"Screening Of Pesticides Residues On Grape Growing Soils In Nashik District"

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Abstract:
Nashik district is known as a capital of Grape which is the highest producer of grapes in Maharashtra an estimated output of 4 lakhs metric tons per year. During 2016-17 export of grapes tunes to about 1.31 lakh metric tons. Chemical pesticides brought a great relief to avoid the loss due to different fungal diseases as well as to improve its yield. The small amounts of it remain and bind with the soil. For the purpose of pesticide residue detection representative soil samples were composed from the major grape growing areas from Nashik district .An analytical multiresidue technique was used for screening of various classes of pesticides at the same time. For this purpose LCMS/MS and GCMS/MS instruments were used on Multi Reaction Monitor (MRM) mode. Ethyl acetate facilitates the extraction of pesticides from the soil. On the basis of retention time pesticides were confirmed.Spiked blank samples were specified as standards. Recovery studies were performed at 10 and 20 ppb concentration levels of each pesticide, all the recoveries are above 70% with a relative standard deviation between 0.31 and 6.4%.In the tested soil samples, residues of various pesticides were found in different concentrations. Concentration and percent contamination of pesticide residues in three soil levels were determined. The results of three soil levels were analyzed and compared by using different statistical tests. The present study monitors the pesticides residues; concentration levels on viticulture of Nashik district, the data will be highly useful to create awareness among the farmers that are capable of maintaining their productivity, commercially competitive and environmentally sound. The right awareness about the use of pesticides will thus be useful for sustainable farming.

Keywords:,Multiresidue pesticide screening, LCMS/MS and GCMS/MS, Statistical analysis.

I. INTRODUCTION

Nashik is well known as "Grape City" in the Maharashtra State.Grapes are an important cash fruit in Nashik with about 3.5 lakh acre land is under grape cultivation.In every year the district recorded 10-15 lakh metric tons production of grapes.Out of 15 tehasils from Nashikdistrict;Nashik,Niphad and Dindori are leaders with 90% production(Raikwaret al,2011).The Yield suffers about 20-10% loss due to common grapevine pests like Mealy bug,Mites,Thrips,Caterpillars,Leafhopper and affect the area with various fungal diseases. Pesticides contribute to be a significant input in a modern agriculture and have to be used for management of pests which are noxtrious,destructive and troublesome organisms.A plant retains only half of the applied spray as the leaf crates a non-wetting interface for the pesticides.The remaining gets adsorbed and degraded in the top soil(Wadhwani and Lall,1972). The retention of pesticides in the soil is affected by the physical and chemical properties of the pesticide, the various properties of the soil such as, presence of clay materials, organic matter, and pH, climate, biology, and other factors (Singh,2001).

In this study, we aim to provide data on contamination of soil collected from different grape growing farms by proper soil sampling technique. For this purpose the soil samples were collected from the selected grape growing farms at three different levels according to period i.e., Lean period (time of pruning), Peak period (time of development) and period of harvesting in a year. Various pesticides residues from collected soil samples were monitored by multiresidue pesticide analysis method. The concentrations of pesticides in different three levels were reported .This paper mainly focused on the withdrawl and separation of target pesticides in soils of vineyards by LC-MS in a single run. The liquid chromatography mass spectrometry technique reported in this work is very acute and speedy.As compared to previously put-out approach this technique allows the determination of higher number of compounds at the same time in a single run.

1. Experimental Work :

Study area: 3 major grape growing tehasils from Nasik district as, Niphad, Dindori, and Nasik was selected because these are leading growers of grapes.

Soil sampling:

Sampling equipments (soil auger,spade bucket, plastic sheet, and plastic bags) are used. 5 soil samples from different villages of each tehasil were collected by proper soil sampling method (Arora and Singh, 2009) in such a way that, 2 samples from export quality grape growing field and 3 samples from random field. Representative soil samples were composed from the study areas in three periods. The composite soil samples were drawn from 0-15 cms depth. Samples were collected from the vine row where most of the vine roots are located using stainless steel auger. A 16-liter bucket was used to put the subsamples, 4-5 samples from each selected vineyard were thoroughly mixed on a plastic sheet so that the soil collected was truly representative of each locality, then air-dried, grounded and sieved through a mesh with a

grain size of 2 mm. Samples were packed in air tight plastic bags, codes are given as, A to O for 15 soil samples and then samples are transported ice preserved to the laboratory until further chemical processing.

