

Performance of Indian laboratories in Ring Test for Residue Analysis in Table Grapes

In India, the pesticide residue monitoring program (RMP) in table grapes for export to the European Union was initiated in 2003-04 season in response to the rapid alerts issued against Indian table grapes in Europe in 2003. The program eventually got transformed into a complete traceability system in for export to the EU countries through a web-based residue monitoring system, viz., *GrapeNet* declaring National Research Center for Grapes, Pune as the National Referral Laboratory. This was introduced in 2007.

Government of India spent close to 10 million Euros to upgrade the laboratory facilities in India and to set up the National Referral Laboratory for monitoring residues in table grapes. In addition, a number of training programmes were organized to ensure that the laboratory staff are fully trained to meet the emerging changes of precision testing of pesticides to meet the EU MRL requirements. With the addition of other chemicals in the grape residue monitoring programme, trainings were also provided to the laboratory analysts in 2010 and one in January 2011 at the Rikilt Institute of Food Safety, Wageningen, The Netherlands to enhance and assess their analytical capability.

Proficiency testing and Ring-Test programmes have also been conducted by the National Referral Laboratory to evaluate the performance of the laboratories for the grape season of 2011. These have been conducted targeting 171 chemicals (including pesticides, growth hormones, heavy metals, etc.). In the ring test, which was conducted in February 2011, eleven (11) Indian laboratories engaged in grape residue testing and 3 European laboratories (AgriQ Group BV, Wageningen, The Netherlands; Eurofins - Dr. Specht Express, Hamburg, Germany; and QTS Analytical Ltd., Kent, UK) participated. Each laboratory was assigned a unique code number to maintain confidentiality. The grape juice used as test material for the analysis was spiked with certain chemicals (11) in bulk (but unknown to the labs.) and then distributed to all of them. They were requested to identify and quantify the residues present in the test material and forward their results with the chromatograms / mass spectra / calculation details, etc. to the National Referral Laboratory within one week.

The National Referral Laboratory has received all the test reports and chromatograms, etc. APEDA is pleased to inform you all that the Z scores of the participating laboratories for all the test chemicals were within the satisfactory range of -2 to +2 for all the spiked agro chemicals. APEDA is also happy to note that the performance of Indian laboratories was 'equivalent to better' in comparison to the performance of the three European laboratories. The report of the ring test conducted by NRL is attached for reference.

In case of any questions, the National Referral Laboratory, whose contact details are given below, can be contacted :

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Ring Test on Grape Juice Test Material

February 2011

Conducted by:

National Referral Laboratory
National Research Centre for Grapes,
Pune – 412 307, INDIA

SUMMARY

1. The test material for the Ring Test was dispatched on 17th February, 2011. Each participant received a grape juice test material to be analyzed for agrochemical residue. The material was distributed amongst 15 participating laboratories (4 in Europe and 11 in India). Out of these 15 participating laboratories, the results were received from 14 laboratories within the time-scale demanded (7 days from the date of receipt of test material).
2. From the list of agrochemicals in Annexure-9 of the 'Residue Monitoring Plan for Export of Table Grapes to the European Union', the participants were required to identify and quantify the chemical residues present in the test material. The grape juice material was spiked with Atrazine, Azoxystrobin, Buprofezin, Chloromequat, Dimethoate, Etofenprox, Flusilazole, β -HCH, Lindane, Metalaxyl and Triazophos.
3. The assigned value (\hat{Y}) was calculated from the most appropriate measure of central tendency of participant's results.
4. The standard deviation (σ) for each pesticide was calculated using the results obtained from the participants and used in conjunction with the assigned value (\hat{Y}) to derive z-score for the participants. The z-scores are considered satisfactory if $|z| \leq 2$.
5. Results for the ring test are summarized as follows:

