ABSTRACT OF THE THESIS

Grape is an important commercial fruit crop, which receives frequent application of large number of agrochemicals, e.g. pesticides etc. throughout the cropping season for management of various pests and diseases. At present, in India grape is grown over an area of 60,000 ha with an annual production of 1.2 million tonnes. It is estimated that around 1200 hectares area with annual production of 9.5 million liters in Maharashtra and about 100 hectares near Bangalore in Karnataka are currently under wine grape cultivation. Indian grape is under constant scrutiny of the environment and health protection agencies worldwide. In India, the cultivation of grapes receive frequent application of large number of pesticides and further, grape is mostly consumed as fresh fruit in intact form without any processing. The residues left on the grapes during harvest can be carried through into the wine. When grapes are processed to raisin or wine, the contaminant molecules are expected to degrade on exposure to various processing treatments. Raisins are prepared by drying the grapes under sun or shade and oven drying whereas wine is prepared through fermentation. Hence during raisin and wine preparation, the agrochemical residues get exposed to various biotic and abiotic factors and may degrade by photo-degradation, chemical degradation and microbial degradation. Thus, processing of grapes may appear to be a major method of decontamination of residues to provide safety to the consumers.

Chapter 1 embodied brief introduction to grape and grape products (raisin and wine). It gives the review about pesticides, antibiotics residue analysis from grapes, raisin and wine. Also it explains the review of literature for journey of pesticides during the processing of grape to wine and raisin.

Chapter 2 explained the validation and uncertainty analysis of a multi-residue method for pesticides in grapes using ethyl acetate extraction and Liquid chromatography tandem mass spectrometry. A method was validated for the multi-residue analysis of 82 pesticides in grapes at ≤25 ng/g level. Berry samples (10 g) mixed with sodium sulphate (10 g) were extracted with ethyl acetate (10 mL); cleaned by dispersive solid phase extraction and the results were obtained by liquid chromatography–tandem mass spectrometry. Reduction in sample size and proportion of ethyl acetate for extraction did not affect accuracy or precision of
analysis when compared to the reported methods and was also statistically similar to the QuEChERS technique. The method was rugged (HorRat<0.5) with <20% measurement uncertainties. Limit of quantification was <10 ng/g with recoveries 70–120% for most pesticides. The method offers cheaper and safer alternative to typical multi-residue analysis methods for grape.

**Chapter 3** has been devoted to a comprehensive GC×GC–TOFMS method was optimized for multiresidue analysis of pesticides using a combination of a non-polar (RTX-5MS, 10m×0.18mm×0.2µm) and a polar capillary column (TR-50MS, 1m×0.1mm×0.1µm), connected in series through a dual stage thermal modulator. The method resolved the co-elution problems as observed in full scan one-dimensional GC–MS analysis and allowed chromatographic separation of 51 pesticides within 24 min run time with library-searchable mass spectrometric confirmation. Four pesticides, viz. chlorpyrifos-methyl, vinclozoline, parathion-methyl and heptachlor could be baseline separated on GC×GC, which were otherwise closely eluting and interfering each other’s detection in 1D GC–MS run. Similarly, it could be possible to separate myclobutanil, buprofezin, flusilazole and oxyfluorfen on GC×GC. Although in 1D GC–MS, these closely eluting compounds could be identified through deconvolution algorithm and ‘peak-find’ option of the Chromatof® software but the spectral purity significantly improved on GC×GC analysis. Thorough optimization was accomplished for the oven temperature programming, ion source temperature and GC×GC parameters like modulation period, duration of hot pulses, modulation-offset temperature, acquisition rate, etc. to achieve best possible separation of the test compounds. The limit of detection significantly improved by 2–12 times on GC×GC–TOFMS against GC–TOFMS because of sharper and narrower peak shapes. The method was tested for grape matrix after preparing the samples using previously described method and recoveries of the entire test pesticides were within 70–110% at 10 ng/g level of fortification. GC×GC–TOFMS was found to be an excellent technique for library-based screening of pesticides with high accuracy and sensitivity.

**Chapter 4** gives an account on a single laboratory validation results reported for multiresidue determination of 78 pesticides at 10ng/g level in wine and raisin. The samples were extracted with ethyl acetate and sodium sulphate. The extract was
cleaned by Primary secondary amine as a sorbent and GCB for color removal. The residue estimation was done in multireaction monitoring mode by liquid chromatography tandem mass spectroscopy. The limit of quantitation varied from 1 to 10 ng/g. The recovery at 2.5, 5, 10 and 25ng/g was in between 80-120%. Matrix effect also evaluated in both the matrix.