2.1 Material and Methods

2.1.1 Sample Extraction Procedure:

Samples are extracted by validated method reported by Zweig (1984),Wang et.al.(2008),Om Prakash et.al. (2004)

2.1.2 a) Sample Extraction for LCMS/MS analysis: 10g soil sample. 5 ml water, 10 ml ethyl acetate and 10 g sodium sulphate anhydrous were homogenizing it for 2 min at high speed and centrifuge for 5 minutes at 50000 rpm. 3 ml of the ethyl acetate phase was taken into a centrifuge tube containing 25mg primary secondary amine Shake vortex for 1 min.Centrifuge at around 5000 rpm for five minutes. 2 ml cleaned supernatant was taken and 0.2 ml 10% diethylene glycol was added with methanol to it .Evaporate it under gentle stream of nitrogen using low volume concentrator at 35^0C.Reconstitute into 1 ml methanol and 1 ml 0.1% acetic acid in water. Centrifuge at 10000 Revolutions per minute for 5 min and filter through 0.2 µm Polyvinylidine fluoride/nylon membrane filter.Inject 10 µl into LCMS/MS.

b) Sample Extraction for GCMS/MS: 1 ml extract was taken and clean it with 25 mg PSA.Centrifuge at 10000 rpm and filter through 0.2 µm PTFE membrane filter.Inject 2 µl (split less injection mode) into GCMS/MS.

***2.1.3Instrumental analysis*:** Samples were analyzed by multiresidue pesticide analysis with the help of GCMS/MS and LCMS/MS.

Chemicals: HPLC Grade Methanol and Water of J T Backer was act as a Mobile phase. Chilled Distilled water helps to maintain the temp. of matrix as increase in temp cause degradation of some pesticides residues and Ethyl Acetate was used to prevent degradation of some PRs in Extraction, Primary secondary amine assists for cleaning of matrix interference in sample, to remove water traces, activated Sodium sulphate was used, Formic Acid aids to acidify extraction solvent,ammoniumformate act as a buffer for mobile phase, all these were purchased from Merck. 10% Di ethylene glycol in methanol apply as an analytes keeper and protector in MS. Acetic acid was used to acidify final volume and PTFE (Polytetrafluoroethylene) Filter of 0.22 µm used to filter final injection volume before going to fill vial.

Pesticide Standard: The certified Pesticides standards were purchased from (Dr. Ehrenstorfer GmbH, Germany).All pesticide standards were more than 95% pure. All standards were tuned to obtain intensified Multi reaction monitor mode and qualifier for confirmation of Pesticide Residue.

Preparation of standards:

5.0 mg of pesticide standards makeup with ethyl acetate for GCMS/MS compounds and methanol for LCMS/MS compounds in to 5 ml volumetric flask.The solution concentration is around 1000 mg / litre prepares the working standard (mix standard) of concentration 1.0 mg/litre and does the subsequent dilution with respective solvent.To obtain desire concentration of Pesticides individual standard stock solutions were mixed appropriately and then stock solution of standard mix was serially diluted with methyl alcohol to 1 µg/ ml.Organochlorines was Separated and Quantified by using GCMS (Perkin Elmer, Clarus500) with auto sampler equipped with an Electron Capture Detector (ECD' 63Ni),while evaluation of other pesticides was carried out using LCMS/MS(Absciex, 4000 Q TRAP).

Analysis on GCMS/MS:

GCMS/MS conditions are as follows

GC conditions:

Column: DB 5, MS 0.25 µm, 30m x 0.25 mm id.Carrier gas: Helium. Constant flow: 1 ml / min.Injection: 2 µl / split. Injector temperature: 250^0C (manual / auto sampler mode).

Analysis on LCMS/MS:

HPLC conditions: Two mobile phases are used

A: 5 mM ammonium formate dissolved in water: methanol (80:20) (157.7 mg ammonium formate dissolved in 500 ml of mobile phase).

