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Investigating The Effect of Postharvest Application of Putrescine and Spermidine on Preserving Quantitative and Qualitative Properties of Grape Cv. Shahroudi

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ABSTRACT

Grape is one of the most important and ancient fruits in the world that have been used by humans. the aim of this study was to investigate the effect of post-harvest application of putrescine and spermidine polyamines on maintaining the quantitative and qualitative characteristics of 'Shahroudi' grapes during storage. Factorial experiment with two polyamine factors including two types of polyamines, putrescine and spermidine, each in 3 concentrations (0.5, 1 and 2 mmol) and storage time (0, 15, 30, 45, 60, 75, 90 and 105 days) based on a completely randomized design in 2019. After treatment, the fruits were stored for 105 days at a temperature of 2 ° C and a relative humidity of 85-95% and their quantitative and qualitative characteristics were evaluated during storage. The results showed that the treatments had a significant effect on fruit firmness, weight loss, anthocyanin content, titratable acidity, vitamin C and total soluble solids at the level of 1% probability and on pH at the level of 5% probability, but on the percentage of fruit juice It had no significant effect. Also, the effect of storage time on all traits was significant at the 1% probability level. However, the interaction effects of treatment and storage time showed a significant effect on total phenol, acidity and Total soluble solids at 1% probability level and a significant effect on pH at 5% probability level. In conclusion, considering maintain different properties using putrescine and spermidine during storage and delaying in senescence and increasing storage life of fruits of 'Shahroudi' grape, polyamines application as a postharvest treatment recommended.

***Keywords*-** Flavor index, Fruit firmness, Grape, Poly amine, Storage.

I. INTRODUCTION

Grape with the scientific name *Vitis vinifera* is a plant of Vitaceae family. And it is one of the most important and oldest garden products in Iran and the world, and its cultivation and production in Iran is very old. However, due to its highly perishable nature, it has always caused serious problems during transportation, storage, after harvesting, and during sale, which has many economic losses. The decrease in the quality of fresh grapes is generally caused by the decrease in weight, rapid softening of the pods, fungal rots, and changes in taste and color, which can accelerate the decrease in quality without proper treatment or storage conditions (Lydakis & Aked, 2007).

Considering the role of polyamines in the field of maintaining the stability of the cell wall of fruits, they increase the hardness of the product, therefore, the use of these compounds is considered in the field of improving the quality of horticultural products (Kramer *et al.*, 1991). There are many reports in this regard. For example, Sadat Hosseini *et al* (2016) reported that the exogenous application of polyamines had a great effect on preserving the firmness of fruit flesh. The decrease in the softening of apple fruit tissue during storage in controlled atmosphere has a close correlation with the increase in the levels of endogenous polyamines. In

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another experiment, with the penetration of polyamines by pressure into the fruit, an immediate increase in the firmness of the apple fruit and a decrease in its softening in zero-degree storage were observed (Asnaashari, Zakaei, & Shahi, 2011). Immersion under pressure with putricine treatment of 1 mmol in Rish Baba grape cultivar and 2 mmol in Al haqhi cultivar was the most effective treatments to maintain firmness during the storage period (Rahimi *et al*., 2014).

The use of spermine, spermidine and putrescine polyamines on mango resulted in maintaining acidity, maintaining fruit firmness, ascorbic acid level and slowing down weight loss during storage without significant reduction in ethylene production. If the total carotenoid content was reduced compared to the control treatment (Malik & Singh, 2005). (Mirdehghan & Rahimi, 2016) also showed that pomegranate fruit changed color during storage and the use of polyamine preserved the color of the fruit during storage. The use of these compounds preserves the appearance of the product, reduces mechanical damage, improves the quality of the product, and increases the shelf life of the fresh product. During the investigations, exogenous polyamines improved the post-harvest life and fruit quality by maintaining fruit firmness, delaying color changes, soluble solids and titratable acidity, as well as protecting different fruits. They improve against frost damage and mechanical damage (Valero *et al*., 2002).

