Establishing Good Agricultural Practices for the Use of Chlormequat Chloride in Grapes to Improve Vine Vigour, Fruitfulness, and Revision of MRL

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Chlormequat chloride (CCC), a gibberellin biosynthesis inhibitor, is widely used in vineyards to regulate vegetative growth and promote berry elongation in grapes. However, its low maximum residue limit (MRL) of 0.05 mg/kg poses challenges for grape exports due to stringent food safety regulations. This study aimed to enhance the maximum residue level (MRL) and revise good agricultural practices (GAP) for CCC application to manage grapevine vigour, enhance fruitfulness, and generate residue data for MRL revision and risk assessment. Field trials were conducted across three locations in Maharashtra, India, during the 2021-2022 and 2022-2023 grape seasons. CCC was applied at rates of 250–2000 g/ha at various growth stages of Thompson Seedless grapevines. Residue analysis using liquid chromatography tandem mass spectrometry (LC-MS/MS) demonstrated that revised CCC treatments significantly reduced shoot length and internodal growth while increasing cane diameter compared to current GAP recommendations. The proposed GAP includes foliar applications of CCC at 1500 g/ha (11-12 leaf stage) and 2000 g/ha (15-16 leaf stage) after foundation pruning, followed by 250 g/ha (3-5 leaf stage) after fruit pruning. This regimen achieved a maximum residue of 0.2 mg/kg, supporting its safety for consumer health. The study will allow growers to achieve higher yields of grapes with better and safer quality subject to revision of the national MRL to 0.2 mg/kg without causing any appreciable health risks to consumers.

INTRODUCTION

Grape (*Vitis vinifera* L.) is an important commercial fruit crop of India covering an area of 175.93 thousand hectares with an average productivity of 21 MT/ha. Grape is mostly produced in peninsular India [Maharashtra (70.67%), Karnataka (24.49%), Tamil Nadu (1.43%), Andhra Pradesh (1.34%)], and also in Madhya Pradesh (1.02%) and Mizoram (0.50%) (Sharma *et al.,* 2023). In 2022–2023, India exported 343,982.34 MT of grapes for a total value of INR 3460.70 crores (APEDA,2023).

During the growth of grapevines, branches are usually pruned to remove the secondary tips (Di Lorenzo *et al*., 2011), which not only consume nutrients but also affect light distribution, air movement, and berry quality. Thus, removal of the secondary tips is an important cultural operation, which is labour-intensive and a time-consuming activity. Bioregulators have various applications in the management of vegetative and reproductive growth of grapevines, and such chemicals are mostly used for cluster thinning, faster maturity, increasing berry size, and restricting vegetative growth (Jegadeeswari, 2008). Chlormequat Chloride (CCC) is a chemical compound (2-Chloroethyltrimethyl ammonium chloride) that exerts its biological effects by inhibiting a specific step in the gibberellin biosynthesis pathway, a key process in the growth regulation of plants (Lone *et al.,* 2010; Pertot *et al.,* 2017). Thus, its application reduces the excess vigour thereby shoot length of grapevines as reported for the cultivar Tas-A-Ganesh (Rademacher, 2000), a mutant of Thompson Seedless.

In India, the south-western part of Maharashtra state is known for high-quality grape production. The grape farmers use CCC to minimize the problem of foliage and sustain the production of grapes during adverse climatic conditions. However, CCC is known to have a tendency to build up its residues in plant system. The European Food Safety Authority (EFSA) concluded that the residues of CCC in table grapes are not likely to pose any consumer health risk (acute toxicity) if the measured concentration is within 1.06 mg/kg (EFSA, 2010). But, its maximum residue limit (MRL) in

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both the EU as well as India is set at the analytical limit of quantification (LOQ) of 0.05 mg/kg (FSSAI, 2020), which is creating the problems of rejection of grape consignments due to detection of CCC residues above the MRL. If this MRL is increased to a higher level, that would facilitate regulatory compliance and, in turn, enhanced the sale of grapes. To assist grape growers in enhancing the production and quality of compliant grapes, it is necessary to raise the MRL to a higher value. Revising the MRL requires adjusting the Good Agricultural Practices (GAP) recommendations. To address this issue, bio-efficacy and residue studies on CCC in grapes were conducted systematically in multiple grape-growing locations representing diverse agro-climatic conditions.