B: 5 mM ammonium formate dissolved in methanol: water (90:10) (157.7 mg ammonium formate dissolved in 500 ml of mobile phase) .Analytical column: Zorbex (Eclipced plus-C18)3.5µ, 4.6 x 100 mm (API 4000).Flow rate: 0.6 ml/min.

Mass Conditions: Interface: ESI + ve .Source température: 450^0C

2.1.4 Analytical quality control:

The certified Pesticides standards were purchased from (Dr. Ehrenstorfer GmbH Germany) used for calibration of the instruments. The certified Pesticides standards were used for calibration of the instruments. The identification of pesticides was done by comparing retention indices of the standard solution peaks with those of the samples. Calibration curve was constructed for determination of concentrations of analyte. These curves were set by tracing peak areas in accordance with the concentration of analyzed pesticide .Calibration was done by linear regression method .The correlation coefficient of calibration curves were ranked from 0.9980 to 0.9990. Results above Limit of Detection (LOD) were taken for calculations and below (LOD) were assumed as zero (0) in the calculations.

2.1.5 Validation of the methodology:

The method was confirmed in soil samples by analysis of spiked samples. the target pesticides was identified by searching in the appropriate retention time windows (RTWs), The evaluation of the samples was carried out by injecting blank sample extracts spiked with the pesticides at five different concentration levels. Spiked blank samples were functioned as standards.Confirmation of pesticides were done on the basis of their retention times, recovery studies were performed at 10 and 20 ppb concentration levels of each pesticide, the recoveries are above 70% with a relative standard deviation between 0..31 and 6.4%.

***2.1.6 Calculation*:**

Concentrations i.e. contaminations of pesticide residues were determined by using peak area of standard and sample, concentration of standard as well as weight of sample.

$$\textbf{Concentration} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Concentration of standard}}{\text{weight of sample}} \times \text{Dilution Factor}$$

2. ResultsAndDiscussions

Several studies Kumar Bhupender,(2011) reported the detection of pesticides and herbicides in soils from farms of Delhi region and most frequent pesticides detected were organochlorine group which is more persistent and decomposed very slowly.InJalgaon district the presence of pesticide residues was reported by Bharambe and Mahulikar,(2010)using technique GCMS.Variousmultiresidue methods for a large number of pesticides, by gas chromatography (GC) coupled to mass spectrometry (MS) have been reported appropriate for soil samples. However, the mentioned liquid chromatography (LC) coupled to mass spectrometry (MS) multiresidue methods relevant to environmental samples can find out fewer pesticides in a single run than GC-MS methods. Furthermore there are very few reported multiresidue methods using LC with a single quadrupole .

3.1 Pesticide Residues at soil level 1; samples collected at the time of pruning (Lean Period)

The percentage of pesticide residues detected in the soil samples collected in the month of August at the time of pruning are reported in Figure 1. Altogether 15 soil samples (A-O) were collected from different locations of Nashik district and Monitor for its percent residues of target pesticides.

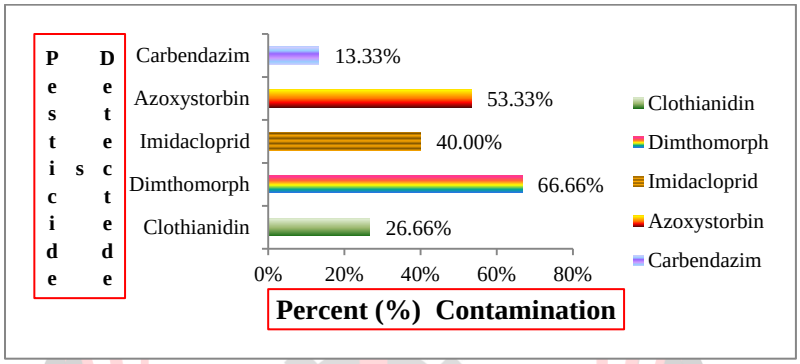

figure 1: graph showing percent (%) contamination of pesticides in soil samples collected at the time of pruning.