Since polyamines are known as anti-aging agents, researchers have paid special attention to the role of external polyamines in harvested fruits. So that maintaining the firmness of the fruit and reducing the speed of ripening and aging have been reported in various products such as apples, kiwi, strawberries, lemons, peaches, tomatoes and other products (Asghari *et al*., 2011).

II. MATERIALS AND METHODS

The grapes needed in this research, Shahroudi grapes, were obtained from a 12-year-old vineyard located in Shahroud city, Semnan province. The fruits were picked according to their commercial maturity in the early hours of the morning and were placed in a row in plastic boxes, and then they were transported to the Physiology Laboratory of Horticultural Sciences Department, Gorgan University of Agricultural Sciences and Natural Resources under full supervision. In the laboratory, healthy, uniform and free fruits from any mechanical damage and microbial contamination were isolated. Testing in a factorial format with two polyamine factors including two types of polyamine putrescine and spermidine each in 3 concentrations (0.5, 1 and 2 mmol) and storage time (0, 15, 30, 45, 60, 75, 90 and 105 days) based on a completely randomized design in three replications in 2019. The fruits were immersed in the above solutions for 10 minutes. After the treatment, the fruits were exposed to normal air in the room to dry. Control fruits were without any treatment and treated with distilled water. After packing the fruits in disposable containers, they were transferred to the cold storage of the Department of Horticultural Sciences, Gorgan University of Agricultural Sciences and Natural Resources and stored for 105 days at a temperature of 2±1 degrees Celsius and a relative humidity of -85 95% were kept and their quantitative and qualitative characteristics such as the firmness of the berries, weight loss percentage, fruit juice volume, anthocyanin, vitamin C, titratable acidity (TA), total soluble solids (TSS), pH of fruit extract, total phenolic compounds and Total sugar was measured once every 15 days.

To measure the percentage of fruit juice before juicing, the weight of the fruit and the volume of fruit juice were measured and the percentage of fruit juice (weight of fruit juice compared to the total weight of the fruit) was determined (Mashayekhi & Atashi, 2015). The firmness of the grape fruit tissue was measured by a firmness meter (model FT 327, made in Italy) with a 2 mm probe in terms of kilograms per square centimeter. The pH value of the juices was measured using a pH meter (model, MTT65, made in Japan). Electrical conductivity was measured by an electrical conductivity measuring device. (Model, using Ceit device. A digital refractometer model (Ceit-060279) was used to measure the amount of dissolved solids in fruit juices at room temperature. The ratio of soluble solids to acidity was considered as an indicator of taste. To determine the titratable acidity, the amount of acidity was determined through titrasone with sodium hydroxide based on the percentage of citric acid (Seicuk & Ercan, 2015). Keshap and Gautam (2012) method was used to measure the amount of vitamin C in grape juice. Wanker (1989) method was used to measure the amount of anthocyanin. Measurement of total phenolic content of fruit juices based on the method of Singleton and Rossi (1965). The whole was done using the method of Makarkarbedi et al (1950).

The current research was conducted in factorial form in a completely randomized design in three replications. All the obtained data were analyzed using SAS software, and the average traits were compared based on the LSD test. Also, the process of changes in quantitative and qualitative characteristics of fruit during storage was drawn using Excel software.

III. RESULTS AND DISCUSSION

The results of analysis of variance of treatment effect and storage time as well as their mutual effect are shown in Table 1. The results of the analysis of variance showed that the treatment had a significant effect on fruit firmness, weight loss, anthocyanin content, titratable acidity, vitamin C and total soluble solids at the 1% probability level, and significant effect on the yield at the 5 probability level. It showed the percentage. Also, the effect of storage time on all traits was significant at

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the 1 percent probability level. However, the interaction effects of treatment and storage time showed a significant effect on total phenol, acidity and total soluble solids at a probability level of 1%, and a significant effect on yeast at a probability level of 5%.