In the current experiment, the effects of CCC on the morphological characteristics of grapevines were investigated. Additionally, the harvest day residues of CCC were evaluated following its applications at different crop growth stages, varying dosages, and across three different locations.

MATERIALS & METHODS

Experimental details

The experiment was conducted at three different locations *i.e.* Nashik (20.28°N and 73.91°E), Solapur (17.62°N and 75.33°E), and Pune (18.32°N and 73.51°E) districts of Maharashtra, India, during 2021-22 and 2022-23 on the grape cultivar Thompson Seedless trained on an extended Y trellis system at a spacing of 2.74 m × 1.52 m. Vines of uniform canopy, structure, and size were selected for the study. The experiment was laid out in a randomized block design (RBD) with five treatments, each having four replications. The standard package of practices was followed for the management of the vineyard at each location. CCC was sprayed at different growth stages during both foundation and fruit pruning as per the treatment details presented in Table 1.

Morphological parameters

The morphological parameters *viz.,* shoot length (cm), internodal length (cm), cane diameter (mm) and leaf area (cm^2) were recorded on the 90th day after foundation and fruit pruning at each location using a measuring tape and digital vernier caliper (0–300 mm RSK™). Fruitfulness was estimated by dissecting the buds under a stereoscopic microscope to count the tiny bunch primordia that get converted into grape bunches after forward pruning. All buds from ten randomly selected canes (from each replicate of the respective treatments) were analyzed under a microscope and were expressed as good, small, absent, joint, and necrotic. The fruitfulness percentage was calculated by using the following formula (Iland *et al*., 2011).

$$\text{Fruitfulness}(\%) = \frac{\text{Well developed cluster primordial}}{\text{Total number of buds}} \text{ x } 100$$

Bunch yield and quality parameters

Bunch yield was calculated by weighing grape bunches from a composite sample of each vine and expressed as kg/vine. Total Soluble Solids (TSS) were determined using a digital refractometer and expressed as °Brix. Titratable acidity (%) was estimated by titrating 5 ml of grape juice (diluted to 100 ml) with 0.1 N NaOH using phenolphthalein as an indicator, with the endpoint indicated by a light pink color (Ranganna, 2011).

TABLE 1
Treatment and application details of Chlormequat Chloride

Treatment	Pruning season	Crop stage	Dose (g a.i./ha)
*T_1 (Standard check)	Foundation pruning	3-5 leaf stage	500
		5- 7 leaf stage	1000
	Fruit pruning	3-5 leaf stage	250
T_2	Foundation pruning	3-5 leaf stage	500
		After first sub- cane (11-12 leaf stage)	1000
		15- 16 leaf stage	1500
	Fruit pruning	3-5 leaf stage	250
T_3	Foundation pruning	3-5 leaf stage	1000
		After first sub- cane (11-12 leaf stage)	1500
		15- 16 leaf stage	2000
	Fruit pruning	3-5 leaf stage	500
T_4	Foundation pruning	After first sub- cane (11-12 leaf stage)	1500
		15- 16 leaf stage	2000
	Fruit pruning	3-5 leaf stage	250
T_5 (control)	Untreated	-	-

*T_1 (Current GAP of CIB & RC)

Sample collection for residue studies

To analyze the residues of CCC in grapes, approximately 500 g of grape berries were randomly collected from each replicate of both CCC-treated and untreated control plots. The samples from each location were transported to the laboratory in dry ice and stored at 0 (± 2) °C. Further, the grape berries were separated from their pedicels, crushed, homogenized, and analyzed for CCC without washing or any pre-treatment.

Chemicals and Reagents

The certified reference material (CRM) of Chlormequat chloride (99.55% purity) was obtained from Dr Ehrenstorfer GmbH (Augsburg, Germany). Water (HPLC grade), methanol, and formic acid (LC-MS-grade from J.T. Baker, Radnor, Pennsylvania), ammonium formate (99%) and formic acid from Fisher Scientifics, Mumbai were utilized during the sample preparation.

Preparation of reference standard solution for residue analysis

The stock solution of CCC was prepared by dissolving 10 (± 0.1) mg of CRM in 10 mL of LC-MS grade methanol solvent, resulting in a concentration of 1000 mg/L. The solution was stored at -20 °C for further experimentation. Intermediate stock (10 mg/L) and working standard solutions (1 mg/L) were prepared by serial dilution of the primary stock and intermediate solutions, respectively, in methanol, using 10 mL volumetric flasks. The calibration standards, ranging from 1 to 100 ng/g, were prepared by diluting the working solution in a 1:1 mixture of methanol and water. The matrix-matched calibration standards at the same concentrations were concurrently prepared by spiking blank grape matrix extracts to account for matrix effects during analysis.