Contamination of pesticide residues shows variation in concentration levels for different soil samplesbecause persistence of these pesticides depends upon physicochemical parameters of different soil samples and sorption and desorption of pesticides in soil[1].Dimethomorph was found in ten samples with highest percent contamination of 66.6%.Azoxystorbin was the second most often detected pesticide investigated in seven samples with percent contamination of 53.33% followed by imidacloprid, clothianidin and carbendazim with percent contamination of 40.0%, 26.66%, 13.33% respectively. In soil samples collected shows highest contamination with concentration of 42.1 µg kg^{-1} in soil sample 'M' followed by 31.6 µg kg^{-1} in 'K', 24.13 µg kg^{-1} in 'B', 23.6 µg kg^{-1} in 'N',8.12 µg kg^{-1} in 'A',7.2 µg kg^{-1} in 'C' and 2.9 in µg kg^{-1} 'J' The mean of ∑ pesticides was found to be 13.386 µg $kg.^{-1}$

3.2 Pesticide Residues in soil samples collected at the time of fruit development (Peak period)

Soil samples were collected from the selected grape farms in the month of November at the time of fruit development period. Most of the soil samples were found to be contaminated with fifteen pesticides residues at various concentration levels.

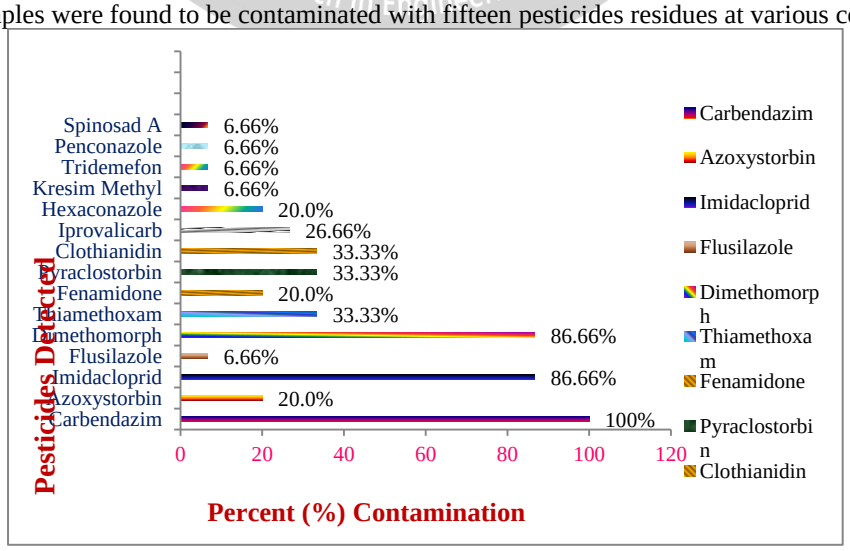

figure 2: graph showing percent (%) contamination of pesticides in soil samples collected at the time of fruit development.

The mean of ∑ pesticides was found to be 113.18 µg kg^{-1} at 95% confidence level. Soil sample 'H' shows highest contamination with concentration of 439.07 µg kg^{-1} followed by 291.33 µg kg^{-1} in 'I', 136.17 µg kg^{-1} in 'F', 128.13 µg kg^{-1} in 'M' , 119.48 µg kg^{-1} in 'G', 100.71 µg kg^{-1} in 'B', 92.15 µg kg^{-1} in 'K', 81.21 µg kg^{-1} in 'E', 71.17 µg kg^{-1} in 'J', 68.99 µg kg^{-1} in 'L', 59.74 µg kg^{-1} in 'A', 47.89 µg kg^{-1} in 'C', 26.92 µg kg^{-1} in 'N', 22.86 µg kg^{-1} in 'O',11.96 µg kg^{-1} in 'D' respectively.Out of fifteen pesticides carbandazim was detected in all soil samples relatively in high concentration with highest percent contamination of 100.0% .Dimethomorph and imidacloprid were the second most often detected pesticides investigated in thirteen soil samples with percent contamination of 86.66 % followed by thiamethoxam, pyraclostorbin,clothianidin with percent contamination of 33.33% detected in six soil samples. Iprovalicarb found in four soil samples with percent contamination of 26.66 %.while fenamidone andhexaconazole were detected in three soil samples with percent contamination of 20%.Remaining five pesticides such as flusilazole ,kresim methyl, tridemefon, spinosad-A andpenconazole were detected in only one soil sample with low percent contamination of 6.66% .