**Chapter- 5** contains a confirmatory method by liquid chromatography tandem mass spectrometry (LC-MS/MS) for the determination of tetracycline, oxytetracycline, doxycycline and chlortetracycline in grapes, wine and raisin. The samples were cleaned on an Oasis HLB solid-phase extraction cartridge and analysed by LC-MS/MS in multiple reaction monitoring. The method was validated at three different levels (10, 25 and 50 ng/g). The limit of quantitation was varied from 1-5 ng g\(^{-1}\). The recovery values were varies from 70-110% with RSD <20%. Moreover, the matrix effects encountered during the LC-MS/MS analyses were also studied in spiked experiments from blank samples.

**Chapter -6** is on the fate of thiamethoxam, tetraconazole, difenoconazole, tebuconazole, trifloxystrobin, buprofezin, fipronil and mandipropamid in grapes. The residue dynamics of thiamethoxam in grape berries was investigated. The residues of thiamethoxam were found to dissipate following first order rate kinetics with half-life ranging between 6-9 days for 3 different rates of foliar applications. The pre-harvest intervals (PHI) were found to be 17, 19 and 30 days corresponding to the application rates of 12.5, 25 and 50g.a.i./ha, respectively. The aerobic degradation was explored in 2 different soils of India with different physico-chemical properties. The half-life was found to be 47 and 51 days for the clay and red-sandy soils, respectively.

Degradation kinetics of commonly used fungicides (tetraconazole, difenoconazole, tebuconazole and trifloxystrobin) in table grapes was explored pertaining to their most critical usage pattern at recommended and double doses, applied through agricultural formulations in separate experimental vineyards. The residue data were fitted to different kinetic models viz. linear 1\(^{st}\) order and non-linear 1\(^{st}\) + 1\(^{st}\) order kinetics to estimate their half-life and pre-harvest interval (PHI) with reference to the MRL applicable for the European Union. The PHI recommendations out of these experiments were successful in minimizing their residues in table grapes at harvest.
To determine the preharvest interval (PHI) of buprofezin to minimize its residues in grapes and thereby ensure consumer safety and avoid possible non-compliance in terms of residue violations in export markets. Furthermore, the residue dynamics in three grapevine soils of India was explored to assess its environmental safety. Residues dissipated following non-linear two-compartment $1^{st} + 1^{st}$ order kinetics. In grapes, the PHI was 31 days at both treatments (312.5 and 625 g a.i./ha), with the residues below the maximum permissible intake even 1 h after foliar spraying. Random sampling of 5 kg comprising small bunchlets (8–10 berries) collected from a 1 ha area gave satisfactory homogeneity and representation of the population. A survey on the samples harvested after the PHI from supervised vineyards that received treatment at the recommended dose showed residues below the maximum residue limit (MRL) of 0.02 mg/kg applicable for the European Union. In soil, the degradation rate was fastest in clay soil, followed by sandy loam and silty clay, with a half-life within 16 days in all the soils. The recommendation of the PHI proved to be effective in minimizing buprofezin residues in grapes. Thus, this work is of high practical significance to the domestic and export grape industry of India to ensure safety compliance in respect of buprofezin residues, keeping in view the requirements of international trade.