Sample preparation

The grape samples were extracted using the earlier methodology reported by Oulkar *et al*. (2011). The homogenized grapes (10 ± 0.1 g) were extracted with HPL grade acidified methanol (20 mL + 1% formic acid). After that, the extract was centrifuged at 5000 rpm for 5 min. next, an aliquot of the supernatant (500 µL) was mixed with 500 µL of LC-MS-grade methanol in a vial. Finally, the solution was filtered through a nylon membrane (0.2 µm), and then measured for CCC residues by LC-MS/MS.

Liquid chromatography tandem mass spectrometry (LC-MS/MS)

For the analysis of Chlormequat chloride (CCC) in grapes, a prominence UFLC XR system (Shimadzu) was coupled with an API 4000 hybrid triple quadrupole/linear ion trap (QqQLIT) mass spectrometer. UFLC chromatographic separation was performed on a Luna HILIC analytical column (150 mm × 2.0 mm, 3 µm particle size). The mobile phase consisted of (A) water containing 10 mM ammonium formate and 0.1% formic acid, and (B) acetonitrile. A gradient elution was applied with the following program: 10% B (0–1 min), 10–95% B (1–2.5 min), 95% B (2.5–7 min), 95–10% B (7–8 min), and 10% B (8–10 min). The mobile phase flow rate was set at 0.7 mL/min, and the column oven temperature was maintained at 40 °C.

CCC detection was performed using electrospray ionization (ESI) in positive mode with a multiple reaction monitoring (MRM) method, employing a dwell time of 100 ms. The mass transitions for CCC were monitored at 122/58 m/z (quantifier ion) and 122/59 m/z (qualifier ion). The declustering potential (DP), collision energy (CE), and cell exist potential (CXP) were 68 (V), 37 (V) for quantifier ion and 26 (V) for qualifier ion, and 14 (V), respectively. Key source parameters included a nebulizer gas pressure of 50 psi, heater gas pressure of 50 psi, ion source temperature of 500 °C, and an ion spray voltage of 4.5 kV in positive ionization mode. Data acquisition and processing were performed using Analyst software version 1.7.1.

Method validation

The performance of the analytical method was evaluated as per the DG SANTE guideline of Europe, SANTE/11312/2021 (SANTE, 2021). Accordingly, limit of detection (LOD), limit of quantification (LOQ), matrix effect, linearity, precision, and accuracy were evaluated. At LOD, the signal-to-noise ratio (S/N) of CCC was 3, whereas at LOQ, S/N was ≥ 10 in the spiked sample matrix. The linearity of CCC was determined by plotting a calibration graph with different concentrations (1-100 ng/g) of standards in solvent as well as matrix extract. The precision in terms of repeatability in recovery was calculated as relative standard deviation (RSD, %). Accuracy (recovery, %) was estimated at 0.01, 0.05, and 0.1 mg/kg levels. The matrix effect (ME) was calculated by using the following formula.

ME% = (Peak area of matrix matched standard - Peak area of solvent standard) × 100 Peak area of matrix matched standard.

Statistical Analysis

Using SAS software, version 9.3, significant differences among the variables were determined using analysis of variance (ANOVA). Means of the variables were separated using the least significant difference (LSD) at $p \leq 0.05$ when the F test result was significant. For residue data analysis, Microsoft Excel was used to calculate the RSD, ME, and to calculate the mean recovery values from different replications.

RESULTS

Effect of CCC on grapevine vigour after foundation pruning

The grapevine vigour was measured in terms of shoot length, internodal length, leaf area and cane diameter. The pooled results (2021-2022 and 2022-2023) showed that an increase in dose of CCC after foundation pruning caused a significant reduction in shoot length, internodal length, and leaf area, with an increase in cane diameter at all the selected locations *i.e*., Location-I: Nashik, Location-II: Solapur, and Location-III: Pune (Table 2). Among the different CCC treatments, T_4 after foundation pruning + 250 g per ha (at 3-5 leaf stage) after fruit pruning recorded the minimum shoot length at selected locations [(I, 114.64 cm), (II, 104.51 cm), (III, 112.38 cm), respectively. This was followed by T_3 (115.77 cm; 110.14 cm and 115.74 cm), respectively, for

