J. of Plant Production, Mansoura Univ., Vol. 14 (2): 97 - 106, 2023

Journal of Plant Production

Journal homepage & Available online at: www.jpp.journals.ekb.eg

Effect of some Safe Alternatives on Yield, Fruit Quality and Gray Mold Disease Control of King Ruby Grape

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ABSTRACT

This research was held during 2020 and 2021 seasons in a private vineyard in Menoufia governorate, Egypt; with the goal of improving the quality of King Ruby grape and controlling gray mold disease (*Botrytis cinerea* P.) using different potassium salts as monopotassium phosphate (MKP), dipotassium phosphate (DKP), and tripotassium phosphate (TKP). Four sprays of each salt, at 1% and 3% doses were applied every 15 days, starting after fruit set and ending two weeks before harvest. The three potassium salts were effective in enhancing yield and both physical and chemical properties significantly, however, firmness and adherence slightly declined with increasing K element concentration. After a month of cold storage, a clear deterioration occurred; but the salts' treatments, especially TKP, still performing better than the control in all aspects definitely showing the least cluster weight loss and berry decay compared to the control, in both seasons of the study. As for the *In vitro* study, it reveal that the three salts significantly reduced the growth of *Botrytis* pathogenic fungus than the control; especially TKP, showed the highest mycelium growth reduction. Furthermore, DKP had stronger inhibition effect than MKP. In conclusion, the three potassium phosphate salts have the potential to improve grape yield, quality, and prevent gray mold infection, making them safe growth promoters and fungicides, especially TKP at 3%.

Keywords: Grape; Monopotassium phosphate; Dipotassium phosphate; Tripotassium phosphate; *Botrytis cinerea*.

INTRODUCTION

King Ruby (Emperor x Pirovano 75) is a mid- to late- season, red seedless flavored table grape. This cultivar is distinct in many ways as it matures' an abundant yield and sunburn resistant, but its clusters' compactness make them rot susceptible (Rehman *et al.*, 2018). Therefore, as all grapes, gray mold (*Botrytis cinerea* P.) is a serious postharvest disease for this cultivar since it develops at low temperatures and goes from berry to berry. This fungus first turns berries to brown, then loosens berry skin and finally develops masses of gray spores, subsequently huge economic losses (Crisosto and Smilanick, 2021). Thus it should be controlled, however, traditional synthetic plant fungicides' frequency usage led to fungi resistant population development, which increases production cost and environment and human disorders'. So, many researchers investigated various compounds' effects on different crops' characters and pathogens such as salts. Biocidal products like potassium phosphates' salts were examined since they have double action on plant; as nutrients and as antifungal agents depending on low doses, thus are offering an eco-friendly effective plant protection approach'. Regarding phosphorus (P) necessity, Farouk (2011); Moyer *et al.* (2018) stated that it is the second most limiting macronutrient affecting grapes anthocyanin accumulation, early maturity plus berry size increase and pathogens' resistance induction. Furthermore it is essential in many biological compounds; such as nucleic acids, enzymes, phosphate sugars and energy-rich phosphate compounds. Concerning Potassium (K) importance, it is essential for a number of plants' biochemical processes subsequently contributing to adequate berry maturation, sugars' accumulation, phenols' synthesis and pH and acidity regulation of grapevines (Mpelasoka *et al.*, 2003; Ben Yahmed and Ben Mimoun, 2019). Also, it can increase various abiotic and biotic stresses' tolerance (Anschütz *et al.*, 2014; Shabala, 2017; Hasanuzzaman *et al.*, 2018). Working on various grape cultivars, (Reuveni and Reuveni, 2008; Thakur *et al.*, 2008; Raath, 2012; Nieves-Cordones *et al.*, 2019; Villette *et al.*, 2020) mentioned (K) as a necessary element for 4 vital roles for membrane maintenance and growth namely; cellular membrane transportations and assimilates translocation, enzyme activation, anion neutralization and osmotic regulation. Thus foliar sprays of MKP at 1 and 1.5% at different periods (every 10, 20 or 30 days) had improved bunch weight and led to the maximum SSC and minimum acidity values. Also, Aly *et al.* (2015) showed that spraying Superior grape with foliar K and B combination recorded the highest yield with the lowest compactness value and the heaviest cluster weight. Furthermore, (Kashyap and Dhiman, 2009; Arslan, 2015) evaluated the antifungal activity of (MKP) and (DKP) as natural fungicides for 10 phytopathogenic fungi control. Mycelial growth inhibition ranged from 0 to 52.2% and 0 to 100% for (MKP) and (DKP), successively. Spore germination and germ tube elongation inhibition ranged from 0 to 100% for both compounds; in addition, DKP at 2% inhibited mycelial growth completely. Both salts could become natural alternatives to synthetic fungicides since (1%) foliar applications provided significant disease control.

