



Preharvest application of methyl jasmonate for improving postharvest quality of 'Pusa Navrang' grapes

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ABSTRACT

Exogenous application of elicitors is a good strategy to induce physiological changes in grapevines. Methyl jasmonate is one of the potential elicitor trigger the synthesis of phytochemical compounds in grapes. Therefore, an experiment was conducted to study the influence of preharvest application of methyl jasmonate (MeJA) on nutritional quality of grapes cv. Pusa Navrang (*Vitis vinifera* L.). The experiment was laid out in factorial RBD with 3 replications the treatments were designated with four levels of MeJA (0, 5, 10, 15 mM) and three time of application. The results clearly showed a negligible effect of MeJA on TSS, titratable acidity, juice pH and TSS/TA ratio. Whereas, significant effect was observed for total phenolics (504.58 GAE, mg /100g), total flavonoids (418.33 QE, mg /100g), and total monomeric anthocyanins (1434 C₃GE mg/kg fresh weight), with the application of methyl jasmonate (10 mM) at veraison stage. This resulted in the accumulation of higher antioxidant activity in terms of FRAP assay (19.44 TE, μ mol /g) with the same treatment combination.

Key words: *Vitis vinifera*, TSS, phenolics, flavonoids, mineral nutrients.

INTRODUCTION

Grape (*Vitis vinifera* L) is one of the most important fruit crops grown worldwide. India is also playing significant role in the quality grape production with maximum productivity per unit area (21 t/ha) and annually production of about 2.74 million (NHB, 6). Globally, it is mainly used for wine making (80%) and rest is being used for table, juice and resin purposes. In contrast, India's produce is mainly used for table purpose and small quantity goes in to processing. Due to wider adaptability, it is grown commercially in different parts of India (Chanana and Gill, 2; Sahoo *et al.*, 13, 14; NHB, 6) covering temperate, subtropical and tropical climatic regions in India. Grapes contain a variety of phytochemicals such as phenolic acids, stilbenes, anthocyanins, and proanthocyanidins, all of which are strong antioxidants. The phytochemical composition of grapes, however, varies greatly among different varieties (Yang and Xiao, 16). Because of health benefits, grapes consumption has been increased many fold among all the age groups. The phytochemical composition of grapes primarily varies according to genetical, physiological and agronomical factors (Yang and Xiao, 16). However, the amount of phytochemicals can also be

improved through the use of elicitors (Ruiz-Garcia and Gomez-Plaza,8). The elicitor treatments trigger the synthesis of phytochemical compounds in fruits. Specific elicitor application can be used to increase metabolite production in the plant and to enhance its qualitative value for fresh produce. Methyl jasmonate is one of the potent elicitors which plays an important role as a signaling molecule mediating intra- and inter-plant communications and modulating plant defense responses, including antioxidant systems (Creelman and Mullet, 3). Application of methyl jasmonate affects various plant processes include plant growth, physiological activities and biochemical processes (Creelman and Mullet, 3).

The pre-harvest application of methyl jasmonate has been reported to increase the nutritional quality of fruits in terms of phenolics, flavonoids, anthocyanins and antioxidant capacity in grapes (Ruiz-Garcia *et al.*, 9; Ruiz-Garcia and Gomez-Plaza, 8; Gil-Munoz *et al.*, 4; Portu *et al.*,7). In grapes, MeJA application enhances anthocyanin accumulation and total phenols (Portu *et al.*,7). Balanced use of fertilizers increases the yield, but excessive applications can have negative effects on fruit quality. Moderate nitrogen supplies before bloom favour the synthesis of polyphenols in the grape. However, an increase in yield is often associated with a berry size increase, so an increase in the pulp/skin ratio causes dilution of the anthocyanins and tannins.

Grape variety 'Pusa Navrang' is a high yielding grape variety, which is ideal for making juice and

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coloured wine. Its juice can be used as a coloured additive in fruit and vegetable juices and also for blending juice of white varieties of grapes. Hence, we attempted to observe influence of preharvest application of MeJA on postharvest quality of Pusa Navrang grapes.