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TABLE 2
Effect of CCC on grapevine vigour after foundation pruning (Pooled data 2021-22 and 2022-23)

Treatments	Location-I (Nashik)				Location-II (Solapur)				Location-III (Pune)			
	Shoot length (cm)	Internodal length (cm)	Cane diameter (mm)	Leaf area (cm^2)	Shoot length (cm)	Internodal length (cm)	Cane diameter (mm)	Leaf area (cm^2)	Shoot length (cm)	Internodal length (cm)	Cane diameter (mm)	Leaf area (cm^2)
*T_1	119.60	6.10	7.23	182.52	115.51	5.83	7.50	179.75	122.86	6.05	7.25	178.85
T_2	117.31	5.82	7.74	180.70	113.63	5.55	8.26	177.30	118.08	5.80	7.60	175.45
T_3	115.77	5.13	8.03	178.19	110.14	5.41	8.39	175.82	115.74	5.26	7.81	172.50
T_4	114.64	5.04	8.55	175.08	104.51	5.05	8.68	174.44	112.38	4.95	8.32	168.08
T_5	124.87	6.33	6.65	187.30	122.87	6.16	7.14	184.75	127.80	6.31	6.78	181.83
S.E. (m)±	1.05	0.05	0.07	0.30	1.13	0.06	0.07	0.63	1.10	0.08	0.09	1.52
CD @ 5%	3.08	0.16	0.19	0.94	3.31	0.16	0.21	1.97	3.22	0.23	0.26	4.72

*T_1 (Current GAP of CIB & RC).

Nashik, Solapur, and Pune. The highest shoot length was recorded in control (T_5), followed by T_1 i.e., the current GAP as per Central Insecticides Board and Registration Committee (CIB & RC, 2020), Government of India.

Similarly, internodal length was the minimum in T_4 at all three locations [5.04 cm (Nashik); 5.05 cm (Solapur) and 4.95 cm (Pune)], while maximum internodal length was recorded in control [T_5: 6.33 cm; 6.16 cm and 6.31 cm in Nashik, Solapur and Pune, respectively], followed by T_1. The leaf area was the minimum in T_4 at selected locations [175.08 cm^2 (Nashik); 175.44 cm^2 (Solapur), and 168.08 cm^2 (Pune)], while the maximum leaf area was recorded in the control, followed by T_1. The cane diameter was the highest in T_4 at selected locations [8.55 mm (Nashik), 8.68 mm (Solapur), and 8.32 mm (Pune)]. Similar to the earlier instances, the lowest cane diameter was noticed in T_5 [6.65 mm (Nashik); 7.14 mm (Solapur), and 6.78 mm (Pune)], followed by T_1.

Effect of CCC on grapevine vigour after fruit pruning

During fruit pruning, grapevine vigour was measured in terms of shoot length, internodal length, leaf area, and cane diameter. The results of two years of different locations were pooled, which showed significant effects of different concentrations of CCC on shoot length, internodal length, leaf area, and cane diameter at different locations (Table 3). Similar to the results obtained after foundation pruning, among the different treatments, T_4 recorded the minimum shoot length at selected locations [112.74 cm (Nashik), 90.94 cm (Solapur), and 76.32 cm (Pune)]. However, the highest shoot length was recorded in control T_5 (125.12 cm; 106.51 cm and 105.64 cm), followed by the current GAP (T_1). Similarly, internodal length was the minimum in T_4 at all the selected locations, while the maximum internodal length was recorded in control T_5, followed by T_1. The leaf area was the minimum in T_4 at selected locations, while the maximum leaf area was recorded in the control, followed by T_1. The cane diameter was the highest in T_4 at all locations. And, the lowest cane diameter was noticed in control (T_5), followed by T_1.

Effect of CCC on grapevine fruitfulness

The results related to the influence of CCC on grapevine fruitfulness recorded after fruit pruning are presented in Table 4. Pooled results of two-year studies showed significant effects of different concentrations of CCC on grapevine fruitfulness. Among the different treatments T_4 recorded the highest fruitfulness at the selected locations [84.22% (Nashik), 83.20% (Solapur) and 83.50% (Pune)]. As expected, the lowest fruitfulness was recorded in control [T_5: 71.79% (Nashik), 70.44% (Solapur), and 70.8% (Pune), followed by T_1.