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DOI: 10.21608/jpp.2023.189786.1212

Moreover, Obenland *et al.,* (2015) applied aqueous potassium solutions to five grape cultivars at veraison and three weeks later. They reported a consistent increase in berries' SSC, differs among various sources, while they inconsistently affect firmness. An inverse relationship between the rapid berry weight loss and the largest SSC increase and color intensity by various salts occurred. Additionally, a dual K application enhanced SSC more than a single one. These cultivars commercial maturity dates were advanced from 4 to 11 d also. Oppositely, Moss (2016) revealed that, high K concentrations have potentially lowered grape pH and color stability. Furthermore, MKP and DKP have been found to be efficient and safe alternatives to synthetic fungicides in suppressing diseases (Taskin, 2016). In these respects, Moyer *et al.* (2018) mentioned DKP as a very low salt index and water soluble fertilizer can be safely applied with high rates. Also, provides readily available P and K remains on the leaf longer offering foliar P uptake essential requirement. Aly *et al.* (2020) applied a pre-harvest treatment of potassium phosphate to Crimson seedless grape and revealed that, yield components, total sugars and anthocyanin contents had improved. In addition, treatments raised berry weight and firmness. Moreover, Fekry and Aboel-Anin (2020) compared different K sources as foliar fertilizers for Early Sweet grape. They found that using micro carbon technology K (K-MCT) 500 ppm was very effective for vegetative growth, leaves nutritional status and fruiting characters enhancer. In grape postharvest field, Irene Romero *et al.* (2020) revealed that preserving grape firmness depends on cell wall genes coding degrading enzymes; whereas, most postharvest treatments served to control lipid peroxidation which been activated during cold storage. This study show that various treatments promote increased levels of the final compounds such as anthocyanins and flavanols, through gene expression modulation.

Regarding King Ruby's susceptibility to *Botrytis cinerea* P. fungus, as one of the common natural enemies, which affect its' productivity and quality. Thus, looking for safe alternative antimicrobial agents is very important. Hence, potassium- phosphate salts are not phytotoxic thus they could be considered biocompatible fungicides and efficient foliar fertilizers.

So, this study main point was maintaining a sufficient yield of King Ruby grape showing better quality and free of gray mold disease (*Botrytis cinerea* P.) by using MKP, DKP and TKP salts' various concentrations. Also, a dual *in vitro* culture technique will be conducted to determine these salts' efficiency against (*Botrytis cinerea* P.).

MATERIALS AND METHODS

This work was conducted in a private vineyard located in Elkhatatba district, Menoufia governorate, Egypt. The experiment was carried out on 10 years-old King Ruby grapevines, trellised by a Spanish Baron trellising system with line spacing about 2 x 3 m, during 2020 and 2021 seasons. The vines were grown in a sandy soil with drip irrigation system, and were trimmed with a total vine load of 44 eyes/ vine. A total of 63 uniform vigor vines were selected for this experiment, 7 treatments x 3 clusters x 3 vines/ replicate; furthermore, common horticultural practices were adopted.

Chemical agents applied

Two concentrations of the following salts were sprayed on clusters at four different times, reapplied every 15 days. The application started when berry's diameter was about 2-3 mm, at veraison stage, two weeks later and ended two weeks before harvest:

1. Control (tap water)
2. Monopotassium phosphate (KH_2PO_4): MKP at 1% & MKP at 3%.
3. Dipotassium phosphate (K_2HPO_4): DKP at 1% & DKP at 3%.
4. Tripotassium phosphate (K_3PO_4): TKP at 1% & TKP at 3%.

The treatments were sprayed on the selected vines using a hand pressure sprayer until completely wet at a rate of 3 L/ vine.

At harvesting time, Clusters were randomly collected when they are fully red colored with satisfying SSC values, around 17- 18 %, according to Tourky *et al.* (1995). Harvested grapes were immediately transferred to The Viticulture Department Laboratory, Horticulture Research Institute, Giza.

Fresh samples

The collected clusters samples in the harvest day, fresh samples, containing 6 clusters/ vine x 3 vines/ treatment; were subdued for various measurements detailed below:

Yield and clusters physical measurements

Yield/ vine (kg), cluster weight (g), berry volume (cm^3) sized by a graded cylinder, berry firmness measured by a penetrometer (FT011) expressed as (g/ cm^2) and berry adherence force with mechanical push-pull dynamometer (Model FD101) and expressed as (g/ cm^2).

Berries chemical measurements

Juice SSC measured using a hand refractometer, total titratable acidity as tartaric acid (%) was calibrated according to (AOAC., 1990) and subsequently SSC/ acid ratio was calculated. Skin anthocyanin content (mg/ 100 g F. W) was evaluated as described by Yieldiz and Dikmen (1990). Total phenolic content (mg/ 100 g D. W) was estimated according to Malick and Singh (1980) method.

Leaves mineral content

Leaf content of macro nutrients: K (%) was measured according to Balo *et al.* (1988), and P (%) was estimated as described by Temminghoff and Houba (2004).

Cold storage samples

Fresh clusters samples for cold storage were kept into coded cardboard boxes (4 boxes/ treatment); each one contains (6 clusters / replicate) and labeled then divided for quality parameters. Grapes stored into an adopted temperature of 0±2 °C and a relative humidity of 90 to 95% (Crisosto and Smilanick, 2021).

To study the effect of storage on the grape clusters resulting from conducting all treatments, weekly samples till a month of storage were taken to estimate the berries' chemical measurements as previously mentioned. In addition, the clusters' physical measurements as berry firmness and adherence (g/ cm^2) were estimated; also, weight loss % and berry decay %, were calculated by the following equations:

Cluster weight loss (%) = Initial cluster weight – Final cluster weight after each storage period/ Initial cluster weight x100
Berry decay (%) = Weight of decayed berries/ Initial cluster weight x 100

Experimental design and statistical analysis

Both fresh and cold stored samples results, were tabulated and statistically analyzed as a randomized complete block design (RCBD). The statistical analysis of the data was carried out according to the method described by Snedecor and Cochran (1990). Comparisons among treatments means were held using the new L.S.D. values at 5 % level.