MATERIALS AND METHODS

Ten-year-old 'Pusa Navrang' grape vines (*Vitis vinifera* L.) at the orchard of Division of Fruits and Horticultural Technology, ICAR- Indian Agricultural Research Institute, New Delhi were used for the study during 2016-17 and 2017-18. The experiment was laid out in factorial RBD with 3 replications. Three vines were randomly selected and the treatments were designated with four levels of MeJA (0, 5, 10, 15 mM) and three time of application. The treatment was given at veraison, 3 days after veraison and 6 days after veraison. The control vines were sprayed with normal water only. Berry quality, phenolic content and antioxidant activities were recorded after harvest maturity. Three lots of 100 g of undamaged berries were randomly selected from each treatment and were then used for physico-chemical analyses. Bunch and berry weight was determined using the electronic precision balance (Citizen, Made in India) in gram (g). Bunch length and width with pedicel was determined by using measuring scale in centimeter (cm). Berry diameter and length was determined by using of Digimatic caliper (Mitutoyo, Japan) in millimeter (mm). Seed number was determined by counting of seed number per berry. The juice recovery was measured and expressed in percent. The total soluble solids (TSS) of grape juice were measured with the help of digital refractometer (HI 96801, Hanna, Romania). Data were expressed as equivalent Brix. The digital pH meter was used to measure the pH of juice extracted from each sample. The pH meter was calibrated before taking the observations with buffer solution of known pH viz., 4.00, 7.00 and 9.00. The titratable acidity of the samples was determined by titration against 0.1N NaOH solution (AOAC,1). The content of the ascorbic acid was also analyzed by titration method (AOAC,1).

The total phenolic content was determined, using Folin-Ciocalteu reagent (FCR) method (Singleton *et al.*, 12) and measured through use of the spectrophotometer (Double beam UV-VIS Spectrophotometer, UV 5704SS, ECIL, India) and using Folin-Ciocalteu reagent and gallic acid as a standard. The total flavonoid content was measured spectrophotometrically (Zhishen *et al.*,17) and expressed as Quercetin equivalent (QE). The quantity of the total monomeric anthocyanin was measured by

UV-VIS Spectrophotometer (UV 5704SS, ECIL, India) using the pH-differential method (Wrolstad *et al.*, 15) and expressed as cyanidin-3-glucoside mg per kg of fresh weight. The antiradical capacity of the sample extract was measured using DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) with spectrophotometer at 515 nm (Sanchez-Moreno *et al.*, 10) and expressed in terms of Trolox equivalents. The significant differences among treatments and for each variable were assessed by analysis of variance (ANOVA) using SAS version 9.3 software (SAS, 11). LSD test was used to separate the means ($p = 0.05$) when ANOVA test was significant.

RESULTS AND DISCUSSION

The methyl jasmonate concentration as well as time of application independently has failed to produce any significant effect on TSS content of berries. However, the highest TSS was reported from MeJA (15 mM) with a value of 18.39°B (Table 1). Among the three application dates, minimum TSS was obtained when the berries were treated at 6 days after veraison. Combination of the two i.e., elicitor and application date also exerted a non-significant effect on TSS character. Almost all the treatments showed TSS values, those were statistically at par with each other. The above findings are in agreement with Karaman *et al.* (5), they reported in plum that MeJA treated fruits have lowest TSS as compared to control. Data pertaining to total titratable acidity indicated that neither methyl jasmonate levels nor time of application had significant effect in creating variation in total titratable acidity (Table 1). Treatment with MeJA @ 5mM helped to accumulate the highest acidity (0.91%) in the grape berries. It is quite interesting to note that use of elicitor (methyl jasmonate) and time of application induced significant variation for juice pH values. The highest pH (3.99) was noticed in MeJA (15mM) treated berries, whereas the lowest pH (3.86) was in case of control. The maximum juice pH was 4.03 in case of 15 mM MeJA at veraison. On the other hand, the juice from control berries showed relatively lower juice pH compared to various treatments. The above findings are in agreement with Karaman *et al.* (13), they reported that in MeJA treated fruits the amount of TSS, TSS/TTA was low and acidity was found high as compared to control. Grape berry ascorbic acid did not get affected significantly within the different time of application (Table 2). On the contrary, methyl jasmonate (5 mM) had significantly different effect on the ascorbic acid content of berries compared to control treatment. The ascorbic acid content of 3.76 mg/100 ml of juice with 5 mM MeJA at 6 days after veraison was significantly different from 6.79