Fipronil is a phenylpyrazole group of insecticide having maximum residues limit 0.005 mg/kg for the table grapes set by European countries and proposed mandipropamid MRL is 1.4 mg/kg. The grape and soil samples were extracted in ethyl acetate and methanol respectively followed by cleanup with 50 mg PSA and 25 mg GCB. The method validation was done at three levels by using liquid chromatography with tandem mass spectrometry. The matrix match calibration standards were used for elimination of matrix effect. The dissipation of the fipronil and mandipropamid was studied in soil and grapes. The limit of quantitation for parent and its metabolites were \( \leq 1 \) ng/g and the calibration range 0.5-25 ng/g. The half-lives according to the $1^{st} + 1^{st}$ order model were 21 days for both single and double dose of mandipropamid and 21 days for single dose and 19 days for double dose for fipronil. The half-lives of fipronil according to the $1^{st} + 1^{st}$ order model were 28.5, 55.25 and 15.25 days for clay, sandy-loam, and silty-clay soils respectively. The residue data of mandipropamid to $1^{st} + 1^{st}$ order kinetics, the $DT_{50}$ in clay, sandy-loam, and silty-clay soils was found to be 17.5, 27.5 and 30.0 days.
Chapter 7 explained the fate of agrochemicals during the processing of grapes to wine at various stages. The mini-lot fermentation method was used for the wine preparation. The red wine grapes (Cabernet, Cabernet Sauvignon and Zinfandel) were used for the wine making. During the processing of grapes to wine few pesticides were used for checking the degradation kinetics. After treatment the samples were analysed and quantified the amount of each pesticides at different days. The extractable residues of the pesticides in the wine were measured at regular intervals. The kinetics of the residue data was evaluated by fitting the data into 1st, square root 1st and 1st + 1st order kinetic models, which were furthermore compared for the best fit and R2>0.99. DT50 for all the pesticides was varied from 1-4 days in cabernet and Cabernet Sauvignon whereas 1-60 days in Zinfandel wine.

Chapter 8 explained the fate of agrochemicals during the processing of grapes to raisin at various stages. The grapes (Thompson, Sonaka, Manik Chamman, Tas-A-Ganesh, sharad Seedles etc) were processed into raisin using 3% Potassium carbonate and 1% dipping oil. Under these conditions the treated grapes were selected for the degradation study of few selected chemicals during processing to raisin. In this study, most of the chemicals have DT50 less than 3 days with the correlation coefficient ($r^2$) value varied from 0.96 to 0.99. Fipronil showed fipronil sulfone as a degradation product during the processing. So both the chemicals included for the residue definition.

Chapter 9 described the summary of the thesis on the basis of work carried out under the scheme. The thesis reports extensive research on the pesticide residues in grapes and during processing of grapes to raisin and wine. The work started by developing multiresidue analysis method based on LC-MS/MS and GC-MS. In the beginning, a fast and cheap multiresidue method was developed and validated for simultaneous determination of 82 pesticides in grapes. The method reduced the sample size and minimized the quantity of solvent ethyl acetate for extraction. The method was validated at 10 ng/g and lower levels and was successful in residue monitoring up to the level as low as 2.5 ng/g. Measurement uncertainty was estimated at 10 ppb level for all the target compounds, which was below 15% in all cases. The sample preparation method was sensitive and rugged enough to provide more than 80% recoveries with high precision and accuracy. Considering the success of this
LC-MS/MS method, a GCxGC-TOFMS method was optimized for 54 GC-amenable pesticides. This method essentially utilized the strength of two-dimensional separation involving two capillary columns of different polarity for orthogonal chromatographic separation of several closely eluting and co-eluting compounds, the detection of which was otherwise not possible or uncertain on one-dimensional full-scan GC-MS. This method was also validated at 10 ppb level with signal-to-noise ratio greater than 10:1 for each analyte. The same ethyl acetate extraction based method was extended to 78 pesticides in two processed products viz. wine and raisin.

Considering the application of antibiotics in grape cultivation for bacterial disease control, the LC-MS/MS method was established and validated for tetracyclines. In grape, lots of pesticides are frequently sprayed throughout the cropping season to manage insect pests and diseases. Hence, it becomes essential to study the degradation kinetics of the pesticides and explore their metabolic fate to ensure safe usage of pesticides in viticulture. The thesis reports the dissipation rate kinetics and metabolic fate of several commonly used pesticides at single and double doses with respect to their critical field usage pattern. The residue data were plotted against time and tested for fitness into linear and non-linear kinetics. On the basis of the regression equations, the half-life and pre-harvest intervals were estimated. The results will be helpful to the farmers to stagger the pesticide applications on the basis of their nature of degradation. For example, a pesticide of longer half-life and PHI should not be sprayed before harvest and it should be substituted by chemicals with faster degradation rate. Along with grape, the metabolic fate was also studied in soil, which acts as indirect source of pesticide residues.

Fate of several pesticides was studied during processing of table grapes to raisin and wine. During raisin making, the grapes are exposed to hot air, whereas, while wine making the grape berries are subjected to fermentation by yeast. The degradation rate of the pesticides during processing of grapes was studied with exploration into the possible metabolites.

The thesis ends up with the list of publications and conferences/seminar attended.
List of Publications