***In vitro* pathogens detection**

Plant material and inoculation

Botrytis cinerea was isolated from Crimson Seedless grape variety. Samples were surface sterilized with 75% ethanol for 1 m and then rinsed thrice with water. The fungi, present on samples were isolated by single spore culture technique (Chomnunti *et al.* 2014). The pure isolates obtained were cultured on potato dextrose agar (PDA) plates and incubated for 7–10 days at 25 °C.

Pure culture of *Botrytis cinerea* was isolated from apparently infected grapevine.

Efficacy of (Mono, Di and Tri potassium phosphate salts) on mycelial growth of *Botrytis cinerea* was determined. The proper quantities of each salt were added and autoclaved and cooled PDA medium at 50º C to obtain concentrations of 0.4, 0.8, 0.5, 1, 0.4 and 0.7 % (w/v), respectively. The three salts amended media were dispensed, 10 ml / plate, aseptically into 6 cm diameter Petri plates. A mycelial disc (5 mm-diameter) taken from 7- day- old culture of the respective fungus was placed in the center of each salt amended PDA medium plate. The plates were sealed with Parafilm and incubated at 20º C. Mycelial growth was measured after 7 days of incubation when the growth in the control plates reached the edge of the plates. Free K- salts plates were used as control. Percentage reduction of mycelial growth was determined as described by the equation of Gamliel *et al.* (1989) as the following:

$$\text{Hyphal growth inhibition \%} = 100 - [(r^2/ R^2)] \times 100$$

Where: **r = is the colony radius of the pathogen on PDA medium incorporated with the concentrations of the three salts**

R= is the colony radius of the fungal pathogen on PDA medium without potassium salts three replicates were used for each concentration of the 3 salts.

Experimental design and statistical analysis

Obtained data were statistically analyzed according to the General Linear Models procedure of Statistical Package for Social Sciences (SPSS, 2008) Version 17.0.0 software. Significant treatment differences were evaluated by using Duncan's Multiple Range Test (P=0.05).

RESULTS AND DISCUSSION

Fresh grape samples

Yield and cluster physical measurements

Yield/ vine (Kg) and cluster weight (g)

At harvest day, Table 1 claims that total yield of a vine and average cluster weight had increased gradually in parallel with increasing K concentrations compared to the control. So, the vines sprayed with TKP 3 % significantly recorded the highest yield and cluster weight at both seasons. Whereas, the control vines recorded the lowest yield and cluster weight values during the two seasons.

Berry volume (cm^3)

Results displayed in Table 1 show that 3% of TKP attained the largest berry volume compared to the other treatments and the untreated vines (control), which recorded the lowest volume with significant differences observed among all treatment in both seasons.

Berry firmness (g/cm^2)

Berries firmness was increased significantly due to salts' additions but turned down with high concentrations (Table 1). The firmest berries were obtained with DKP 3% compared to the control which showed the softest ones, in the both seasons.

Berry adherence (g/cm^2)

Results illustrated in Table 1 clear that berry adherence was significantly increased by all of the adopted treatments compared to the control since spraying DKP 1% salt was significantly enhanced berry adherence, meanwhile the untreated vines resulted in the weakest adherence values in the both seasons of the study.

Table 1. Yield and cluster physical measurements of King Ruby grape at harvest as affected by MKP, DKP and TKP sprays in 2021 and 2022 seasons

Treatment	Total Yield/ Vine (kg)		Cluster weight (g)		Berry volume (cm^3)		Berry firmness (g/cm^2)		Berry adherence (g/cm^2)	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Cont.	20.4	21.5	421.6	445	3.58	3.65	180.7	192.2	170.0	185.5
MKP 1%	21.5	22.5	443.5	464.5	4.42	4.57	213.2	217.0	220.3	225.2
MKP 3%	22.2	23.0	458.7	476.1	4.46	4.59	212.3	215.0	215.5	228.0
DKP 1%	22.5	23.1	464.1	497.6	4.49	4.65	214.2	216.5	223.5	230.0
DKP 3%	23.8	24.5	492.5	506.7	4.58	4.70	215.4	218.0	221.3	220.0
TKP 1%	23.9	24.1	493.4	540.3	4.62	4.74	200.0	202.3	210.5	210.2
TKP 3%	24.7	25.4	549.1	575.8	4.69	4.87	200.5	203.0	205.2	212.0
$LSD_{at\ 0.05}$	1.53	1.48	30.2	28.8	0.20	0.21	14.33	15.21	12.23	11.40

Berries chemical measurements

Total soluble solids (SSC %), Titratable acidity (%) and SSC / acid ratio

All treatments had a positive impact on juice chemical properties with significant differences between them and the control at both seasons, as shown in Table 2. The foliar sprays of TKP at 3% were found to be the most effective in increasing juice SSC and reducing total acidity values; subsequently recording the highest SSC/Acid ratio. On contrary, results noticed that the untreated clusters had the lowest SSC and the highest total acidity values accordingly calculated SSC/Acid ratio was the least.

Anthocyanin content (mg/ 100 g F. W)

It is clear from Table 2 results that, various treatments had a positive significant effect on increasing berry color compared to the control. As TKP 3 % application gained the highest anthocyanin content of berry skin; on the other hand, the control application gave the lowest content significantly.

Total phenols (mg/ 100 g D. W)

Concerning the total phenols content, the different treatments had a significant impact compared to the control; where Table 2 claim that spraying vines with TKP 3 % resulted in the maximum phenols accumulation Whereas, the control sprays scored the minimum content at both seasons. Similar observations by Mona, Soliman and El-Mohamedy (2018) explained phenols content gradual increment parallel with increasing K-salts concentration.