3.3 Pesticide Residues in soil samples collected at the time of harvesting.

The concentration of pesticide residues detected in the soil samples collected in the month of February-March at the time of harvesting are reported. All collected soil samples were found to be polluted with eighteen pesticides residues differ widely in contamination levels. Some pesticides such as imidacloprid, dimethomorph shows large variation with relatively high concentration values for different soil samples. While other pesticides were found to be detected with low concentration which reduces the possibility of biomagnifications of pesticides in grapes and avoid contamination in grapes.

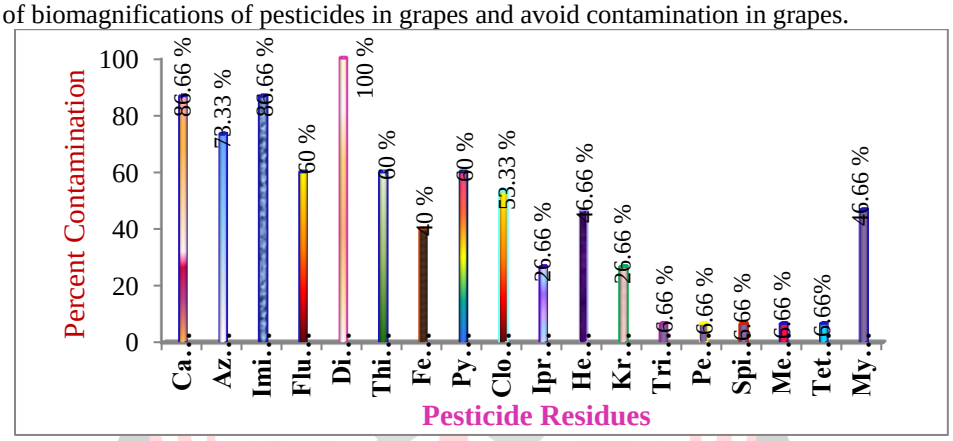

figure 3 : graph showing percent (%) contamination of pesticides in soil samples collected at the time of harvesting

Out of eighteen pesticides detected dimethomorph, imidacloprid and carbendazim were the most often detected pesticides found to be contamination levels relatively in high concentration with highest percent contamination of 100.0%, 86.66 %, 86.66% respectively shown in figure 28. This indicates that use of these pesticides in grape growing farms was on higher side throughout the year.

Azoxystorbin was investigated in eleven soil samples with percent contamination of 73.33%. followed by pyraclostorbin, thiamethoxam and flusilazole with 60.0 % while clothianidin with 53.33% .Pesticide residues of hexaconazole and myclobutanil detected in seven soil samples with percent contamination of 46.66% followed by fenamidone with 40%, iprovalicarb and kresim methyl contaminates four soil sample with percent contamination of 26.66%. Remaining five pesticides such as, tridemefon, penconazole, metalaxyl, tetraconazole, and spinosad A were detected in only one soil sample with low percent contamination of 6.66%.

The mean of ∑ pesticides was found to be 290.67µg kg^{-1} at 95% confidence level. Soil sample 'A' shows highest contamination with concentration of 840.30 µg kg^{-1} followed by 826.60 µg kg^{-1} in 'K', 714.8 µg kg^{-1} in 'I', 655.10 µg kg^{-1} in 'L', 273.10 µg kg^{-1} in 'M', 232.60µg kg^{-1} in 'H', 212.40µg kg^{-1} in 'O', 179.80 µg kg^{-1} in 'B', 174.60 µg kg^{-1} in 'F', 89.80 µg kg^{-1} in 'C', 66.90 µg kg^{-1} in 'N', 50.60 µg kg^{-1} in 'G', 34.90 µg kg^{-1} in 'J', 4.70 µg kg^{-1} in 'D', 3.9 µg kg^{-1} in 'E' respectively.

3.4 Statistical Analysis

In this study, the statistical methods for qualitative data, frequency count (N) and percentage were set up in a tabular and graphical form. For quantitative data, descriptive statistics was presented by frequency count (N), Mean, Standard Deviation and Range. To analyze the data, appropriate statistical tests were applied such as General Linear Model (GLM) of repeated measures was used for analysis of data. Statistical packages for the social sciences software (version 16.0) was used for statistical anlysis. Tables and graphs were prepared by Microsoft excel (Windows 7)

The profile plots show the model-estimated means for the 18 pesticides for each of the soil levels of the study.

