcd | 4.96 abcdef | 18.44 abcd | 27 hij | 5 defg |
| CPPU | 10 | 30 days after INF | 116.37 abc | 4.93 abcdef | 23.66 ab | 21 ij | 3 efg |
| CPPU | 20 | 30 days after INF | 109.70 abcd | 6.83 abcd | 17.33 bcde | 32 defghij | 21 a |
| 3,5,6-TPA | 10 | 30 days after INF | 104.66 abcd | 4.29 abcdef | 17.63 bcde | 34 defghi | 12 bc |
| GA ₃ +CPPU | 170+20 | 30 days after INF | 129.88 a | 5.82 abcde | 21.34 abc | 34 defghi | 6 def |
| GA ₃ +CPPU +3,5,6-TPA | 170+10+10 | 30 days after INF | 95.91 bcd | 4.40 abcdef | 14.03 cdefg | 44 bcd | 3 efg |
| GA ₃ +CPPU | 170+20 | All application days | 122.43 ab | 2.88 cdef | 15.82 cdefg | 42 bcdef | 4 efg |
| GA ₃ +CPPU | 170+20 | 20+30 days after INF | 115.54 abc | 6.98 abc | 19.26 abcd | 24 hij | 4 efg |
| Control | | | 104.39 abcd | 6.70 abcd | 19.53 abcd | 27 hij | 1 fg |
| P-value | | | 0.0205 | 0.0028 | <0.0001 | <0.0001 | <0.0001 |

^aValues in a vertical column followed by different letters are significantly different at the specified *P*-value by Fisher's protected LSD test.

Table 2. Effect of GA₃ (85 mg L⁻¹), CPPU (10 mg L⁻¹) and GA₃ combined with CPPU applied at the initiation of exponential embryo growth (initiation of nut fill, INF) (5 July), 15 days after INF (20 July), 30 days after INF (7 August), 45 days after INF (21 August) on total fruit fresh weight and split nut dry weight per branch and nut quality of 'Kerman' pistachio (Year 2).

| Treatment | Application time | Total fruit fresh wt. (g/branch) | Split nuts dry wt. (g/branch) | Embryo dry wt. (mg/nut) | Split nuts (%) | Aborted embryos (%) | Blank nuts (%) |
|-----------------------|--------------------|----------------------------------|-------------------------------|-------------------------|----------------|---------------------|----------------|
| Control | | 85.04 bc ^z | 13.99 abcde | 6.78 | 43 abc | 39 bcde | 0 |
| GA ₃ | INF | 70.23 bc | 8.05 ef | 7.76 | 23 def | 50 abc | 0 |
| CPPU | INF | 88.28 bc | 10.79 cdef | 8.68 | 25 cdef | 47 abcd | 0 |
| GA ₃ +CPPU | INF | 58.96 c | 7.07 ef | 4.58 | 30 bcdef | 58 a | 0 |
| GA ₃ | 15 days after INF | 77.31 bc | 17.91 ab | 3.95 | 52 a | 35 cde | 0 |
| CPPU | 15 days after INF | 73.81 bc | 11.37 bcdef | 5.20 | 40 abcde | 36 cde | 0 |
| GA ₃ +CPPU | 15 days after INF | 77.42 bc | 13.62 abcde | 5.04 | 38 abcde | 42 abcde | 0 |
| GA ₃ | 30 days after INF | 76.92 bc | 13.94 abcde | 4.56 | 45 ab | 41 abcde | 0 |
| CPPU | 30 days after INF | 82.00 bc | 13.19 abcdef | 6.55 | 41 abcd | 42 abcde | 0 |
| GA ₃ +CPPU | 30 days after INF | 120.99 a | 19.56 a | 9.40 | 42 abcd | 33 de | 0 |
| GA ₃ | 45 days after INF | 97.48 ab | 16.92 abc | 8.45 | 48 ab | 26 e | 0 |
| CPPU | 45 days after INF | 80.06 bc | 15.28 abcd | 7.58 | 49 a | 37 cde | 0 |
| GA ₃ +CPPU | 45 days after INF | 89.44 b | 13.80 abcde | 6.33 | 43 abc | 41 abcde | 0 |
| GA ₃ | INF + 30 after INF | 79.33 bc | 13.54 abcdef | 5.46 | 46 ab | 35 cde | 0 |
| CPPU | INF + 30 after INF | 87.60 bc | 6.64 f | 13.15 | 16 f | 58 a | 0 |
| GA ₃ +CPPU | INF + 30 after INF | 72.51 bc | 9.31 def | 5.49 | 22 ef | 56 ab | 0 |
| P-value | | 0.0776 | 0.0078 | 0.2791 | 0.0018 | 0.0148 | 0.2333 |

^zValues in a vertical column followed by different letters are significantly different at the specified P-value by Fisher's protected LSD test.

Table 3. The effect of CEPA (100, 500 and 1,000 mg L⁻¹), MJA (10 and 50 mg L⁻¹), ABA (250 and 500 mg L⁻¹), TIBA (125 and 250 mg L⁻¹) and the combination of ABA (250 mg L⁻¹) plus TIBA (250 mg L⁻¹) applied to branches of ‘Kerman’ pistachio 14, 10 and 4 days before commercial harvest by mechanical shaking on the proportion of fruit harvested, fruit that abscised after treatment but before harvest, and fruit remaining on the tree after harvest.

| Treatment | Concentration (mg L ⁻¹) | Application time (no. of days before harvest) | Harvest fruit (%) | Remaining fruit (%) | Abscised fruit (%) |
|-----------------|--|---|-------------------------|---------------------------|--------------------------|
| CEPA | 100 | 14 | 92 | 6 | 2 |
| CEPA | 500 | 14 | 79 | 20 | 1 |
| CEPA | 1000 | 14 | 85 | 11 | 4 |
| MJA | 10 | 14 | 94 | 4 | 2 |
| MJA | 50 | 14 | 82 | 8 | 10 |
| ABA | 250 | 14 | 81 | 17 | 1 |
| ABA | 500 | 14 | 78 | 21 | 1 |
| TIBA | 125 | 14 | 84 | 10 | 6 |
| TIBA | 250 | 14 | 89 | 5 | 6 |
| CEPA | 100 | 11 | 92 | 7 | 1 |
| CEPA | 500 | 11 | 74 | 25 | 1 |
| CEPA | 1000 | 11 | 79 | 18 | 3 |
| MJA | 10 | 11 | 89 | 8 | 3 |
| MJA | 50 | 11 | 89 | 11 | 0 |
| ABA | 250 | 11 | 82 | 16 | 2 |
| ABA | 500 | 11 | 88 | 12 | 0 |
| TIBA | 125 | 11 | 86 | 9 | 5 |
| TIBA | 250 | 11 | 72 | 24 | 4 |
| ABA+TIBA | 250+250 | 11 | 71 | 25 | 4 |
| CEPA | 500 | 4 | 94 | 3 | 3 |
| CEPA | 1000 | 4 | 89 | 10 | 1 |
| MJA | 10 | 4 | 83 | 14 | 3 |
| MJA | 50 | 4 | 90 | 10 | 0 |
| ABA | 250 | 4 | 86 | 12 | 2 |
| ABA | 500 | 4 | 77 | 20 | 3 |
| TIBA | 125 | 4 | 98 | 1 | 1 |
| TIBA | 250 | 4 | 91 | 8 | 1 |
| Control | | | 83 | 15 | 2 |
| <i>P</i> -value | | | 0.7703 | 0.5767 | 0.7985 |

^zValues in a vertical column followed by different letters are significantly different at the specified *P*-value by Fisher’s protected LSD test.