Generally, all harvest day results reveal that K-salts applied in this study have various positive effects on grape definitely enhanced cluster weight, berry volume, due to expansion depending on osmotic regulation, sugar accumulation and cellular growth and free tartaric acid decrease, a main organic acid in grape, sub sequentially decreasing juice pH that alters red color quality (Obenland *et al.,* 2015 and Wu *et al.,* 2021). Moreover, berries firmness and adherence readings are in harmony with Khalifa and Thabet (2014) and Riya, Johnson *et al.* (2022) findings. Also, repetitive sprays had positive effects; since K acts for metabolism aspects thus it improves growth, yield and quality characteristics (Zaree *et al.,* 2015; Karimi, 2017 and Ben Yahmed and Ben Mimoun, 2019). Furthermore, Irene Romero *et al.* (2020) revealed that some postharvest treatments responses are cultivar-dependent.

Table 2. Berries chemical measurements of King Ruby grape at harvest as affected by MKP, DKP and TKP sprays in 2021 and 2022 seasons

Treatment	SSC (%)		Titratable acidity (%)		SSC/ acid ratio		Anthocyanin content (mg/100 g F.W)		Total phenols (mg/100g D.W)	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Cont.	17.40	17.52	0.69	0.68	25.21	25.76	30.61	30.90	0.310	0.315
MKP 1%	17.88	17.97	0.66	0.63	27.09	28.52	36.90	37.43	0.363	0.366
MKP 3%	18.15	18.29	0.61	0.57	29.75	31.00	38.57	39.06	0.371	0.377
DKP 1%	18.80	19.10	0.56	0.55	32.98	34.10	40.22	40.87	0.391	0.398
DKP 3%	19.36	19.83	0.52	0.49	35.85	37.41	41.45	42.11	0.407	0.411
TKP 1%	19.20	19.72	0.55	0.52	33.10	35.85	40.71	41.24	0.400	0.406
TKP 3%	19.51	20.44	0.48	0.45	38.25	40.88	43.10	43.85	0.413	0.417
LSD at 0.05	1.05	0.92	0.052	0.048	4.22	3.87	4.58	3.11	0.028	0.031

Leaves P and K contents (%)

Table 3 present the impact of pre-harvest sprays of potassium salts on two leaf macro nutrients' contents, namely P and K. All treatments significantly enhanced both than the control; where findings confirmed that TKP 3% recorded the maximum percentages, while the control showed their minimal percentages at the two seasons. Such results mentioned by Obenland *et al.* (2015).

Table 3. Leaves P and K contents (%) of King Ruby grape as affected by MKP, DKP and TKP sprays in 2021 and 2022 seasons

Treatments	P %		K %	
	1st Season	2nd season	1st Season	2nd season
Control	0.24	0.26	1.38	1.42
MKP 1%	0.36	0.38	2.04	2.09
MKP 3%	0.37	0.40	2.10	2.16
DKP 1%	0.40	0.44	2.21	2.30
DKP 3%	0.42	0.45	2.29	2.33
TKP 1%	0.46	0.50	2.35	2.42
TKP 3%	0.49	0.54	2.47	2.56
LSD at 0.05	0.056	0.018	0.079	0.056

Cold stored grape samples

Physical measurements

Cluster weight loss and Berry decay (%)

Cluster weight loss and berry decay percentages were significantly influenced by the pre-harvest applications of the 3-k salts during prolonging cold storage period till the 4th week compared to the control at both seasons. Results presented in Tables 4 & 5 pointed out a significant decrement in both measurements progressively occurred parallel with K increment, since the minimum values during storage period were realized with TKP 3%. However, the maximum weight loss and decay percentages were found with the control.

Similarly, the storage period had a significant effect on both physical characters, as the lowest weight loss and decay records were scored by the one week stored clusters; meanwhile the month stored ones showed the highest percentages at both seasons.

Concerning the interaction, MKP 3% and TKP 1% at the first and the second season consecutively were very efficient in reducing the weight loss percentage. Meanwhile, both DKP and TKP totally suppressed berries decay until the 2nd week of cold storage; furthermore, TKP 3% maintained the least decayed berries till the 4th week at both seasons. On the contrary, the control showed the biggest weight loss and decay percentages at the two seasons.

All salts' treatments had a pronounced effect in attaining the least decay percentages compared to the untreated vines, where P- salts act as foliar fertilizers and exert a direct effect on plant resistance since it sequester apoplastic Ca, altering membrane integrity and influencing apoplastic enzymes activity, that releasing elicitor-active oligogalacturonides from plant cell walls . In addition, activate peroxidase which involve in disease defense (Reuveni and Reuveni, 2008 and Mona Soliman and El-Mohamedy, 2018). Also, because of potassium's ability to rise stresses resistance by ROS detoxification contribution and cells repair; as it has a role in membrane stabilization, maintenance turgor and phloem transport into grape berries (Anschütz *et al.,* 2014, Shabala, 2017 and Rogiers *et al.,* 2017). Furthermore, DKP and MKP ability in regulation and promoting fungus resistance through K function in activating defense genes and total lignin enzymes and producing resistance compounds had explained by Khalifa and Thabet, (2014) and Riya, Johnson *et al.* (2022).