Table 1. Effect of methyl jasmonate on total soluble solids (TSS), TSS: Acid ratio, juice pH and total titratable acidity (TTA) in 'Pusa Navrang' grapes.

Methyl jasmonate levels	TSS (°Brix)	TSS: Acid ratio	Juice pH	TTA (%)
MeJA 5 mM	18.36 ^a	20.32 ^a	3.98 ^b	0.91 ^a
MeJA 10 mM	18.29 ^a	21.31 ^a	3.93 ^c	0.86 ^a
MeJA 15 mM	18.39 ^a	21.94 ^a	3.99 ^a	0.84 ^a
Control	18.00 ^a	21.80 ^a	3.86 ^d	0.83 ^a
LSD (0.05%)	0.45	2.06	0.01	0.08
Time of application				
At veraison	18.37 ^a	21.46 ^a	3.97 ^a	0.86 ^a
3 days after veraison	18.27 ^a	21.49 ^a	3.91 ^c	0.86 ^a
6 days after veraison	18.15 ^a	21.08 ^a	3.93 ^b	0.87 ^a
LSD (0.05%)	0.39	1.78	0.01	0.07
MeJA × Time of application				
MeJA 5 mM at veraison	18.25 ^a	20.17 ^a	3.94 ^f	0.91 ^a
MeJA 5 mM at 3 DAV	18.35 ^a	20.58 ^a	3.94 ^{ef}	0.91 ^a
MeJA 5 mM at 6 DAV	18.48 ^a	20.21 ^a	3.97 ^{bc}	0.92 ^a
MeJA 10 mM at veraison	18.68 ^a	20.97 ^a	3.96 ^{de}	0.90 ^a
MeJA 10 mM at 3 DAV	18.12 ^a	21.56 ^a	3.88 ^g	0.84 ^a
MeJA 10 mM at 6 DAV	18.08 ^a	21.40 ^a	3.94 ^f	0.85 ^a
MeJA 15 mM at veraison	18.53 ^a	21.82 ^a	4.03 ^a	0.85 ^a
MeJA 15 mM at 3 DAV	18.37 ^a	21.67 ^a	3.99 ^b	0.85 ^a
MeJA 15 mM at 6 DAV	18.28 ^a	22.34 ^a	3.97 ^{cd}	0.83 ^a
Control at veraison	18.00 ^a	22.87 ^a	3.88 ^g	0.79 ^a
Control at 3 DAV	18.25 ^a	22.14 ^a	3.84 ^h	0.83 ^a
Control at 6 DAV	17.75 ^a	20.38 ^a	3.84 ^h	0.88 ^a
LSD (0.05%)	0.78	3.56	0.01	0.14

Abbreviations: MeJA= methyl jasmonate, DAV = Days after veraison, LSD = Least significant difference. Different letters in the same row indicate significant differences according to the LSD test ($p < 0.05$).

mg/100 ml of juice with 10 mM MeJA at veraison. The treatment combination of 10 mM MeJA at veraison stood significantly distinct from the rest two application dates in combination with it.