Effect of CCC on bunch weight and yield per vine

The results related to the influence of CCC on bunch weight and yield per vine recorded after fruit pruning are presented in Table 4. The bunch weight was the highest in T_4 at selected locations (355.7 g, 347.4 g and 354.5 g in Nashik, Solapur and Pune, respectively), while the lowest bunch weight was recorded in T_5, followed by T_1. Bunch yield per vine was

TABLE 3
Effect of CCC on grapevine vigour after fruit pruning (Pooled data 2021-22 and 2022-23)

Treatments	Location-I (Nashik)				Location-II (Solapur)				Location-III (Pune)			
	Shoot length (cm)	Internodal length (cm)	Cane diameter (mm)	Leaf area (cm^2)	Shoot length (cm)	Internodal length (cm)	Cane diameter (mm)	Leaf area (cm^2)	Shoot length (cm)	Internodal length (cm)	Cane diameter (mm)	Leaf area (cm^2)
*T_1	119.73	6.02	7.55	183.42	102.65	5.75	7.65	180.32	93.95	5.94	7.52	179.29
T_2	117.17	5.81	8.14	181.14	99.05	5.54	7.97	178.61	87.38	5.70	7.71	176.30
T_3	113.88	5.11	8.46	179.26	94.24	5.38	8.10	176.97	83.02	5.21	8.21	173.27
T_4	112.74	5.05	8.79	176.19	90.94	5.15	8.41	174.89	76.32	5.13	8.46	169.63
T_5	125.12	6.23	7.45	189.30	106.51	6.14	7.31	185.51	105.64	6.21	7.58	183.45
S.E. (m)±	1.51	0.06	0.08	0.85	0.59	0.05	0.07	0.79	0.95	0.08	0.10	1.11
CD @ 5%	4.43	0.16	0.22	2.65	1.73	0.16	0.19	2.45	2.78	0.23	0.30	3.46

*T_1 (Current GAP of CIB & RC)

TABLE 4
Effect of CCC on grapevine fruitfulness, bunch weight, yield and quality parameters (Pooled data 2021-22 and 2022-23)

Treatments	Location-I (Nashik)					Location-II (Solapur)					Location-III				
	Fruitfulness %	Bunch weight (g)	Bunch yield (kg/vine)	TSS (°Brix)	Acidity (%)	Fruitfulness %	Bunch weight (g)	Bunch yield (kg/vine)	TSS (°Brix)	Acidity (%)	Fruitfulness %	Bunch weight (g)	Bunch yield (kg/vine)	TSS (°Brix)	Acidity (%)
*T_1	76.48	310.30	14.26	18.30	0.76	75.41	306.60	12.26	18.30	0.76	75.20	329.90	13.19	18.47	0.73
T_2	78.17	324.30	14.77	18.30	0.74	77.66	364.20	14.57	18.40	0.75	77.00	337.20	13.49	18.54	0.72
T_3	82.17	341.50	15.41	18.50	0.72	81.47	332.30	13.29	18.60	0.71	81.00	347.20	13.89	18.68	0.70
T_4	84.22	355.70	15.96	18.70	0.69	83.20	347.40	13.89	18.80	0.69	83.50	354.50	14.18	18.76	0.68
T_5	71.79	336.10	13.32	18.00	0.78	70.44	280.20	11.21	18.30	0.78	70.80	317.70	12.71	18.32	0.76
S.E. (m)±	0.74	10.30	0.19	0.15	0.01	0.73	12.50	0.50	0.09	0.00	0.61	2.40	0.09	0.14	0.01
CD @ 5%	2.17	30.30	0.56	0.45	0.03	2.14	36.50	1.46	0.26	0.01	1.70	6.90	0.28	0.40	0.03

*T_1 (Current GAP of CIB & RC).

also the highest in T_4 [15.96 kg/vine (Nashik), 13.89 kg/vine (Solapur) and 14.18 kg/vine (Pune)]. The lowest bunch yield per vine was recorded in control [T_5: 13.32 kg/vine (Nashik), 11.21 kg/vine (Solapur), and 12.71 kg/vine (Pune)], followed by T_1.