Table 4. Cluster weight loss (%) of King Ruby grape as affected by MKP, DKP and TKP sprays in 2021 and 2022 seasons

	First Season					Second season				
Storage period (B)	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**
Treat (A)										
Cont.	0.47	0.78	1.13	1.72	1.03	0.46	0.84	1.08	1.44	0.96
MKP 1%	0.4	0.71	1.16	1.43	0.93	0.48	0.73	0.98	1.17	0.84
MKP 3%	0.15	0.37	0.64	1.38	0.64	0.64	0.84	0.95	0.99	0.86
DKP 1%	0.16	0.42	0.74	1.22	0.64	0.3	0.57	0.94	1.4	0.80
DKP 3%	0.17	0.42	0.82	1.33	0.69	0.29	0.65	0.83	1.29	0.77
TKP 1%	0.2	0.61	0.64	1.04	0.62	0.21	0.51	0.94	1.37	0.76
TKP 3%	0.22	0.44	0.52	0.81	0.50	0.26	0.56	0.89	1.25	0.74
Mean B	0.25	0.54	0.81	1.28		0.38	0.67	0.94	1.27	
	LSD (A) = 0.04					LSD (A) = 0.02				
	LSD (B) = 0.02					LSD (B) = 0.03				
	LSD (A×B) = 0.07					LSD (A×B) = 0.06				

Table 5. Berries decay (%) of King Ruby grape as affected by MKP, DKP and TKP sprays in 2021 and 2022 seasons

	First Season					Second season				
Storage period (B)	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**
Treat (A)										
Cont.	0.00	1.85	3.75	4.72	2.58	0.00	1.80	3.61	4.55	2.49
MKP 1%	0.00	0.95	1.74	3.14	1.46	0.00	0.68	1.58	3.10	1.34
MKP 3%	0.00	0.88	1.68	2.25	1.20	0.00	0.73	1.60	2.18	1.13
DKP 1%	0.00	0.00	1.51	1.92	0.86	0.00	0.00	1.36	1.87	0.81
DKP 3%	0.00	0.00	1.40	1.57	0.74	0.00	0.00	1.37	1.53	0.73
TKP 1%	0.00	0.00	1.10	1.33	0.61	0.00	0.00	0.90	1.26	0.54
TKP 3%	0.00	0.00	0.79	0.91	0.43	0.00	0.00	0.70	0.78	0.37
Mean B	0.00	0.53	1.71	2.26		0.00	0.46	1.59	2.18	
	LSD (A) = 0.53					LSD (A) = 0.52				
	LSD (B) = 0.23					LSD (B) = 0.27				
	LSD (A×B) = 0.81					LSD (A×B) = 0.87				

Berry firmness (g/ cm^2)

Data of Table 6 illustrate that, K treatments significantly strengthen berries' firmness compared to the control since MKP 3% resulted in the firmest berries; whereas, the control treatment recorded the lowest firmness value significantly at the two studied seasons.

Moreover, storage period significantly affected firmness where a week stored clusters showed the firmest berries; however, firmness reduced significantly when clusters stored for a month at both studied seasons.

Regarding both afore factors; one week stored MKP's and DKP 1% clusters had scored the highest values; meanwhile, the month stored controls recorded the lowest values at the two seasons.

Table 6. Berry firmness (g/ cm^2) of King Ruby grape as affected by MKP, DKP and TKP sprays in 2021 and 2022 seasons

	First Season					Second season				
Storage period (B)	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**
Treat (A)										
Cont.	180	178	167	163	172	187	183	177	171	180
MKP 1%	210	203	197	183	198	214	211	205	198	207
MKP 3%	210	208	200	194	203	213	211	209	201	209
DKP 1%	210	205	198	187	200	214	213	208	197	208
DKP 3%	202	200	196	188	196	210	200	200	190	200
TKP 1%	200	195	190	187	193	201	198	194	189	195
TKP 3%	200	188	185	180	188	201	188	185	178	188
Mean B	201.64	196.71	190.44	183.20		205.67	200.43	196.93	189.23	
	LSD (A) = 17.9					LSD (A) = 18.1				
	LSD (B) = 9.3					LSD (B) = 9.7				
	LSD (A×B) = 24.6					LSD (A×B) = 25.2				

Berry adherence (g/ cm^2)

As shown in Table 7, all treatments were superior to the control since DKP 1% resulted in the strongest adherence force; whereas, the control weakened detachment force to the least values at both seasons.

In addition, storage period significantly lowered adherence as the one-week stored clusters recorded the highest values; while, the 4 weeks stored ones had the lowest value at the two seasons.

Regarding the interaction, the strongest adherence values were measured with DKPs at the first season and DKP 1% at the second season both clusters were stored for a week. Meanwhile the weakest value was recorded with a month stored control clusters at both seasons.

Table 7. Berry adherence (g/ cm^2) of King Ruby grape as affected by MKP, DKP and TKP sprays in 2021 and 2022 seasons

	First Season					Second season				
Storage period (B)	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**
Treat (A)										
Cont.	180	165	150	135	157.50	178	170	158	147	163.38
MKP 1%	217	190	185	165	189.30	222	203	189	172	196.68
MKP 3%	217	205	190	175	196.75	219	201	190	175	196.38
DKP 1%	220	200	192	187	199.75	224	201	191	177	197.96
DKP 3%	220	210	190	177	199.30	218	200	191	178	196.68
TKP 1%	210	200	180	155	186.25	208	195	187	172	190.58
TKP 3%	201	190	172	150	178.25	210	200	182	165	189.25
Mean B	209.29	194.31	179.89	163.43		211.19	195.73	184.03	169.56	
	LSD (A) = 20.4					LSD (A) = 18.7				
	LSD (B) = 10.3					LSD (B) = 9.5				
	LSD (A×B) = 31.6					LSD (A×B) = 25.6				

Chemical measurements

SSC (%)

Data of Table 8 indicate that, various treatments had influenced SSC content significantly as increasing K had increased it than the control.

