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Studies on the Residue of Hydrogen Cyanamide in Grape Berries

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Abstract Multilocational field trials were conducted in grapevines at four different locations by applying hydrogen cyanamide 50% SL during 2006–2007. In order to determine the residue of hydrogen cyanamide in grape, hydrogen cyanamide 50% SL was applied to the freshly pruned grapevines at the rate of 1.20% a.i./L, 2.40% a.i./L, 4.80% a.i./L along with untreated control. No residue was detected in grape berries at the time of harvest irrespective of any locations.

Keywords Hydrogen cyanamide · Plant growth regulator · Grape · Harvest time residue

Grape (*Vitis vinifera* L.) is cultivated on all the continents of the world, except Antarctica, and is the most widely distributed fruit crop. Every year, India earns a lot of foreign exchange by exporting grape to European countries including Middle East and the Far East. In India, most of the grape cultivation spread over states, viz. Maharashtra, Karnataka and Andhra Pradesh etc. For grape cultivation chilling climatic condition is essential. But in number of situations bud breaking is the unique problem in India, as chilling climate is the basic requirement for good grape cultivation. A number of plant growth regulators are being used to stimulate bud burst, causing a more uniform and increase percentage of bud break. Among them hydrogen cyanamide is one of them. It has been used for a number of years on fruit crops to replace lack of winter chilling, induce uniform bud break, enhance production efficiency and to optimize harvest timing and management (Subhadrabandu and Rakngan 1999; Austin et al. 2002; Clayton et al. 2003; Bound and Jones 2004; Stringer et al. 2004). It is widely used in grape orchard for enhanced and uniform bud burst, shortened flowering period and increase in yield (Williams 1987; George et al. 1988; Reddy and Shikhamany 1989; Rieden and Gehrmann 1992; Shukla et al. 1995). It was also observed that indiscriminant uses of hydrogen cyanamide may persist residue in grape berries at harvest. As very little information are available on the residues of hydrogen cyanamide in grape berries at harvest under Indian climatic condition, the present study was undertaken to determine the harvest residues of hydrogen cyanamide in grape during 2006–2007.

Materials and Methods

The experiment was laid out at the four different locations of Maharashtra, viz., (1) grape vine farm, Narayangaon, Junnar Taluk, Pune; (2) grape vine farm, Latur; (3) grape vine farm, Sangli; (4) grape vine farm, Nasik in a randomized block design (RBD), replicated thrice with plot containing two rows of grape vines containing 15 plant each and spacing was 10 × 6 ft. Hydrogen cyanamide (50% SL) was applied on 5 years old grape plant [variety: Tas A Ganesh (Narayangaon, Latur, Sangli) and Thompson

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seedless (Nasik)] by swabbing of its solution at the effective application rate of 1.20% a.i./L (T_1), 2.40% a.i./L (T_2) and 4.80% a.i./L (T_3), respectively, on the buds immediately after pruning in September 2006. Untreated control (T_4) was simultaneously maintained.

For residue study at harvest, the berry samples (500 g) were collected randomly from each treatment plot replication wise after the final application. The soil samples (1 kg) were also drawn randomly from the top layer (0–15 cm) of each plot (around basin of the tree) at the stage of harvest to monitor the residues of hydrogen cyanamide, if any. Untreated berry samples were collected at similar way. All the samples were packed with dry ice ($<-20 \pm 2°C$) and brought to laboratory. The residue analysis was carried out at Pesticide Residue Laboratory, Department of Agricultural Chemicals, BCKV, Nadia, West Bengal.

Hydrogen cyanamide reference standard of 99.9% purity was supplied by M/S Canpex Chemicals Pvt. Ltd., Pune. All the solvents were glass distilled before use. Other chemicals used were of analytical reagent grade. Water was double glass distilled. The analytical methodology used for estimation of hydrogen cyanamide was done by modification of previously established method (Sharma and Awasthi 1996; Banerjee et al. 2000).

The grape berry samples (500 g) were crushed in a homogenizer and then a 25 g representative homogenized sample was taken in a centrifuge tube and added 50 mL of water. The mixture was centrifuged for 5 min at 1,200 rpm. The clear supernatant was separated and 4 mL of this was taken in a test tube. It was then evaporated to dryness under nitrogen stream in a low volume concentrator. The residue was re-dissolved in 1.5 mL water and added 0.5 mL of 0.1% acetic acid, then thoroughly mixed by shaking. It was then centrifuged at 10,000 rpm for 5 min. The supernatant thus obtained was decanted and finally passed through 0.2 μm membrane filter paper and subsequently injected to HPLC for estimation of residues. The soil samples (25 g) were analyzed in similar way.

Final analysis of hydrogen cyanamide residue in grape berries and soil were done by high performance liquid chromatography (HPLC, Model JASCO, JAPAN, PU 1575) with UV/VIS variable detector coupled with 3392A integrator. The reversed phase Thermo Hypersil (250 × 4.6 mm) ODS, 5 μ (RPC_{18}) column was used. Ammonium formate (5 mM) in a mixture of methanol and water (2:8, v/v) was used as mobile phase for estimation of hydrogen cyanamide residue. The other parameters like flow, wave length (λ_{max}), retention time, limit of quantification (LOQ) and limit of detection (LOD) were 1 mL/min, 200 nm, 2.50 min, 0.1 μg/g and 0.05 μg/g, respectively.