Analyte	Assigned Value mg/kg	Number of satisfactory z-scores	Total no. of Scores	% satisfactory
Atrazine	0.155	13	13	100.0
Azoxystrobin	0.158	14	14	100.0
Buprofezin	0.165	14	14	100.0
Chloromequat	0.173	13	13	100.0
Dimethoate	0.237	14	14	100.0
Etofenprox	0.204	14	14	100.0
Flusilazole	0.269	14	14	100.0
β -HCH	0.191	14	14	100.0
Lindane	0.204	14	14	100.0
Metalaxyl	0.259	14	14	100.0
Triazophos	0.847	14	14	100.0

6. z-scores of all the participating laboratories were within the satisfactory range of -2 to +2 for all the 11 spiked agrochemicals. Performance of the APEDA-nominated laboratories appeared equivalent to the participating laboratories from Germany, United Kingdom and the Netherlands.

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1. Introduction

1.1 Background of the ring test

In India, the pesticide residue monitoring program (RMP) in table grapes for export to the European Union was initiated in 2003-04 season in response to the rapid alerts issued against Indian table grapes in Europe in 2003. The program was initiated to establish complete traceability of grapes for export to the EU countries from farm to fork levels through a web-based residue monitoring system viz. GRAPENET, declaring National Research Centre for Grapes as the National Referral Laboratory.

A meeting amongst the APEDA-nominated laboratories was conducted on 13th November, 2010 at the NRL, NRC for Grapes, Pune, where as per the demand of the Indian grape exporters, it was decided to conduct a 'Ring Test' involving the Indian vis-a-vis interested laboratories of the EU countries.

This proposed 'Ring Test' would serve as an independent proof of competence relevant to the food testing laboratories in India and Europe. This test would also serve to establish the analytical credibility of the Indian laboratories as well as compare their efficiency with European laboratories that are regularly testing Indian grape samples exported to the EU countries.

This ring test was organized as per the international protocols of IUPAC, where homogenized grapes were spiked with pesticides uniformly in bulk and then distributed among the participating independent laboratories identified with unique code numbers. Samples were dispatched after receiving "in principle confirmation" from the participating laboratories in this ring test. The laboratories were requested to submit their results within one week time from the date of receipt of the sample.

2. Test Material

2.1 Preparation

Sample preparation was carried out by the National Referral Laboratory at Pune. Freshly harvested grapes were obtained from the experimental farm of National Research Centre for Grapes, Pune, India and crushed thoroughly in a blender. Juice was extracted from the crushed material and used for spiking. The spiking solutions of agrochemical standards were added drop

wise to the bulk sample while mixing in a blender. After all the spiking solution had been added, the bulk sample was mixed thoroughly for a further 30 min.

After spiking with agrochemicals, individual sub-samples were measured (at least 100 g) into clean PTFE bottles, labeled with unique lab code numbers and stored at -20 °C in darkness until distribution. The spiked test material was prepared to attain the following approximate concentrations.

Agrochemical name	Approximate spiked concentration mg/kg
Atrazine	0.200
Azoxystrobin	0.150
Buprofezin	0.150
Chlormequat	0.200
Dimethoate	0.300
Etofenprox	0.300
Flusilazole	0.350
β-HCH	0.250
Lindane	0.200
Metalaxyl	0.300
Triazophos	1.400

2.2 Homogeneity

Ten randomly selected test materials were analyzed in duplicate for all the analytes within the National Referral Laboratory. The data established sufficient homogeneity as presented below:

Analyte	RSD of replicates 1-10 (%)
Atrazine	5.9
Azoxystrobin	6.0
Buprofezin	3.1
Chlormequat	6.2
Dimethoate	5.9
Etofenprox	9.0
Flusilazole	4.3
β-HCH	3.8

Analyte	RSD of replicates 1-10 (%)
Lindane	4.2
Metalaxyl	4.5
Triazophos	8.5

2.3 Distribution

The laboratories of India sent their representatives to collect the ring test samples from NRL, Pune. In case of the European laboratories, the samples were sent by DHL Express Courier on 17th February 2011 and all of them received the samples within 4-5 days of dispatch. Each participant received uniquely numbered samples, packed with cooling blocks in an insulated container, together with a covering letter, instructions for submission of results and methods and the results form.