Table 1. Analysis of variance effect of putrescine and spermidine treatments on quantitative and qualitative traits of grape cv. Shahroudi during storage

		average of squares									
Sources of change	**Degrees of freedom**	**fruit hardness**	**The percentage of fruit juice**	Weight Loss	**total sugar**	**anthocyanin**	**titratable acidity**	**Total dissolved solids**	**pH**	**Total phenol**	**Vitamin C**
treatment	7	0.171**	39.02ns	2.05**	48.46ns	0.003**	0.009**	3.64**	0.923*	49.28ns	0.002**
storage time	7	1.86**	670.01**	89.61**	256.33**	0.006**	0.041**	15.36**	2.941**	572.55**	0.015**
T × ST storage	49	0.09ns	19.02ns	0.11ns	45.72ns	0.001ns	0.005**	3.69**	0.014*	44.92**	0.001ns
Test error	128	0.06	20.02	0.111	39.23	0.00084	0.001	1.14	0.009	25.47	0.0008
CV (%)	-	17.84	12.1	11.54	47.46	51.38	9.93	6.34	2.82	12.1	19.29

* and ** are significant at probability level of 5% and 1% ns: non-significant, respectively

Fruit firmness: The results of comparing the averages on fruit firmness (kg/cm2) showed that the changes of this quantity in the treatments were 4.76-1.97. The highest level of fruit firmness was related to 1 mmol putrescine treatment and the lowest level was related to distilled water treatment. Also, the comparison of the average storage time (Table 2) showed that the hardness of the samples decreased during the storage time, and the maximum hardness was observed at the beginning of the storage and the lowest at the end of the storage. Jafarpour *et al* (2014) reported that strawberry fruits treated with 1 and 1.5 mmol putrescine had more firmness at the end of storage than untreated fruits (control). The obtained results are in line with the researches.

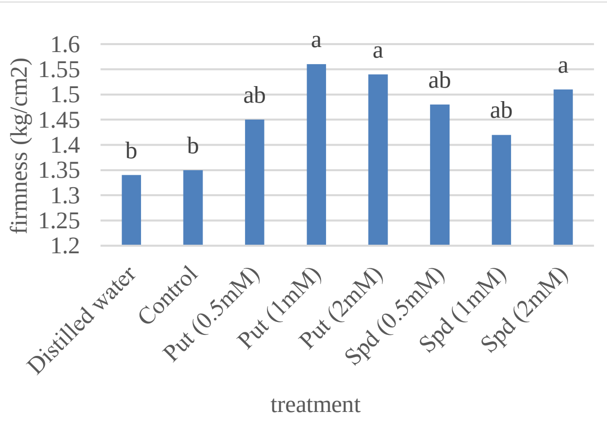

Figure 1: Effect of polyamines on the firmness of grape fruit cv. Shahroudi

Percentage of fruit juice: The percentage of fruit juice in the samples was 38.9-35.58%. The highest value of the percentage of fruit juice related to the putrescine treatment was 0.5 mmol, and the lowest value was related to the control treatment. Also, the results obtained from the analysis of storage time averages in (Table 2) show that the highest percentage of fruit juice was observed on the 30th day of storage and the lowest on the 0th day of storage. Pre-harvest application of putrescine and spermidine (1 and 2 mmol) resulted in maintaining fruit weight and reducing grape juice loss during storage (Mirdehghan & Rahimi, 2016).

Weight Loss: The results of comparing the averages (Figure 3) showed that the highest amount of weight loss belonged to the treatment of 0.5 mmol spermidine, on the other hand, the lowest amount of weight loss was recorded in the treatment of 1 mmol putrescine and distilled water Receipt. Also, the results of the comparison of the storage time averages in (Table 2) showed that the weight loss during the storage time had an increasing trend, and the maximum amount of weight loss was observed at the last stage of sampling and the lowest amount was observed at the beginning of sampling.