TKP 3% resulted in the highest values at the two seasons; however, the lowest values were measured with the control and with MKP 1% at the two seasons, consecutively.

Similarly, storage period significantly affected SSC since it was concentrated by prolonging the period, thus the month stored clusters scored the highest value; while the lowest value was measured with the week stored clusters at both seasons.

As for factors interacting, the highest SSC content was recorded with TKP 3% clusters that stored for a month at both seasons, but the lowest content was found with the one-week stored control clusters at both seasons.

Table 8. SSC % of King Ruby grape as affected by MKP, DKP and TKP sprays in 2021 and 2022 seasons

	First Season					Second season				
Storage period (B)	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**
Treat (A)										
Cont.	17.70	18.61	19.40	19.94	18.91	19.10	19.92	20.55	21.73	20.33
MKP 1%	18.56	19.00	19.70	20.96	19.56	18.80	19.25	20.41	21.50	19.99
MKP 3%	19.50	19.91	20.16	20.93	20.13	19.59	20.70	21.19	21.85	20.83
DKP 1%	19.35	20.28	20.75	21.17	20.39	19.44	19.92	20.77	22.00	20.53
DKP 3%	20.14	20.89	21.61	22.10	21.19	20.13	20.64	21.40	21.94	21.03
TKP 1%	20.11	20.90	21.45	22.13	21.15	20.26	20.71	21.32	22.45	21.19
TKP 3%	20.14	20.92	21.64	22.20	21.23	20.90	21.58	21.98	22.66	21.78
Mean B	19.36	20.07	20.67	21.35		19.75	20.39	21.09	22.02	
	LSD (A) = 1.31					LSD (A) = 1.25				
	LSD (B) = 0.94					LSD (B) = 0.84				
	LSD (A×B) = 2.43					LSD (A×B) = 2.12				

Total titratable acidity (%)

Table 9 claim that acidity had an inverse relationship with K concentration as the least values were detected with TKP 3% sprays; while, the highest value was measured with the control sprays at the two studied seasons.

Furthermore, extending storage period had gradually reduced the total acidity, where the lowest value was measured for one month stored clusters' juice; whereas storing them for a week showed the highest values at both seasons. Such result could be attributed to organic acids degradation and new compounds induced by storage temperature interacting with enzymes activity (Singh and Sharma, 2017).

As for the interaction, the less acidic juice was measured in TKP 3% and in DKP 1% both stored for a month at the first and the second season, sequentially. While, the most acidic juice was found in control samples stored for a week at both seasons.

Table 9. Total titratable acidity (%) of King Ruby grape as affected by MKP, DKP and TKP sprays in 2021 and 2022 seasons

	First Season					Second season				
Storage period (B)	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**
Treat (A)										
Cont.	0.65	0.62	0.60	0.56	0.61	0.64	0.61	0.59	0.54	0.60
MKP 1%	0.62	0.60	0.57	0.54	0.59	0.63	0.61	0.58	0.55	0.59
MKP 3%	0.59	0.56	0.54	0.51	0.55	0.59	0.57	0.54	0.51	0.55
DKP 1%	0.53	0.51	0.49	0.47	0.50	0.54	0.53	0.32	0.30	0.43
DKP 3%	0.51	0.48	0.45	0.42	0.47	0.51	0.51	0.48	0.43	0.48
TKP 1%	0.48	0.45	0.42	0.39	0.44	0.47	0.42	0.41	0.38	0.42
TKP 3%	0.46	0.44	0.41	0.37	0.42	0.46	0.43	0.39	0.35	0.41
Mean B	0.55	0.52	0.50	0.47		0.55	0.52	0.47	0.43	
	LSD (A) = 0.092					LSD (A) = 0.122				
	LSD (B) = N.S					LSD (B) = N.S				
	LSD (A×B) = 0.193					LSD (A×B) = 0.212				

SSC/Acidity

As shown in Table 10, various treatments increased the SSC/ Acid ratio compared to the control since spraying clusters with TKP 3% had raised the ratio to the maximum at the two seasons. Meanwhile, spraying them with water at the first season and with MKP 1% at the second season had lowered the ratio to the minimum.

In addition, a positive relationship between prolonging storage period and SSC/ Acid ratio appeared as the highest ratio was scored by the month stored clusters, while the lowest ratio was recorded with the week stored clusters at both seasons.

Finally, the interaction showed that the highest ratio was calculated for TKP 3% samples at the first season; whereas for DKP 1% samples at the second one both stored for a month. However, the lowest ratios were calculated for the week stored controls at the two studied seasons.

Table 10. SSC/ Acid ratio of King Ruby grape as affected by MKP, DKP and TKP sprays in 2021 and 2022 seasons

	First Season					Second season				
Storage period (B)	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**
Treat (A)										
Cont.	27.23	30.02	32.28	35.54	31.27	29.70	32.50	34.71	40.54	34.36
MKP 1%	29.79	31.61	34.32	38.60	33.58	29.75	31.82	35.31	38.81	33.92
MKP 3%	32.88	35.75	37.13	40.72	36.62	32.98	36.57	39.02	42.68	37.81
DKP 1%	36.30	39.53	42.70	44.66	40.80	35.87	37.30	64.10	73.33	52.65
DKP 3%	39.18	43.25	47.60	52.25	45.57	39.32	40.87	44.40	51.02	43.90
TKP 1%	42.25	46.04	51.28	56.54	49.03	42.83	49.66	53.87	59.32	50.45
TKP 3%	44.17	47.33	51.81	60.00	50.82	45.14	50.66	55.23	64.74	53.12
Mean B	35.97	39.07	42.45	46.90		36.51	39.91	46.66	52.92	
	LSD (A) = 7.22					LSD (A) = 7.14				
	LSD (B) = 4.52					LSD (B) = 4.17				
	LSD (A×B) = 10.65					LSD (A×B) = 11.21				

Anthocyanin content (mg/ 100 g F. W)

Results of Table 11 gave evidence that K sprays increased anthocyanin content significantly than the control where TKP 3% sprays raised anthocyanin content to the maximum value; while the control sprays lowered it to the minimum value at both seasons.