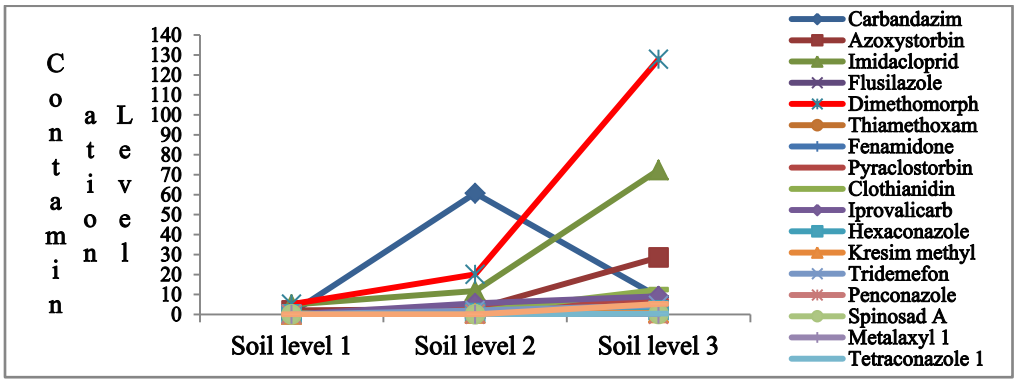

figure 4: mean plot for pesticide-wise contamination at three soil levels

The profile plot for soil level 1 means, soil samples collected at the time of pruning shows that, the contamination levels of pesticides generated slight steady pattern. However, Pesticide residue of dimethomorph shows higher contamination level as compared to others.

In the study, at soil level 2 means soil samples collected at the time of fruit development pesticides contamination levels were shows fairly steady pattern except carbendazim, imidacloprid and dimethomorph detected in higher concentration levels. While at soil level 3 means, soil samples collected at the time of harvesting contamination levels of few pesticides were shows a lot of variation in concentration than other especially dimethomorph, carbendazim,imidacloprid and azoxystorbin This variation is about 27 % , so it is an important effect to model.

Table 1: Repeated contrasts for soil levels

Tests of Within-Subjects Contrasts							
Measure: pesticides							
Source	soil	Type III Sum of Squares	df	Mean Square	F	P value	Partial Eta Squared
Soil levels	Level 1 vs. Level 2	1242.29	1	1242	0.554	0.457	0.002
	Level 2 vs. Level 3	2480.82	1	2481	1.079	0.3	0.004
soil levels* selected grape farms	Level 1 vs. Level 2	4707.07	1	4707	2.098	0.149	0.008
	Level 2 vs. Level 3	11309.4	1	11309	4.917	**0.027**	0.019
soil levels* pesti	Level 1 vs. Level 2	81107.1	17	4771	2.127	**0.007**	0.126
	Level 2 vs. Level 3	230347	17	13550	5.891	**0.0001**	0.285
Error(soil)	Level 1 vs. Level 2	563069	251	2243			
	Level 2 vs. Level 3	577277	251	2300			

The contrasts for the source, soil level versus soil have not significance values of 0.149 (P>0.05) in soil level 1 verses soil level 2, indicating that at the time of pruning these soil levels did not have an effect of the pesticide contamination on selected grape farms .However in soil level 2 verses soil level 3 shows, significant value of 0.027 (P<0.05). This means that, the significant results of the multivariate tests are due to the effect of the pesticide contamination on those soil sampling area .Indicates that grape farms under study area have notable effect of contamination of pesticide residues on their soil .The contrasts for the source, soil level verses pesticides, all have significance values of 0.007 for soil level 1 verses soil level 2 while 0.0001 for comparison between soil level 2 verses soil level 3 (p<0.05), indicating that the pesticides have an effect on the soil levels, since at each time period, the soil of selected grape farms become affected by the contamination due to pesticides.