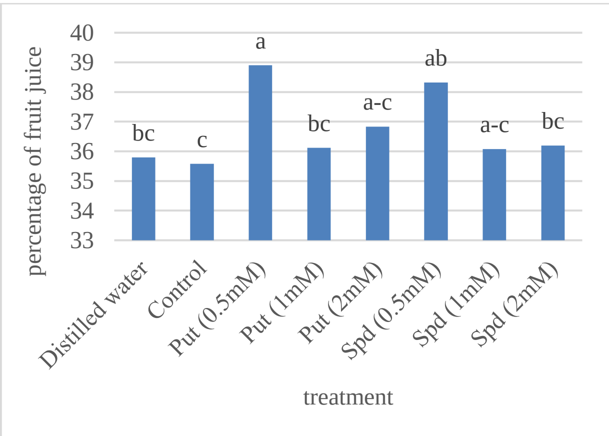

Figure 2: Effect of polyamines on fruit juice volume of grape cv. Shahroudi

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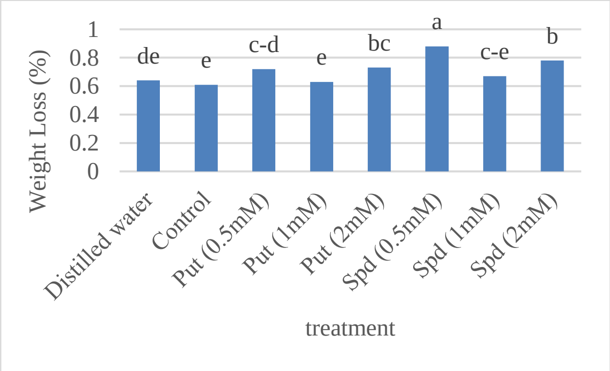

Figure 3 - Effect of polyamines on weight loss of grape cv. Shahroudi

Total sugar: Based on the comparison of the averages between the treatments, the highest amount of total sugar (14.69 mg/g) was related to the distilled water treatment and the lowest amount of total sugar (12.008 mg/g) was observed in the control (Figure 4). As can be seen in (Table 2), the total sugar content increased at the beginning of storage and then decreased at the end of storage. The highest amount of total sugar (17.23 mg/g) was on the 30th day of storage and the lowest amount (8.19 mg/g) was related to the last period of storage.

Anthocyanin: Examining the effects of comparing the averages (Figure 5) shows that the highest amount of anthocyanin (0.075 mg/ml) in the control treatment and the lowest amount of anthocyanin (0.041 mg/ml) ml) corresponding to 1 mmol spermidine treatment was recorded. The comparison of the averages in (Table 2) shows the increase in the amount of anthocyanin during the storage period. According to this figure, the highest amount of anthocyanin (0.085 mg/ml) corresponds to the 105th day of storage and the lowest amount of anthocyanin (0.033 mg/ml) was related to the 15th day of storage.

In grape fruit of Michele Palieri cultivar treated with putrescine, the interaction effects of putrescine and ultrasound maintained the anthocyanin content compared to the control treatment (Bal *et al*., 2017). In the present study, probably due to the loss of water and the concentration of cell sap as well as the low amount of anthocyanin in this fruit, higher amounts of anthocyanin were recorded in the treatment of control and distilled water and 1 mmol putrescine.