On the contrary, extending storage period significantly lowered berry anthocyanin content as the highest value was scored by the week stored clusters, whereas the lowest contents were recorded with the month stored clusters at the two seasons. In this respect, Picariello *et al.* (2019) claimed that anthocyanin decrement is attributed to tartaric acid role in preserving its' oxidative degradation, so wide changes in tartaric/malic acid ratio during storage affect its concentration.

Regarding the interaction, results showed that the major skin content was detected in TKP 3% clusters stored for a week; meanwhile the lowest content was measured in the control clusters stored for a month at both seasons.

Table 11. Anthocyanin content (mg/ 100 g F. W) of King Ruby grape as affected by MKP, DKP and TKP sprays in 2021 and 2022 seasons

	First Season					Second season				
Storage period (B)	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**
Treat (A)										
Cont.	34.15	32.86	31.90	30.69	32.40	34.90	32.67	31.54	31.10	32.55
MKP 1%	39.68	38.12	37.17	37.00	37.99	39.75	38.66	37.70	37.50	38.40
MKP 3%	40.90	39.94	39.41	38.70	39.74	41.84	40.94	40.00	39.17	40.49
DKP 1%	42.61	41.11	40.50	40.28	41.13	43.30	42.52	41.88	40.92	42.16
DKP 3%	44.20	43.60	42.24	41.50	42.89	44.89	43.75	42.90	42.20	43.44
TKP 1%	43.35	41.77	41.00	40.78	41.73	43.82	42.97	42.07	41.33	42.55
TKP 3%	45.86	44.96	43.62	43.16	44.40	46.70	45.27	44.30	43.96	45.06
Mean B	41.54	40.34	39.41	38.87		42.17	40.97	40.06	39.45	
	LSD (A) = 6.24					LSD (A) = 5.55				
	LSD (B) = 3.25					LSD (B) = 2.87				
	LSD (A×B) = 9.11					LSD (A×B) = 8.79				

Total phenols (mg/ 100 g D. W)

As illustrated in Table 12, K- salts had increased phenolic content significantly than the control where spraying TKP 3% strongly raised the content whereas spraying water had sharply lowered phenols content at both seasons.

Regarding storage period, extending time lowered values as the highest content was measured in the week stored clusters, meanwhile this content declined in the month stored ones at the two seasons. Ruiz-García and Gómez (2013) and Pratyusha (2022) had explained phenolic content degradation during storage by linking its' production induce only to stress factors presence like postharvest diseases.

Looking to the interaction, the highest content was recorded in TKP 3% berries stored for a week; however, the lowest value was found in the control stored for a month with significant differences among treatments at both seasons.

A month of clusters cold storage was enough to stop the experiment where berries slightly shrank, declined weight and became loose as a result of water loss and aging due to K / Ca ratio decrement; subsequently phloem and xylem inflow relatively increased (Rogiers *et al.*, 2008). Also, senescence mitigation as mentioned by (Anschütz *et al.*, 2014; Nisansala *et al.*, 2015, Shabala, 2017 and Rogiers *et al.*, 2017).

Table 12. Total phenolic content (mg/ 100 g D. W) of King Ruby grape as affected by MKP, DKP and TKP sprays in 2021 and 2022 seasons

	First Season					Second season				
Storage period (B)	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**	**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**Mean A**
Treat (A)										
Cont.	0.334	0.326	0.319	0.314	0.323	0.345	0.336	0.329	0.321	0.333
MKP 1%	0.393	0.384	0.377	0.368	0.381	0.410	0.395	0.382	0.373	0.390
MKP 3%	0.412	0.395	0.388	0.379	0.394	0.420	0.405	0.390	0.385	0.400
DKP 1%	0.430	0.415	0.404	0.395	0.411	0.433	0.421	0.411	0.402	0.417
DKP 3%	0.434	0.423	0.418	0.411	0.422	0.441	0.430	0.423	0.418	0.428
TKP 1%	0.429	0.420	0.410	0.406	0.416	0.434	0.427	0.418	0.410	0.422
TKP 3%	0.440	0.429	0.421	0.416	0.427	0.448	0.436	0.428	0.422	0.434
Mean B	0.410	0.399	0.391	0.384		0.419	0.407	0.397	0.390	
	LSD (A) = 0.091					LSD (A) = 0.092				
	LSD (B) = N.S					LSD (B) = N.S				
	LSD (A×B) = 0.113					LSD (A×B) = 0.101				

In vitro pathogen detection

Table 13 and Figure 1 obtained results indicated that all potassium salts tested *in vitro* significantly reduced pathogenic fungus growth compared to control treatment. Percentages of *Botrytis cinerea* growth reduction ranged from 0 to 86.2 %. TKP at both doses 3% and 1 % showed the highest mycelium inhibition reduction in fungus growth (86.2 and 76.4 %), respectively. Whilst results cleared that DKP at 3% and 1 % recorded stronger inhibition effect against this fungus than MKP at 3% and 1%, consecutively.