3. Results

Participants were required to report which pesticide residues the grape juice had been analyzed for, together with the residue concentration (in mg/kg, uncorrected for recovery), limit of quantification (LOQ). Pesticide residue concentrations were requested to be reported in terms of the parent compound only, as the parent compounds plus one or more metabolites or as the sum of isomers. The reporting format was indicated in the results form.

On receipt of the sample, the Neutron SpA lab from Italy expressed inability to test the sample because they felt that the sample got fermented during transit! Rest of the three European laboratories submitted their reports within one week time.

4. Statistical Evaluation of Results

The recommended procedure in the IUPAC/ISO/AOAC International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories was utilized.

5. Individual z-Scores

Participant's z-scores were calculated as;

$$z = \frac{(x - \hat{Y})}{\sigma}$$

Where x = the participant's reported result,

\hat{Y} = the assigned value

σ = the target standard deviation

The participant's z-scores for the tested analytes are given in the following table.

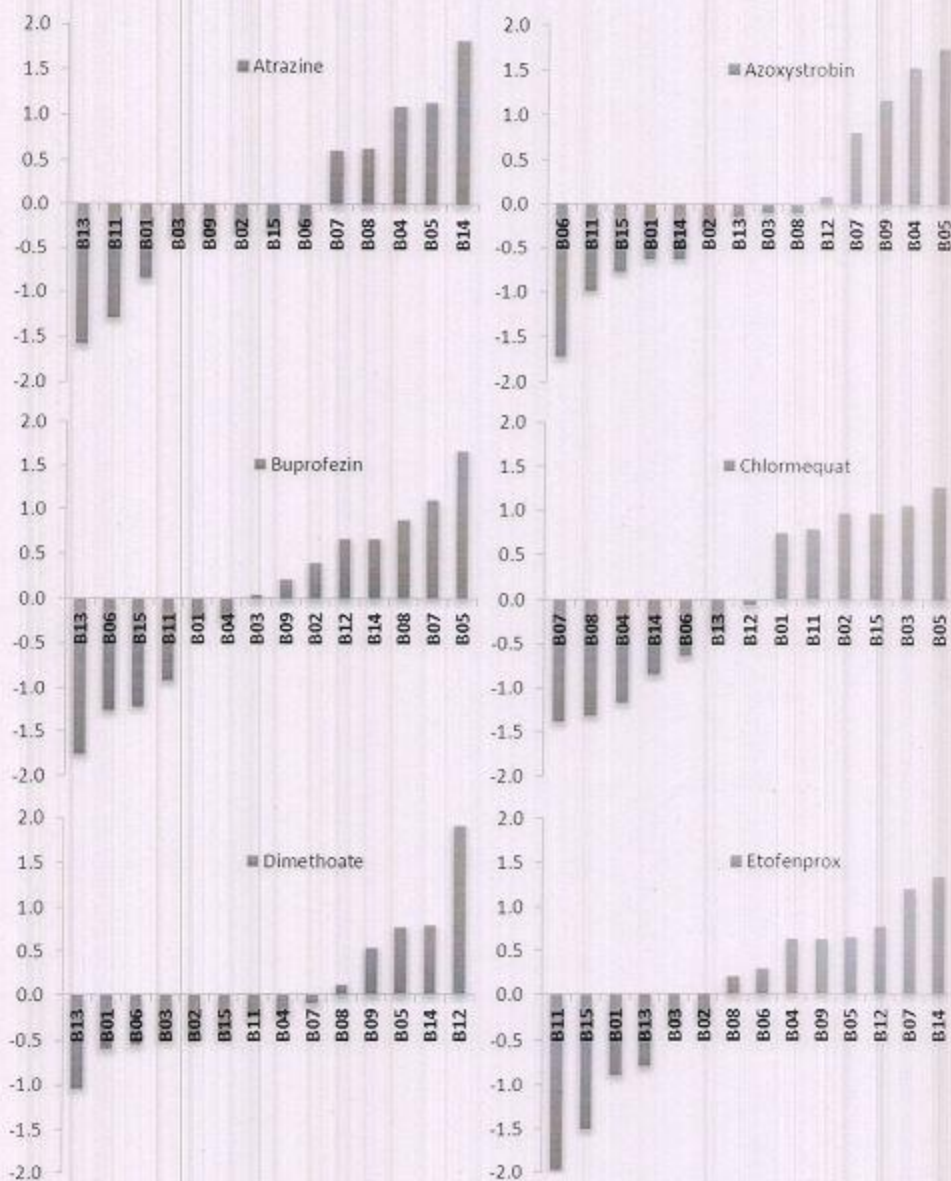
Table 1. z-scores for the 14 participating laboratories