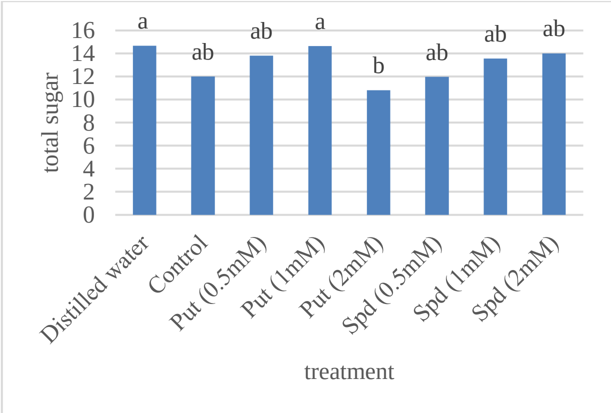

Figure 4 - Effect of polyamines on total sugar of grape cv. Shahroudi

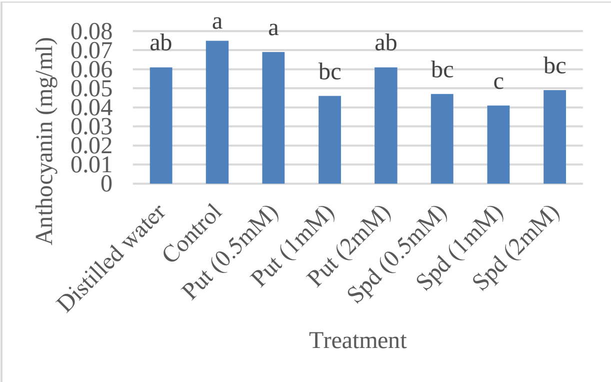

Figure 5- Effect of polyamines on anthocyanins of grape cv. Shahroudi

Table 2. Comparison of the average effect of storage time on the studied traits in grape fruits of Shahroudi variety

time	Hardness	The volume of fruit juice	weight loss	total sugar	Anthocyanin
0	1.84	26.66	0	15.52	0.058
15	1.64	41.33	1.3	14.93	0.033
30	1.69	43.7	1.77	17.53	0.037
45	1.5	40.37	2.49	15.43	0.05
60	1.5	38.38	3.25	13.32	0.064
75	1.22	34.44	4.09	11.02	0.054
90	1.15	35.07	4.84	9.61	0.069
105	1.08	35.85	5.61	8.19	0.085

titratable acidity (TA): According to Table 3, comparing the average interaction effects of treatment and time, the highest titratable acidity (0.513 mg/100 ml) is related to the 2 mmol spermidine treatment and the 45th day of storage, and the lowest The amount of

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titratable acidity (0.276 mg/100 ml) was related to the treatment of distilled water on the 75th day of storage.

In 2007, researchers stated that during storage of fruits in the cold room, titratable acidity decreases over time due to ripening of fruits (Gong *et al*., 2007). Also, (Patel *et al*., 2018) reported that titratable acidity in green bell pepper decreased significantly over time. The above results are consistent with the results of this research.

Total soluble solids: The amount of total solids in the research samples was 19.76-13.13%. The highest amount of total soluble solids was related to spermidine treatment of 2mmol at the 75th day of storage and the lowest amount of total soluble solids was related to the spermidine treatment of 0.5mmol on day 30 of the storage period (table 3). The amount of total soluble solids in Spadona pears increased significantly during storage, but the changes in fruits treated with 1 and 2 mmol putrescine were significantly lower (Hosseini *et al*., 2017).

pH: The test of its value showed that the values of this quantity are between 3.15 and 4.21. The highest pH value was related to the interaction effects of the control treatment and zero time, and the lowest value was related to the interaction effects of 2 mmol spermidine treatment and the 45th day of storage (table 3). The research was consistent with the findings of Rahimi (2011), that showed that the 2 mmol putrescine treatment in Rish-Baba variety and the 2 mmol putrescine and 1 and 2 mmol spermidine treatments in Alhaqi variety had the lowest pH in Comparison with the control showed.

Electrical conductivity (EC): In general, the comparison of the mean interaction effects of treatment and time in Table 3 indicates that the highest electrical conductivity (4.33 mM/cm2) was related to spermidine 2 mM and day 105 of storage, which was not statistically significant with control treatment on day 60 of storage and the lowest amount (2.93 mM/cm2) was related to the control treatment on day 75 of storage, which was statistically significant with Spermidine 0.5 mM on day 15 of storage time showed no significant difference. According to Table 3, the amount of electrical conductivity has increased. The increase in electrical conductivity is likely due to damage to the cytoplasmic membrane, which is another reason for this claim.

In this study, Mohammad Rezakhani and Pakish (2015) investigated the effect of application of proline and ascorbic acid on decreasing the freezing index and hydrogen peroxide on grape cv. Thomson during storage. During storage and in all treatments used ascorbic acid and proline, as well as control treatment, electrolyte leakage has increased.