Effect of CCC on quality

The results related to the effect of CCC on berry quality i.e., total soluble solids (TSS) and acidity are presented in Table 4. At harvest, the highest TSS in the range of 18.7-18.8 °Brix was recorded in T_4 at all locations. This was slightly higher than the control (18.0-18.3 °Brix). Similarly, acidity was significantly lower in T_4 (0.68-0.69%) as compared to control T_5 (0.76-0.78%).

Method validation

CCC was eluted at the retention time (RT) of 5.81 min (Fig. 1). The calibration linearity employing linear regression equation was established with $r^2 \geq 0.999$ for CCC matrix-matched standards (Fig. 2). Average recoveries for CCC were 98.13%, 100.36%, and 102.52% at fortification levels of 0.01 mg/kg, 0.05 mg/kg, and 0.1 mg/kg, respectively, with the corresponding precision-RSDs of 2.71%, 0.96%, and 4.90%. The matrix effect (ME) was recorded in terms of signal suppression (-32%). All the above-mentioned results confirmed that the method is suitable for the analysis of grape matrix according to the validation criteria established by SANTE 11312/2021 guidelines.

Effect of varying dosage and application time on residue concentrations of CCC

The residue data obtained from three locations for 2021-22 and 2022-23 are summarised in Table 5. In Location-III, residue levels ranged from 0.110 mg/kg (T_1) to 0.153 mg/kg (T_3) in 2021-22, and from 0.085 mg/kg (T_1) to 0.135 mg/kg (T_3) in 2022-23. Although these levels were lower than those observed in Location-I (ranged from 0.118-0.142 mg/kg and 0.109-0.158 mg/kg), the residues exceeded the MRL. Similarly, in Location-II, residue concentrations ranged from 0.107 mg/kg (T_1) to 0.169 mg/kg (T_3) in 2021-22, and from 0.096 mg/kg (T_1) to 0.153 mg/kg (T_3) in 2022-23. These values were comparable to those in Location-III but were also above the MRL. Among all treatments, T_3 consistently exhibited the highest residue levels across both years (and in all locations), significantly exceeding the MRL of 0.05 mg/kg.

DISCUSSION

The results showed that increased application rates of CCC significantly reduced grapevine vigor after foundation and fruit pruning at all three locations. Among the CCC treatments, T_4 was significantly superior in controlling vine vigor compared to the current GAP of CIB & RC. Significant reductions in shoot length, internodal length, and leaf area, with an increase in cane diameter, were observed under different CCC treatments during the study, with T_4 being significantly superior to T_1.

CCC inhibits gibberellic acid biosynthesis, influences the source-sink relationship, and stimulates the translocation of photosynthates towards the sink, decreasing shoot and internodal length significantly while increasing reserve food material. This aligns with the findings reported by Ibrahim *et al.* (1996), who mentioned that CCC treatment resulted in short, thick internodes and dark green leaves. Similarly, Kumar *et al.* (2006) noted that treatments of tri-iodobenzoic acid (TIBA), CCC, and mepiquat chloride were more beneficial for the translocation of photoassimilates towards developing reproductive parts compared to growth