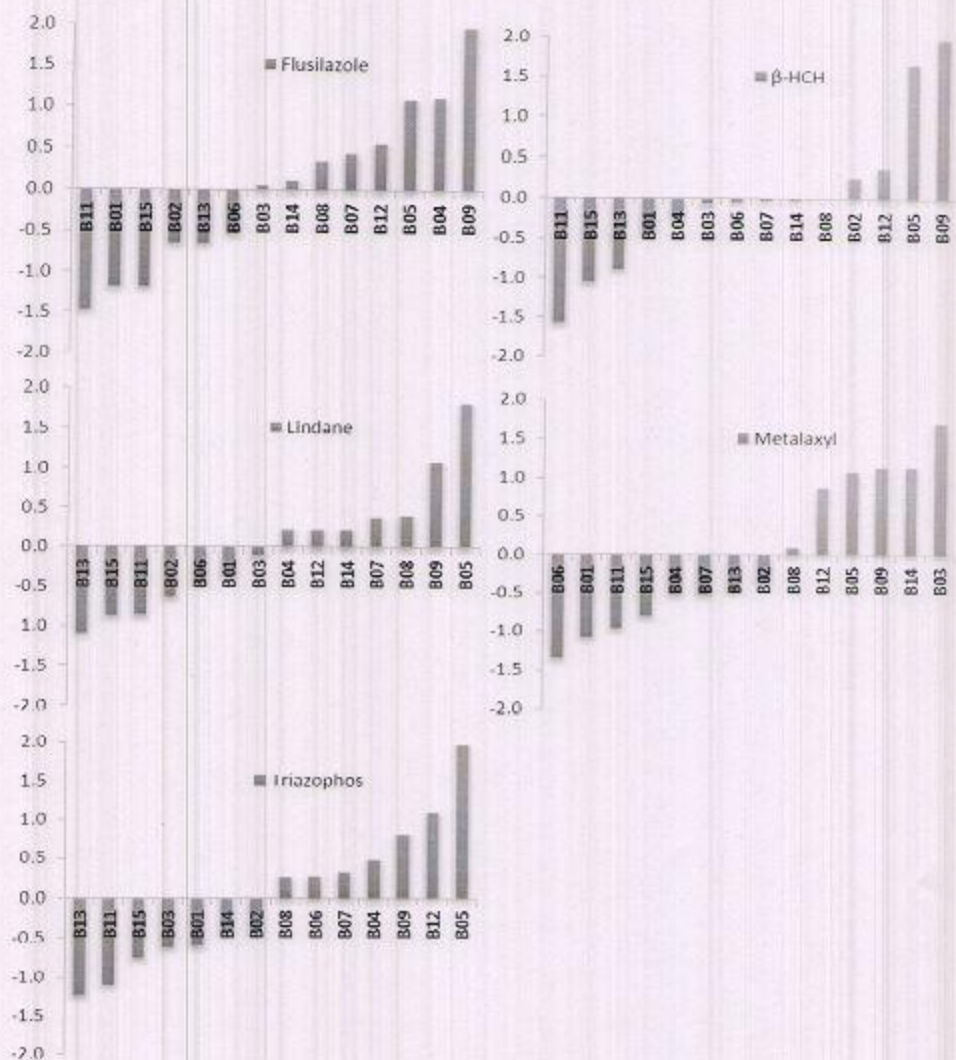
Analyte	Laboratory Code Number													
	B01	B02	B03	B04	B05	B06	B07	B08	B09	B11	B12	B13	B14	B15
Atrazine	-0.85	-0.29	-0.37	1.10	1.14	-0.27	0.61	0.63	-0.37	-1.29	NA	-1.58	1.83	-0.29
Azoxystrobin	-0.64	-0.27	-0.09	1.54	1.76	-1.72	0.81	-0.09	1.18	-1.00	0.09	-0.13	-0.64	-0.78
Buprofezin	-0.22	0.40	0.05	-0.22	1.67	-1.27	1.10	0.88	0.22	-0.92	0.66	-1.77	0.66	-1.23
Chlormequat	0.74	0.96	1.05	-1.17	1.27	-0.64	-1.39	-1.33	NA	0.78	-0.06	-0.34	-0.84	0.96
Dimethoate	-0.59	-0.46	-0.49	-0.21	0.77	-0.54	-0.09	0.11	0.54	-0.24	1.91	-1.04	0.79	-0.45
Etofenprox	-0.89	-0.27	-0.32	0.64	0.65	0.29	1.20	0.21	0.64	-1.98	0.78	-0.79	1.34	-1.51
Flusilazole	-1.19	-0.65	0.06	1.10	1.07	-0.52	0.44	0.36	1.97	-1.48	0.55	-0.64	0.12	-1.19
β -HCH	-0.42	0.26	-0.06	-0.22	1.66	-0.04	-0.02	0.00	1.98	-1.58	0.38	-0.90	-0.02	-1.06
Lindane	-0.34	-0.63	-0.10	0.23	1.81	-0.46	0.37	0.40	1.09	-0.86	0.23	-1.10	0.23	-0.87
Metalaxyl	-1.08	-0.35	1.71	-0.52	1.10	-1.36	-0.52	0.09	1.15	-0.97	0.87	-0.48	1.15	-0.80
Triazophos	-0.60	-0.43	-0.62	0.48	1.97	0.26	0.32	0.26	0.79	-1.10	1.09	-1.24	-0.43	-0.76

NA: Not Attempted

Note: Laboratory code B10 was not considered due to outlier values.

Figure 1. Presentation of z-scores for different analytes





Conclusion

z-scores of the above participating laboratories for all the test chemicals were within the satisfactory range of -2 to +2 for all the 11 spiked agrochemicals.

Performance of the APEDA-nominated laboratories appeared equivalent to the 3 participating laboratories from Germany, United Kingdom and the Netherlands.

Appendix

The list of laboratories submitting the results are as follows.

Name of the Laboratory in Alphabetical order

AgriQ Group BV, Wageningen, Netherlands

Arbro Pharmaceuticals Ltd., New Delhi

Delhi Test House, New Delhi

Eurofins – Dr Specht Express, Hamburg, Germany

Geochem Laboratories Pvt. Ltd., Mumbai

Interfield Laboratories, Cochin

Microchem Laboratory Pvt. Ltd., Mumbai

National Collateral Management Services Limited, Hyderabad

National Horticultural Research and Development Foundation, Nashik

QTS Analytical Ltd., Kent, United Kingdom

Reliable Analytical Laboratories Pvt. Ltd., Mumbai

Sargam Labs, Chennai

SGS India Pvt. Ltd., Chennai

Vimta labs Ltd., Hyderabad