Vitamin C: According to Table 3 comparing the average interaction effects of treatment and storage time, the highest amount of vitamin C (0.230 mg/100 ml) was related to putrescine treatment at 0.5 mmol and day 105 of storage and the lowest The highest amount of vitamin C (0.100 mg/100 ml) related to 2 mmol spermidine treatment and the 30th day of storage was recorded.

The amount of vitamin C in mango fruit decreased significantly during storage, but the amount of changes in the fruits treated with chitosan and spermidine was 2 mmol more than the control treatment (Zahedi *et al*., 2019). (Hossaeini *et al*., 2018) also reported that with the passage of time vitamin C in all pear fruits (Shahmiwah and spadona) treated with 1 and 2 mmol putrescine and untreated fruits (control) significantly You have decreased.

Total phenol: Based on the averages obtained from the mutual effects of treatment and storage time, the highest amount of total phenol (26.43 mg of gallic acid per 100 ml) is related to the distilled water treatment and the 45th day of storage, and the lowest amount (7.50 mg of gallic acid per 100 ml) related to 2 mmol putrescine treatment and day 75 of storage was observed (Table 3).

(Asghari & Ahadi, 2013) reported that the content of total phenol in the fruit of Qezl-Ozom grape variety decreased during storage for 45 days. Also, (Hossaeini *et al*., 2018) showed that the amount of total phenol and total antioxidant activity in all pear fruits of cultivars (Shahmiwah and Spadona) decreased with the passage of the storage period, while in the fruits treated with Putrescine was 1 and 2 mmol higher than the control during the storage period.

IV. CONCLUSIONS

Based on the results obtained from this research, it can be said that the use of putrescine and spermidine had a positive effect on the quantitative and qualitative characteristics of the grape fruit of the Shahroudi variety, and it helped maintain the marketability and increase the life after harvesting of the fruits. In such a way that the use of putrescine and spermidine was able to maintain the firmness of the tissue and significantly reduce the spoilage of the berry during the storage period compared to the control fruits.

Table 3: Mutual effects of treatment and storage time on chemical and biochemical characteristics of Shahroudi grapes during storage

treatment	time (day)	acidity Titleable (%)	Soluble solids (%)	pH	Electrical conductivity (mmohs/cm)	Vitamin C (mg/100ml)	Total phenol (mg Gallic acid/100ml)
Control	0	0.306^{s-v}	17.2^{b-n}	4.21^{a}	3.28^{k-p}	0.113^{mn}	8.35^{p-r}
Distilled water	15	0.330^{p-v}	15.33^{p-s}	3.71^{e-d}	3.35^{h-p}	0.126^{j-n}	10.66^{k-r}
Control	15	0.34^{n-u}	14.96^{rs}	3.99^{b}	3.61^{b-l}	0.133^{h-n}	7.70^{qr}