Total phenolics content of the grape berries was significantly influenced by the different concentrations of methyl jasmonate and also the time of application. Among all the levels, MeJA applied at 10mM was found to be most favourable in enhancing the phenolics content, that reached upto 460.58GAE, mg /100g. The days of application also plays a key role in increasing the total phenolics in grapes. The

veraison and 3 days after veraison stage had more or less similar kind of effect on phenolics, whereas, 6 days after veraison stage remained distinct from the above two. However, the interaction effects were found most synergistic (Fig. 1). Application of MeJA (10 mM) applied at veraison improved the maximum total phenolics (504.58 GAE, mg /100g) by 78.2% as compared to rest of the treatment combinations. Similarly, there was 17.4 %, 65.84 %, 56.83 % increase in total phenolics in 5 mM MeJA at veraison, 10 mM MeJA at 3 days after veraison and 10 mM MeJA at 3 days after veraison respectively. Karaman *et al.* (5) also recorded that MeJA help to improve the total phenolic content of fruits as compared to control. Ruiz-Garcia and Gomez-Plaza (8) and Creelman and Mullet (3) explained the rise in levels due to induction of genes related to polyphenol oxidase by jasmonic acid.

Methyl Jasmonate (15mM) was proved to be the most potent dosage in increasing the flavonoids concentration to the maximum (359.74 QE, mg /100g). Similarly, veraison stage was treated as the most suitable stage for applying treatment for the purpose of obtaining highest total flavonoids. The interaction effect of MJ and days of application resulted in 12 different flavonoids profile, those were clearly distinguishable from each other (Fig. 2). It is quite interesting to mention that, the treatment 10 mM MeJA at veraison, which was found best for phenolics also proved to be best suited for flavonoids to increase their level to the highest peak i.e. 418.33 QE, mg /100g and this increase was more than twice the amount of that in control. However, the lowest amount of flavonoids was seen in control at veraison

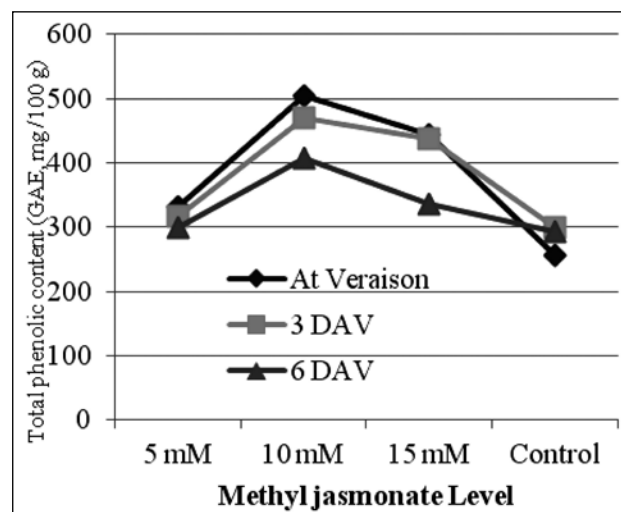


Fig. 1. Effect of different levels of methyl jasmonate on total phenolics content of Pusa Navrang grapes.

Table 2. Effect of methyl jasmonate on ascorbic acid content, total phenolics, total flavonoid, total monomeric anthocyanins content in 'Pusa Navrang' grapes.