Table 13. *In vitro* growth inhibition percentage of *Botrytis cinerea* as affected by MKP, DKP and TKP at various concentrations

Salt	Inhibition %
Control	0.0^{g}
MPK 1 %	13.6^{f}
MPK 3 %	25.4^{e}
DPK 1 %	37.25^{d}
DPK 3 %	60.7^{c}
TPK 1 %	76.4^{b}
TPK 3 %	86.2^{a}

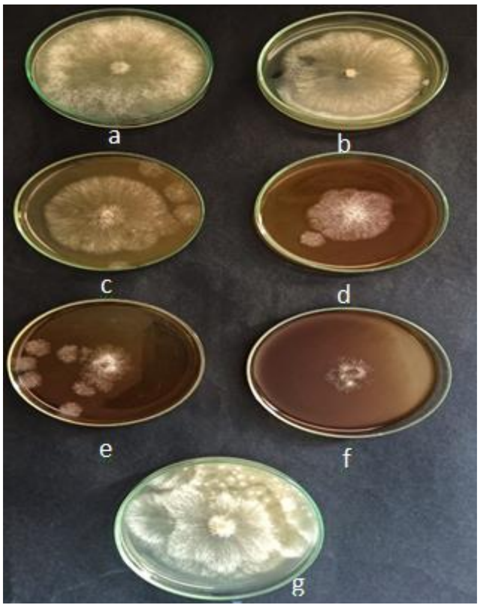

Fig. 1 – a- g. Growth of *Botrytis cinerea* P. on different media- a. MKP 1%; b. MKP 3%; c. DKP 1%; d. DKP 3 %; e. TKP 1 %; f. TKP 3 % and control

These results will be explained by knowing that K is a cofactor for over 40 enzymes involving in many cellular processes lead to disease resistance increase that attributed to several mechanisms like respiration, photosynthesis, defense substance synthesis C supply, stomatal regulation, cell permeability and tissues susceptibility to pathogen maceration and penetration decrease (Arslan, 2015; Santos *et al.,* 2019).

CONCLUSION

The current research conclusively revealed that TKP, DKP and MKP, by order are highly recommended as a safe controlling agents for grape rot *Botrytis cinerea*, they also presents convenient marketable and storable quality of King Ruby grape cultivar. In general, TKP at 3% showed the best overall performance than other treatments on grapes; therefore, it can be recommended to obtain a satisfactory yield in terms of quantity and quality, free from fruit rot, in storage conditions for almost a whole month.

Abbreviations: MKP: monopotassium phosphate; DKP: dipotassium phosphate; TKP: tripotassium phosphate; SSC: soluble solids content; K-MCT: potassium with micro carbon technology.

ACKNOWLEDGMENT

The authors would like to thank the Viticulture Research Department laboratory and the Central Laboratory of Organic Agriculture, Agricultural Research Centre for facilitating this work analysis. Special thanks for Prof. Dr. Raafat S.S. El Gendy for her sincere effort during this work.

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تأثير بعض البدائل الآمنة على المحصول، جودة الثمار و السيطرة على مرض العفن الرمادي في عنب كنج روبي

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[٢] المعمل المركزي للزراعة العضوية- مركز البحوث الزراعية- الجيزة- مصر.

الملخص

أجري هذا البحث في مزرعة خاصة بمحافظة المنوفية، مصر وذلك خلال الموسمين (٢٠٢١- ٢٠٢٢) على كرمات عنب صنف كينج روبي، و ذلك بهدف تحسين الجودة و مقاومة مرض العفن الرمادي (بوترايتس)؛ من خلال رش أملاح مونو، داي و تراي بوتاسيوم فوسفات بتركيزين ١ و ٣ % لكل منهم. تم رش كرمات العنب بالأملاح اربعة مرات خلال الموسم بمعدل كل اسبوعين بدئاً من بعد العقد ووصولاً لما قبل الجمع بأسبوعين. أثبتت الأملاح البوتاسية الثلاثة فعاليتها في زيادة المحصول و تحسين كلاً من الخصائص الفيزيائية والكيميائية معنوياً. كما لوحظ انه بزيادة عنصر البوتاسيوم تحسنت جميع الخصائص محل الدراسة، عدا صلابة الحبات و قوة الشد لها تدهورت بشكل طفيف. بعد شهر من التخزين البارد حدث تدهور واضح في العديد من خصائص العناقيد إلا أن العناقيد المعاملة ظلت أفضل معنوياً من غير المعاملة في جميع الصفات خاصة كأقل نسب لفقد وزن العنقود و عفن الثمار في كلا الموسمين. أما عن الجزء المعملي من الدراسة فقد أظهركفاءة الأملاح الثلاثة في تقليل نمو الفطر معنوياً مقارنة بالكنترول و تحديدا تراي بوتاسيوم فوسفات والذي سبب أقوى تأثير مثبط لنمو الفطر. كما لوحظ أيضاً أن داي بوتاسيوم فوسفات أقوى تثبيطاً لنمو الفطر من مونو بوتاسيوم فوسفات. وبصفة عامة فإن أملاح البوتاسيوم الثلاثة محل الدراسة لها القدرة على تحسين محصول العنب كماً و جودة و مقاومة للإصابة بالعفن الرمادي ، مما يجعلها محفزات للنمو ومبيدات آمنة خاصة تراي بوتاسيوم فوسفات بتركيز ٣%.

الكلمات الدالة: العنب- مونو بوتاسيوم فوسفات- داي بوتاسيوم فوسفات- تراي بوتاسيوم فوسفات- فطر البوترايتس.