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Put (0.5mM)	15	0.376^{i-p}	14.66st	3.75^{cd}	3.31^{i-p}	0.111^{mn}	9.96^{l-r}
Put (1mM)	15	0.303^{s-v}	14.66st	3.72^{de}	3.27^{k-p}	0.146^{e-m}	21.58^{a-g}
Put (2mM)	15	0.346^{m-u}	15.46^{o-s}	3.58^{ef}	3.11^{n-p}	0.150^{e-m}	19.08^{a-j}
Spd (0.5mM)	15	0.340^{n-s}	16.06^{j-s}	3.56^{fg}	3.05^{op}	0.136^{g-n}	17.35^{c-n}
Spd (1mM)	15	0.31^{r-v}	17.2^{b-n}	3.9^{bc}	3.28^{k-p}	0.126^{j-n}	16.55^{e-o}
Spd (2mM)	15	0.343^{n-u}	15.86^{k-s}	3.77^{cd}	3.44^{d-o}	0.146^{e-n}	14.90^{g-r}
Distilled water	30	0.366^{j-s}	16/23^{h-s}	3.21^{k-p}	3.47^{c-o}	0.126^{j-n}	9.03^{-r}
Control	30	0.406^{e-m}	15.53^{n-s}	3.18^{o-q}	3.81^{c-g}	0.140^{h-n}	10.64^{l-r}
Put (0.5mM)	30	0.336^{o-v}	15.56^{m-s}	3.18^{o-q}	3.63^{b-m}	0.130i-n	9.74^{m-r}
Put (1mM)	30	0.370^{i-r}	16.33^{h-s}	3.18^{o-q}	3.29^{j-p}	0.136^{g-m}	11.14^{j-r}
Put (2mM)	30	0.310^{r-v}	17.1^{b-o}	3.26^{h-q}	3.31^{i-o}	0.150^{e-m}	9.68^{n-r}
Spd (0.5mM)	30	0.343^{n-u}	13.13^{t}	3.16^{pq}	3.65^{b-k}	0.113^{mn}	11.79^{i-r}
Spd (1mM)	30	0.423^{e-k}	17.26^{b-m}	3.22^{j-q}	3.85^{b-f}	0.126^{j-n}	16.10^{f-p}
Spd (2mM)	30	0.40^{f-n}	16.16^{i-s}	3.28^{h-q}	3.78^{b-g}	0.100^{n}	15.72^{f-q}
Distilled water	45	0.416^{d-k}	17.73^{b-j}	3.3^{i-q}	3.44^{d-o}	0.206^{a-c}	26.43^{a}
Control	45	0.37^{-r}	15.83^{k-s}	3.35^{i-m}	3.91^{a-c}	0.190^{a-f}	18.80^{a-j}
Put (0.5mM)	45	0.453^{a-g}	15.63^{m-s}	3.22^{j-q}	3.76^{b-i}	0.210^{ab}	26.28^{a}
Put (1mM)	45	0.460^{a-f}	16.86^{h-q}	3.25^{i-q}	3.55^{b-n}	0.203^{a-d}	21.35^{a-g}
Put (2mM)	45	0.376^{i-p}	18.7^{ab}	3.33^{h-o}	3.57^{b-m}	0.115^{e-m}	26.04^{ab}
Spd (0.5mM)	45	0.506^{ab}	15.1^{rs}	3.2^{j-q}	3.74^{b-j}	0.150^{e-m}	24.98^{a-d}
Spd (1mM)	45	0.39^{h-p}	14.66st	3.33^{h-o}	3.44^{d-o}	0.143^{f-m}	23.38^{a-f}
Spd (2mM)	45	0.513^{a}	18.66^{ab}	3.15^{q}	3.52^{c-n}	0.193^{a-e}	24.99^{a-d}
Distilled water	60	0.313^{r-v}	17.1^{b-o}	3.34^{h-n}	3.91^{a-c}	0.160^{c-m}	17.27^{c-n}
Control	60	0.333^{o-v}	14.66st	3.27^{h-q}	3.99^{ab}	0.176^{b-i}	14.63^{h-r}
Put (0.5mM)	60	0.276^{uv}	18.1^{a-e}	3.22^{j-q}	3.71^{b-k}	0.146^{e-n}	21.86^{a-h}
Put (1mM)	60	0.370^{i-r}	18^{b-f}	3.27^{h-q}	3.64^{b-l}	0.116^{l-n}	18.10^{b-l}
Put (2mM)	60	o.373^{j-q}	18.26^{a-e}	3.34^{h-n}	3.64^{b-l}	0.150^{e-m}	17.66^{c-m}