Methyl jasmonate levels	AAC (mg/100 ml of juice)	Total Phenolics (GAE, mg /100 g)	Total flavonoids (QE, mg /100 g)	TMA (C ₃ GE mg/kg fresh berry wt.)
MeJA 5 mM	4.18 ^b	316.24 ^c	245.99 ^c	1058.56 ^c
MeJA 10 mM	5.88 ^a	460.58 ^a	355.29 ^b	1286.83 ^a
MeJA 15 mM	6.34 ^a	406.27 ^b	359.74 ^a	1250.07 ^b
Control	5.61 ^a	283.14 ^d	197.52 ^d	826.78 ^d
LSD (0.05%)	0.90	4.69	2.47	4.35
Time of application				
At veraison	5.70 ^a	384.66 ^a	317.05 ^a	1172.87 ^a
3 days after veraison	5.47 ^a	380.61 ^a	299.04 ^b	1109.81 ^b
6 days after veraison	5.33 ^a	334.41 ^b	252.82 ^c	1034.00 ^c
LSD (0.05%)	0.78	4.07	2.14	3.77
MeJA × Time of application				
MeJA 5 mM at veraison	4.27 ^{cd}	332.50 ^e	222.86 ^h	1074.33 ^g
MeJA 5 mM at 3 DAV	4.51 ^{bcd}	316.11 ^f	266.02 ^f	1067.14 ^g
MeJA 5 mM at 6 DAV	3.76 ^d	300.12 ^g	249.09 ^g	1034.22 ^h
MeJA 10 mM at veraison	6.79 ^a	504.58 ^a	418.33 ^b	1434.43 ^a
MeJA 10 mM at 3 DAV	5.24 ^{abcd}	469.58 ^b	353.61 ^d	1317.11 ^c
MeJA 10 mM at 6 DAV	5.63 ^{abcd}	407.56 ^d	293.93 ^e	1108.95 ^f
MeJA 15 mM at veraison	6.32 ^{abc}	444.05 ^c	439.31 ^a	1344.28 ^b
MeJA 15 mM at 3 DAV	6.62 ^{ab}	437.58 ^c	379.58 ^c	1237.93 ^d
MeJA 15 mM at 6 DAV	6.07 ^{abc}	337.20 ^e	260.32 ^f	1167.99 ^e
Control at veraison	5.42 ^{abcd}	257.50 ^h	187.68 ^k	838.45 ^j
Control at 3 DAV	5.52 ^{abcd}	299.17 ^g	196.94 ^j	817.05 ^j
Control at 6 DAV	5.87 ^{abc}	292.76 ^g	207.93 ^j	824.85 ^j
LSD (0.05%)	1.55	8.13	4.28	7.53

Abbreviations: MeJA= Methyl jasmonate, DAV = Days after veraison, AAC = Ascorbic acid content; TMA = Total monomeric anthocyanin, C₃GE = Cynidin-3-glucoside equivalent, GAE = Gallic acid equivalent, QE = Quercetin equivalent, LSD = Least significant difference. Different letters in the same row indicate significant differences according to the LSD test ($p < 0.05$).

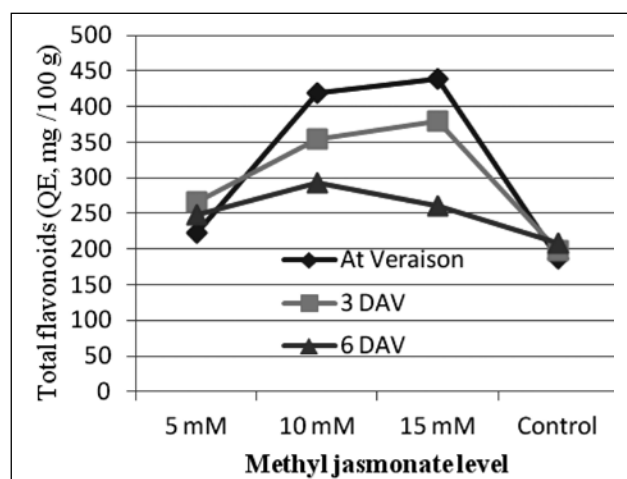


Fig. 2. Effect of different levels of methyl jasmonate on total flavonoid content of Pusa Navrang grapes.

(187.68 QE, mg/100g). Ruiz-Garcia and Gomez-Plaza (8) also reported that, methyl jasmonate levels affects widely with different concentrations through activation of the enzymes involved in flavonoid biosynthesis pathways.

Our data revealed that the methyl jasmonate significantly influenced total anthocyanin content in the grape berries (Table 2). The maximum content was accumulated by MeJA 10 mM (1286.83 C₃GE mg/kg fresh weight). Interestingly, the days of application also exerted a significant influence. The maximum anthocyanin concentration gets accumulated in the berries, when these were treated at veraison stage (Fig. 3). The highest TMA was recorded for treatment 10 mM MeJA applied at veraison stage (1434.43 C₃GE mg/kg fw). The TMA content for control berries was 838.45 C₃GE mg/kg fw at veraison stage. Henceforth, the best treatment

for enhancing the berry with anthocyanins was found to be 10 mM MeJA at veraison stage, which caused 73.49 % increase in TMA content. Similarly, there was 29.94%, 59.30% and 62.59% increase in TMA content in the treatments 5 mM MeJA at veraison, 10 mM MeJA at 3 days after veraison, 15 mM MeJA at veraison respectively. Ruiz-Garcia *et al.* (9) also reported in grapes that, MeJA application increased the anthocyanins by increasing the enzymatic activity of phenylpropanoid pathway.