In order to establish the efficiency of the method employed for recovery of hydrogen cyanamide, fresh untreated grape berries and soil (25 g each) were fortified with three concentration levels separately, i.e., limit of quantification (LOQ) of 0.1 μg/g, 0.3 μg/g (3 × LOQ) and 0.5 μg/g (5 × LOQ). The percent recovery was calculated for all three fortification levels and the data are presented in Table 1 for grape and soil, respectively.

Table 1 Recovery of hydrogen cyanamide in grapes and soil

Levels of fortification (μg/g)	Percent (%) recovery	
	Grapes	Soil
0.1	82.3	88.5
0.3	83.9	90.7
0.5	89.9	91.4

Results and Discussion

No detectable residue (Tables 2, 3) of hydrogen cyanamide was present in the grape and soil samples at harvest following the treatment with T_1, T_2 and T_3 to the freshly pruned canes irrespective of location and dose. No residue was detected in the untreated control samples through out the study. Goldbach et al. (1988) conducted a field study with ^{14}C labeled cyanamide to understand the metabolism of Cyanamide in young shoots of *Vitis vinifera* when applied on cut ends. It was reported that the one-third of activity was decreased as $^{14}CO_2$ within 20 h of application. The highly rapid metabolism of ^{14}C labeled showed that it was first transformed to urea and then degraded to $^{14}CO_2$. In the present study the interval between the last application and sampling was 137 days, so it might be stated that hydrogen cyanamide degraded during this period. Similar findings are also recorded by Sharma and Awasthi (1996).

Table 2 Harvest time residues of hydrogen cyanamide in grape

Dosages	Location			
	Narayangaon	Latur	Sangli	Nasik
T_1 (1.20% a.i./L)	BDL	BDL	BDL	BDL
T_2 (2.40% a.i./L)	BDL	BDL	BDL	BDL
T_3 (4.80% a.i./L)	BDL	BDL	BDL	BDL

Table 3 Harvest time residues of hydrogen cyanamide in soil

Dosages	Location			
	Narayangaon	Latur	Sangli	Nasik
T_1 (1.20% a.i./L)	BDL	BDL	BDL	BDL
T_2 (2.40% a.i./L)	BDL	BDL	BDL	BDL
T_3 (4.80% a.i./L)	BDL	BDL	BDL	BDL

BDL below detectable level (<0.01 ppm)

The MRL of hydrogen cyanamide is 0.1 mg/kg in fresh grape berries (BIS, 1994). As no residue was detected in the harvest sample of grape irrespective of any dose, it might be stated that hydrogen cyanamide will not pose any residual toxicity problem in berries at harvest.

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References

Austin PT, Hall AJ, Snelgar WP, Currie MJ (2002) Modelling kiwifruit budbreak as a function of temperature and bud interactions. Ann Bot 89:695–706. doi:10.1093/aob/mcf113

Banerjee K, Ramteke SD, Somkuwar RG (2000) Terminal residues of hydrogen cyanamide in grape (*Vitis vinifera* L.) berries. The Indian J Agric Sci 70:481

BIS (1994) Proceedings of the third meeting of pesticide residue analysis sectional committee. Bureau of Indian Standards, New Delhi, India, p 26

Bound SA, Jones KM (2004) Hydrogen cyanamide impacts of flowering, crop load and fruit quality of red 'Fuji' apple (*Malus domestica*). N Z J Crop Hort Sci 32:227–234

Clayton M, Biasi WV, Agar IT, Southwick SM, Mitcham EJ (2003) Post harvest quality of 'Bing' cherries following preharvest treatment with hydrogen cyanamide, calcium ammonium nitrate of gibberellic acid. Hort Sci 38:407–411

George AP, Nissen RJ, Baker JA (1988) Effects of hydrogen cyanamide in manipulating budburst and advancing fruit maturity of table grapes in South Eastern Queensland. Aust J Exptl Agric 28:533–538. doi:10.1071/EA9880533

Goldbach HT, Wunseh A, Ambergen A (1988) Decomposition of 14C–labelled cyanamide in *Vitis vinifera* cuttings. J Plant Physiol 133:299–303

Reddy NN, Shikhamany SD (1989) Effect of hydrogen cyanamide and thiourea on budbreak and bloom of Thompson seedless grapevines under tropical conditions. Crop Res 2:163–168

Rieden G, Gehrmann H (1992) Experiences with dormancy breaking agent dormax on grapevines. Proceedings of international symposium recent advances in viticulture and oncology, Hyderabad, India, p 157–162

Sharma D, Awasthi MD (1996) Harvest time residues of hydrogen cyanamide in grape berries. Pestic Res J 8:84–86

Shukla B, Tripathi SP, Kumar R, Misra SP (1995) A short note on early bud burst in grape cv. Perlette. Recent Hortic 2:156–157

Stringer SJ, Marshall DA, Sampson BJ, Spiers JM (2004) The effect of chill hour accumulation on hydrogen cyanamide efficacy in rabbiteye and southern highbush blueberry cultivars. Small Fruits Rev 3:339–347. doi:10.1300/J301v03n03_11

Subhadrabandu S, Rakngan J (1999) Effect of hydrogen cyanamide and time of application on bud breaking and fruit quality of kiwifruit cv. Bruno in Thailand. Thai J Agric Sci 32:161–170

Williams LE (1987) The effect of cyanamide on bud break and vine development of Thompson seedless grapevines in the San Joaquin valley of California. Titis 26:107–113