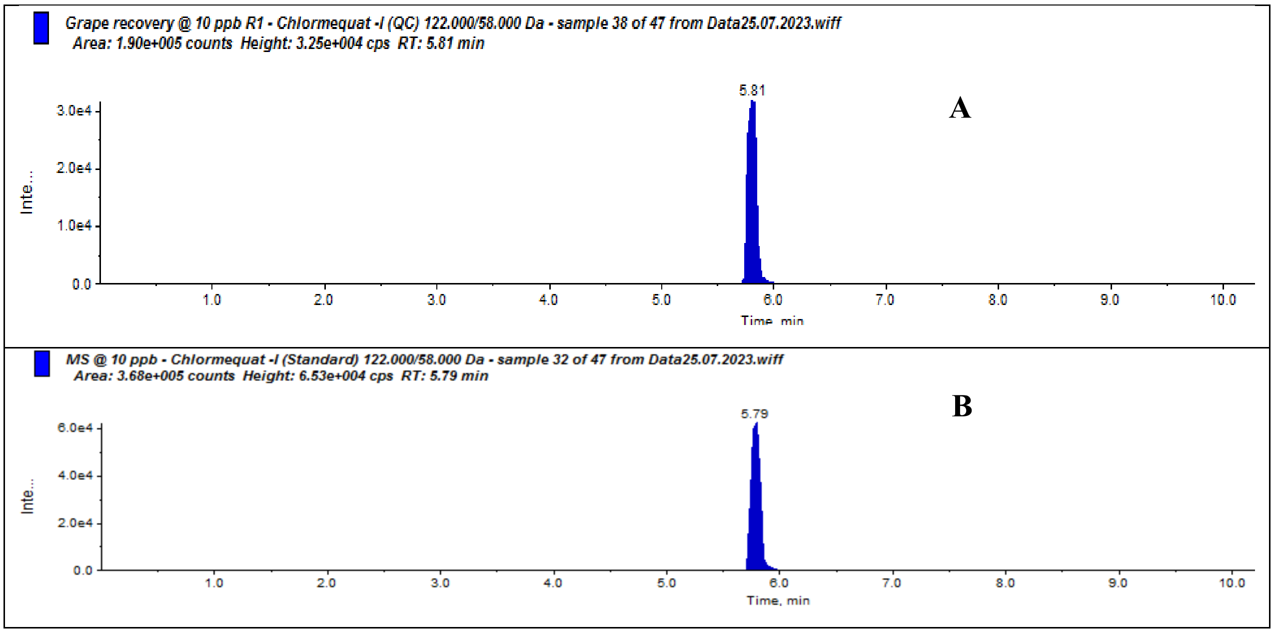
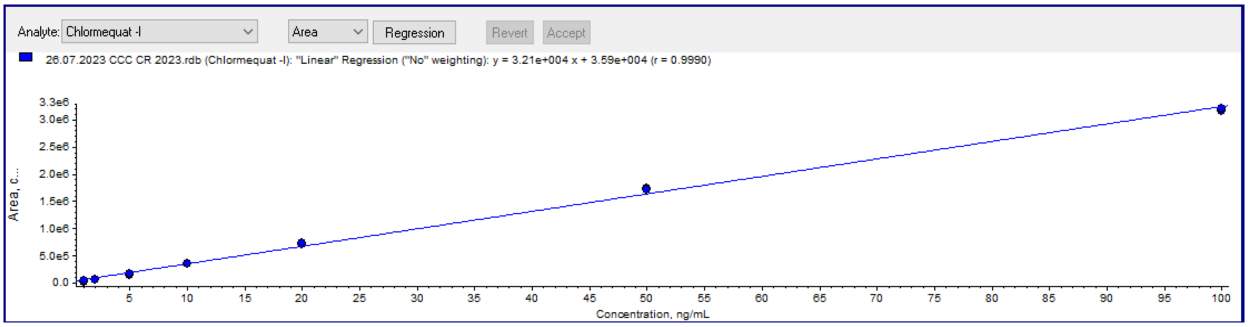


FIGURE 1
LC-MS/MS extracted ion chromatogram for Chlormequat chloride at 10 mg/kg; A: Matrix matched standard; B: Grape spiked sample.


Analyte: Chlormequat -I

Area

26.07.2023 CCC CR 2023.rdb (Chlormequat -I): "Linear" Regression ("No" weighting): y = 3.21e+004 x + 3.59e+004 (r = 0.9990)

FIGURE 2
Matrix-matched standard calibration curve for Chlormequat chloride.

TABLE 5
The residue concentration of CCC during grape season 2021-22 and 2022-23

Treatments	Residue concentration (mg/kg)						EU-MRL (mg/kg)
	2021-22			2022-23			
	Location-I (Nashik)	Location-II (Solapur)	Location-III (Pune)	Location-I (Nashik)	Location-II (Solapur)	Location-III (Pune)	
*T_1	0.103	0.107	0.110	0.109	0.096	0.085	0.05
T_2	0.142	0.118	0.127	0.158	0.109	0.098	
T_3	0.345	0.169	0.153	0.362	0.153	0.135	
T_4	0.157	0.127	0.136	0.161	0.119	0.112	

*T_1 (Current GAP of CIB & RC).

promoters. Increased carbohydrates due to optimal leaf area resulted in a better carbon to nitrogen (C:N) ratio, leading to better differentiation of vegetative buds into fruiting. Ramteke and Somkuwar (2005) also reported that CCC application reduced vine vigor, which is consistent with the findings in okra by Thanopoulos *et al*. (2013).