FRAP antioxidant activity was found significant among all the treatment combinations (Table 3). Both levels viz., MeJA 10 mM and MeJA 15 mM were significantly different from the rest two i.e. MeJA 5 mM and control. However, the maximum FRAP activity was 16.75 TE, $\mu\text{mol/g}$ in case of MeJA (10 mM). Veraison stage was supposed to be the ideal stage for obtaining highest antioxidant activity by using elicitation process with methyl jasmonate.

However, the two days of application i.e. veraison and 3 days after veraison exhibited almost similar kind of effect on enhancing antioxidant activity in terms of FRAP. The FRAP antioxidant activity was found to be maximized by the application of 10 mM MeJA at veraison to the extent of 19.44 TE, $\mu\text{mol/g}$ (Fig. 4). The corresponding FRAP antioxidant activity for 10 mM MeJA at 3 days after veraison (17.54 TE, $\mu\text{mol/g}$), 15 mM MeJA at veraison (18.05 TE, $\mu\text{mol/g}$) and 15 mM MeJA at 3 days after veraison (16.87 TE, $\mu\text{mol/g}$) were considerably higher than the control berries. The positive effect of MeJA for improving antioxidant activity in terms of FRAP assay was also reported by Karaman *et al.* (5).

This study demonstrated the, the higher bioactive compounds accumulated in grape cv. Pusa Navrang berries after treated with MeJA. The evaluation of phenolics, flavonoids and

Table 3. Effect of methyl jasmonate on antioxidant activity in terms of FRAP assay and leaf nutrient content (N, P, K) in 'Pusa Navrang' grape.

Methyl jasmonate levels	FRAP (TE, $\mu\text{mol/g}$)	Leaf Nitrogen (%)	Leaf Phosphorus (%)	Leaf Potassium (%)
MeJA 5 mM	10.43 ^b	2.70 ^b	0.32 ^b	1.82 ^b
MeJA 10 mM	16.75 ^a	2.45 ^c	0.25 ^c	1.40 ^c
MeJA 15 mM	16.15 ^a	1.47 ^d	0.18 ^d	1.09 ^d
Control	10.05 ^b	2.85 ^a	0.38 ^a	2.17 ^a
LSD (0.05%)	0.48	0.10	0.03	0.12
Time of application				
At veraison	14.33 ^a	2.58 ^a	0.31 ^a	1.81 ^a
3 days after veraison	14.05 ^a	2.31 ^b	0.27 ^b	1.59 ^b
6 days after veraison	11.66 ^b	2.22 ^b	0.26 ^b	1.46 ^b
LSD (0.05%)	0.41	0.08	0.02	0.10
MeJA \times Time of application				
MeJA 5 mM at veraison	9.75 ^{fg}	2.88 ^b	0.35 ^{ab}	2.12 ^a
MeJA 5 mM at 3 DAV	10.92 ^e	2.68 ^{bcd}	0.31 ^{bc}	1.80 ^b
MeJA 5 mM at 6 DAV	10.63 ^{ef}	2.55 ^{cde}	0.29 ^{cd}	1.53 ^{bcd}
MeJA 10 mM at veraison	19.44 ^a	2.54 ^{cde}	0.28 ^{cd}	1.62 ^{bc}
MeJA 10 mM at 3 DAV	17.54 ^{bc}	2.46 ^{de}	0.23 ^{def}	1.37 ^{cde}
MeJA 10 mM at 6 DAV	13.26 ^d	2.37 ^e	0.24 ^{ef}	1.21 ^{ef}
MeJA 15 mM at veraison	18.05 ^b	1.72 ^f	0.21 ^{efg}	1.26 ^{def}
MeJA 15 mM at 3 DAV	16.87 ^c	1.42 ^g	0.17 ^{fg}	1.01 ^f
MeJA 15 mM at 6 DAV	13.54 ^d	1.27 ^g	0.15 ^g	0.99 ^f
Control at veraison	10.08 ^{efg}	3.17 ^a	0.39 ^a	2.22 ^a
Control at 3 DAV	10.86 ^{ef}	2.69 ^{bc}	0.38 ^a	2.16 ^a
Control at 6 DAV	9.20 ^g	2.69 ^{bc}	0.38 ^a	2.13 ^a
LSD (0.05%)	0.82	0.17	0.05	0.20