4. Conclusions

The findings of this study do not confirm the widespread assumption that, grape growing soils in the study area are severely contaminated with pesticides because, most of the soil samples were found to be contaminated by pesticides with very low concentration. The foregoing results have shown that, the major pesticide used by farmers in combating the effects of pests and diseases on their grape farms are of fungicides which, do not have any severe negative impacts on soil .Most of the soil samples were found to be contaminated by fungicides of azole group such as, Flusilazole, Hexaconazole, Penconazole, Propiconazole, tebuconazole, myclobutanil, carbendazim, tridemefon. It may be because of their longer half life period in the soil of 14 to 420 days (Kookana et.al, (1998)

The persistence of pesticide residues in the soil is related to their chemical structure, half life period, hydrophobicity, bioavailability for degradation and physicochemical parameters of soil(Komarek et .al,(2010) &Gevao

et.al,(2000)Only five pesticide residues were detected in soil level 1.The mean of ∑ pesticides was found in very low concentration, *i.e.* 13.386 µg kg^{-1}, at the time of pruning, before application of pesticides in grape farm indicates persistence of fungicides in the soil from one season to the next season.Total fifteen pesticide residues of various groups were detected in soil level 2 *i.e.* at thetime of fruit development with mean of ∑ pesticides 113.18 µg kg^{-1} shows the application of multiple agrochemicals during growing season by farmers.

It was found that, most of the soil samples have higher contamination at soil level 3 *i.e.* at the time of harvesting as compared to soil level 1 and 2. The mean of ∑ pesticides was found to be 290.67µg kg^{-1}.The observed higher levels of pesticide residue concentration can be attributed to the current application of these chemicals a few days prior to sampling, means farmers did not follow Pre Harvest Interval (PHI) during application of pesticides. It is a good sign of awareness observed among the farmers that, not a single soil sample was found to be contaminated with organochlorine compounds. The results of statistical analysis shows highly significant values ($p<0.05$), indicates the interaction between pesticides and soil samples at each level.

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References:

[1] RaikwarMukeshKumar,BhardvajVikas,SawantUrmila and VoraVirendra (2011). Study of Contamination Level of Pesticide Residues in Grapes (*Vitisvinifera*) in Maharashtra; Green Pages: 1-4.

[2]Wadhwani A.M.andLallJ.J. (1972) "Harmfuleffectsof pesticidesonagriculturallandin India",*Indiancouncilof agriculturalresearch*NewDelhiP.N.44.

[3] Singh, R.P. 2001. Comparison of organochlorine pesticide levels in soil and groundwater of Agra, India. *Bull. Environ. Contam.Toxicol.* 67: 126-132.

[4] Arora P.K. and GaganjyotB.Singh (2009)" Persistence of imidaclloprid pesticides on grape growing soil in Ludhiana (Punjab)" bulletin of environmental contamination and toxicology 82 P.N. 239-242

[5] Zweig G (1984),Analytical methods for pesticides and plant growth regulator, In; *Academic press,New York* (Eds.Sharma J.).Pp.143-146

[6] Wang,X., Wang,D.,QinX.andXu,X.(2008),Residuesof organochlorine pesticides in surface soils from college school yardsinBeijing,China.*J.Environ.Sc.*,20:1090-1096.

[7] Om Prakash, Suar, M., Raina, V., Dogra, C., Pal R. and Rup Lal. (2004), Residues of Hexachlorocyclohexane isomers in soil and water samples from Delhi and adjoining areas. Curr. Sci., 87 (1):73-77

[8] KumarBhupander.,(2011) ,Residues of Pesticides and Herbicidesinsoilsfrom Agriculture AreasofDelhi Region,India

[9]Bharambe S. and Mahulikar P. (2010) contamination level of pesticides residues and physiochemical properties of soil from cottonrowing area and farmer awareness journal natural resource management for sustainable development "

[10] Kookana, R S., Baskaran, S. and Naidu, R. (1998). Pesticide fate and behaviour in australian soils in relation to contamination and management of soil and water: a review. Australian Journal of Soil Research; Vol.36 (5) : 715-764.

[11] Komarek, M., Cadkova, E., Chrastny, V., Bordas, F. and Bollinger,C. (2010).Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. Environment International; Vol.36 : 138-151.

[12] Gevao,B.,Semple,K.T. and Jones,K.C. (2000).Bound pesticide residues in soils:a review.Environmental pollution; Vol.108: 3-14.