The physiological role of CCC is related to choline, which is involved in lipid metabolism and methylation reactions, resulting in shorter and thicker cane growth. Data showed that application of increased CCC concentrations led to thicker canes, which were more vigorous and better matured, as opposed to thin canes that are deficient in reserve food material. The findings of the current study conform to the findings of Koutroubas *et al*. (2014), who reported that CCC applications increased stem thickness Similarly, Morandi *et al*. (1984) noted a logarithmic relationship between stem shortening and CCC doses, reducing stem length and node number in soybean. CCC (as Cycocel) sprays did not significantly influence the vigor of Tas-A-Ganesh vines grafted on Dogridge rootstock, but there was a significant reduction in mean shoot length when CCC was applied multiple times with topping and side shoot removal. Increased fertile buds due to CCC applications were also reported by Motoike *et al*. (1996).

To determine potential bunch numbers, the developed shoots were examined and counted, the dormant buds were dissected, and examined microscopically. This method is beneficial for quickly determining potential bunches and detecting primary bud activity or necrosis, as well as the presence of bud mites, if any (Iland *et al*., 2011). Data showed that increase in concentration of CCC led to higher bud fruitfulness. Ahlawat and Daulta (1981) reported that CCC at 500 and 1000 ppm improved berry set and quality in Kishmish grapes. Bhat (1992) found that CCC spraying (at 1000 ppm) 21-25 days after pruning in April improved grape quality. Similarly, Ramteke and Somkuwar (2005) and Clingeleffer *et al*. (2001) noted that bunch numbers per grapevine accounted for 60-70 percent of seasonal yield variations.

In the EU, several cases of enhancing the MRLs of agrochemicals for trade facilitation have been noted in the recent past. These include increasing the MRL of tricyclazole in rice from 0.01 mg/kg to 0.09 mg/kg, backed by EFSA's thorough risk assessments deeming the higher level of safety to consumers (Bellisai *et al*., 2023), increase in MRL of mandipropamid in papaya from 0.01 mg/kg to 0.8 mg/kg, MRL enhancement of fenpyroximate in blackberry and raspberries, from 1 mg/kg to 1.5 mg/kg, to name some (EFSA, 2013). Such regulatory shifts align with the findings of the current experiments with CCC in Thompson Seedless grapes, supporting an MRL revision in India from the current value of 0.05 mg/kg to 0.2 mg/kg (actually 0.161 mg/kg, rounded to 0.2 mg/kg), the highest residue across the treatments, years, and locations. Based on this MRL revision, India will be in a position to request the EU authority to decide an India-specific tolerance limit for CCC in grapes (at above 0.05 mg/kg) to facilitate grape export. The revised MRL would ensure consumer safety while benefiting grape growers by allowing more effective CCC use, enhancing grape quality and yield, and leading to better compliance and economic outcomes for the grape industry.

The residue analysis indicates that despite following good agricultural and effective management practices, CCC residues exceeded the current MRL value across all treatments and locations, suggesting a need to reconsider the current EU-MRL for CCC in grapes to achieve regulatory compliance. Since EFSA has reported that up to 1.06 mg/kg of CCC residues do not pose any acute toxicity risk to human being, the MRL may be reset at 0.2 mg/kg, which is the highest residue across the doses, years, and study locations.

CONCLUSION

The current multi-location study on Thompson Seedless grapes in 2021-22 and 2022-23 showed a significant reduction in shoot length and internodal length with an increase in cane diameter during both foundation and fruit pruning in response to CCC treatments. Significant increase in fruitfulness was observed in CCC treatment T_4 when compared to T_5 (control) and T_1 (the current GAP of CIB & RC). Thus, the results revealed that the treatment T_4 [foliar application of CCC 1500 g per ha (after 1st sub cane i.e. 11-12 leaf stage) + 2000 g per ha (at 15- 16 leaf stage) + 250 g per ha after fruit pruning (3-5 leaf stage)] performed the best to control the vine vigor and improve fruitfulness in Thompson Seedless.

The residue data indicate similar results in all locations, which are higher than the current FSSAI (Indian) and EU MRL of 0.05 mg/kg. Thus, the highest residue of 0.2 mg/kg may be considered for the risk assessment to decide the new MRL. The revised GAP will effectively address grape growers' current needs. Increasing the FSSAI's MRL for CCC from 0.05 mg/kg to 0.2 mg/kg would greatly benefit the grape industry by facilitating domestic marketing as well as export. This change would enable growers to use CCC efficiently while ensuring compliance with the MRL at harvest. The study highlights that this adjustment will help growers achieve higher yields and better-quality of grapes, leading to enhanced financial benefits.

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