Abbreviations: MeJA= Methyl jasmonate, DAV = Days after veraison, FRAP = Ferric reducing antioxidant power, TE = Trolox equivalent, LSD = Least significant difference. Different letters in the same row indicate significant differences according to the LSD test ($p < 0.05$).

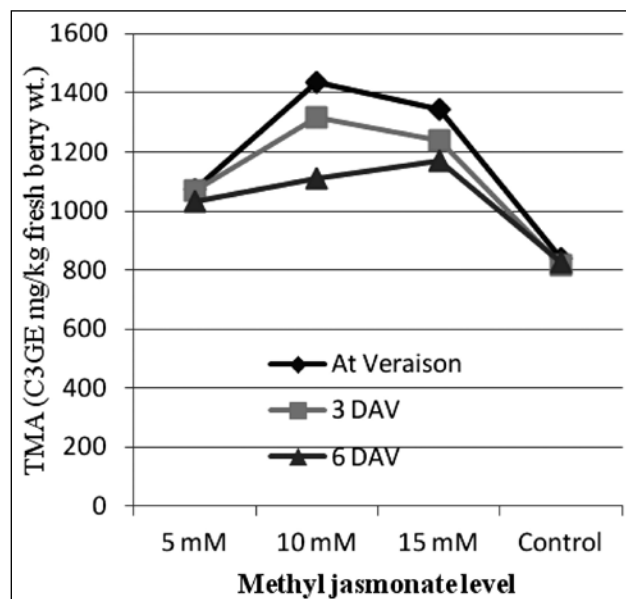


Fig. 3. Effect of different levels of methyl jasmonate on total monomeric anthocyanin (TMA) content of grapes cv. Pusa Navrang.

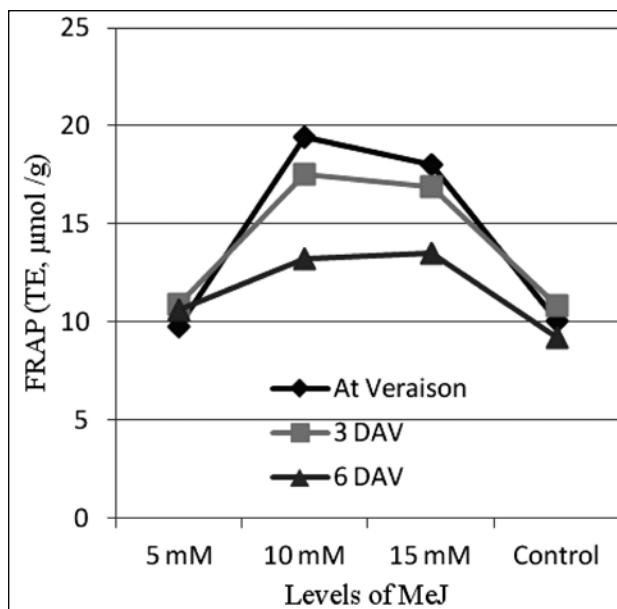


Fig. 4. Effect of different levels of methyl jasmonate on antioxidant activity (FRAP assay) of grapes cv. Pusa Navrang.

anthocyanins as the responsible components for the higher antioxidant activity. These compounds have been reported to show high activity of nutraceutical compounds. Thus, MeJA has potential application in pre-harvest treatment as a useful tool for the induction of health benefitting chemicals in the plant diet.

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