Spd (0.5mM)	60	0.370^{i-r}	18.6^{a-c}	3.24^{i-q}	3.54^{b-n}	0.143^{f-m}	24.60^{a-e}
Spd (1mM)	60	0.446^{b-h}	16.4j-r	3.25^{i-q}	3.87^{b-d}	0.140^{h-n}	15.60^{f-r}
Spd (2mM)	60	0.440^{c-h}	17.36^{b-k}	3.26^{h-q}	3.42^{h-n}	0.140^{h-n}	15.64^{f-r}
Distilled water	75	0.276^{v}	17^{b-o}	3.41^{gh}	3.89^{a-d}	0.230^{a}	22.11^{a-g}
Control	75	0.373^{j-q}	18.6^{a-c}	3.26^{h-q}	2.93^{p}	0.170^{b-j}	16.38^{f-p}
Put (0.5mM)	75	0.393^{h-o}	18.6^{a-c}	3.28^{h-q}	3.19^{l-p}	0.183^{a-g}	19.70^{a-i}
Put (1mM)	75	0.460^{a-f}	17.23^{b-n}	3.37^{h-j}	3.37^{g-p}	0.153^{e-m}	10.48^{l-r}
Put (2mM)	75	0.490^{a-c}	15.6^{m-s}	3.22^{j-q}	3.47^{c-o}	0.166^{b-k}	7.50^{r}
Spd (0.5mM)	75	0.433^{c-i}	17.73^{b-j}	3.39^{hi}	3.44^{d-o}	0.173^{b-j}	18.84^{a-j}
Spd (1mM)	75	0.426^{e-j}	18.23^{a-d}	3.37^{h-q}	3.57^{b-m}	0.176^{b-i}	23.64^{a-f}
Spd (2mM)	75	0.410^{e-l}	19.76^{a}	3.34^{h-n}	3.19^{l-p}	0.150^{e-m}	25.27^{a-c}
Distilled water	90	0.333^{p-v}	17.23^{b-n}	3.38^{hi}	3.72^{c-k}	0.193^{a-e}	18.86^{a-j}
Control	90	0.373^{j-q}	17.91^{b-h}	3.25^{i-q}	3.39^{h-o}	0.180^{b-h}	19.93^{a-i}
Put (0.5mM)	90	0.393^{h-o}	16.93^{c-q}	3.3^{h-q}	3.43^{e-o}	0.206^{a-c}	22.91^{a-g}
Put (1mM)	90	0.433^{c-i}	16.83^{h-q}	3.3^{h-p}	3.56^{b-m}	0.163^{b-l}	17.06^{d-o}
Put (2mM)	90	0.476^{a-d}	16.13i-s	3.2^{j-q}	3.69^{b-k}	0.156^{d-m}	11.22^{j-r}
Spd (0.5mM)	90	0.433^{c-i}	17.85^{b-g}	3.3^{h-q}	3.58^{b-m}	0.156^{d-m}	17.40^{c-n}
Spd (1mM)	90	0.386^{h-p}	18.36^{a-e}	3.32^{h-o}	3.72^{b-k}	0.160^{c-m}	20.49^{a-g}
Spd (2mM)	90	0.386^{h-p}	16.6^{i-s}	3.35^{h-m}	3.56^{b-m}	0.143^{f-m}	21.58^{a-h}
Distilled water	105	0.393^{h-o}	17.66^{b-j}	3.35^{h-m}	3.55^{b-n}	0.160^{c-m}	15.62^{f-r}
Control	105	0.370^{i-r}	17.23^{b-n}	3.25^{i-q}	3.85^{b-f}	0.193^{a-e}	23.48^{a-f}
Put (0.5mM)	105	0.393^{h-o}	15.26^{q-s}	3.31^{h-p}	3.66^{b-k}	0.230^{a}	26.12^{ab}
Put (1mM)	105	0.406^{e-m}	16.43^{h-r}	3.25^{i-q}	3.75^{b-i}	0.180^{b-h}	23.63^{a-f}
Put (2mM)	105	0.456^{a-f}	16.66^{e-r}	3.19^{n-q}	3.91^{a-c}	0.150^{e-m}	14.94^{g-r}
Spd (0.5mM)	105	0.426^{e-j}	17.96^{b-f}	3.2^{j-q}	3.72^{b-k}	0.140^{h-n}	15.96^{f-o}
Spd (1mM)	105	0.353^{l-q}	18.5^{a-d}	3.37^{h-j}	3.86^{b-f}	0.143^{f-m}	17.30^{c-l}
Spd (2mM)	105	0.363^{k-t}	17.46^{b-k}	3.37^{h-j}	4.33^{a}	0.143^{f-m}	17.88^{c-m}

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