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Pesticide risk assessment in integrated apple (*Malus* × *domestica*) farming systems of dry temperate and cold desert region of Indian Himalaya

RAJESHWAR S CHANDEL[1], I D SHARMA[2], R C MISHRA[3], PRAMOD KUMAR[4] and DEEPALI BAKSHI[5]

Y S P University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh 173 230

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ABSTRACT

Horticulture has an important role in the socio-economic context of people of remote and inaccessible Himalayan mountains. The intensive cultivation of horticultural crops however, has led to heavy usage of different pesticides resulted into the contamination of environment. Pesticide residues were determined in soil and water samples from integrated apple farming systems falling under dry temperate and cold desert (Zone-IV) of Himachal Pradesh, a north-west Himalayan state of India. The pesticide residues of HCH and DDT were detected in soil samples, despite their non-use history in apple (*Malus* × *domestica* Borkh.) orchards for the last 15 years. Σ-endosulfan, dicofol, Σ-HCH and Σ-DDT were detected as the major contaminants among organochlorines, whereas, chlorpyrifos was the only organophosphate pesticide present in the soils. Endosulfan sulphate (16.67%) was the most encountered soil contaminant in Zone-IV followed by β-endosulfan, chlorpyrifos, α-HCH, β-HCH and p,p-DDE' each in all 2 samples (8.33%) with a maximum concentration of 0.015, 0.008, 0.006, 0.006, 0.007 and 0.008 mg/kg, respectively. The monitoring of potable water revealed presence of no pesticide contaminants in natural resources used as potable water. The microbial counts were found higher in the soils of apple farming systems in Kinnaur district than in Lahaul & Spiti.

Key words: Environmental risks, Residue accumulation, Soil contaminants, Water residues

Pesticides overuse and misuse has been an increasing problem in developing countries especially in the Hindu-Kush Himalayas of India (Neupane 2002). If the increased fruit production has provided livelihood opportunities to the poor mountainous farmers, it has also been resulting into serious debits in terms of serious health and environment implications. The environmental impact of pesticides is often greater than what is intended by those who use them. During the past 5-6 decades, there has been a substantial increase in the use of pesticides in terms of both volume and value. The widespread contamination and the toxic effects of the organic chemicals have become serious environmental concerns to soil by direct treatment or through washed off from the plant surface during rainfall. An appreciable quantity of pesticides and their degraded products have been reported to accumulate in the soil ecosystem (Kumari *et al*. 2008), that adversely affected the soil health and productivity (Kammenga *et al*. 2000). The physico-chemical characteristics mainly, hydrophobicity and resistance to degradation, have accumulated the chemicals in soils and sediments (Hu *et al*. 2010). Moreover, higher bioaccumulation potential, ubiquity, persistence, deleterious effect and toxicity to non-targeted organisms have been a major environmental issue (Hao *et al*. 2008). Recent investigations have shown the persistence of the residues of Hexachlorocyclohexane (HCHs) and dichlorodiphenyltrichloroethane (DDT) in soil rhizosphere (Wang *et al*. 2007, Zhang *et al*. 2006). The persistence of toxic pesticides has been reported in aquatic life and drinking water (Kumar *et al.* 1995).

Himachal Pradesh is known as 'the fruit bowl of India', is a mountainous State in the north-west Himalayas. Apple (*Malus* × *domestica* Borkh.) is known as the King of temperate fruits in India, occupies a total cultivable area of 109853 ha, with annual production of 754954 MT (Anonymous 2016), which is providing livelihood options to the mountainous rural and tribal farming community. Earlier reports indicated a large scale switching of mountain farmers to the cultivation of apple with the indiscriminate use of pesticides (Partap 2010), and the recommendations apart, the chemicals are being used indiscriminately without considering scientific recommendations. The improvement in fruit yield however, is sometimes concomitant with the occurrence and persistence of pesticide residues in soil and water (Ware and Whitacre 2004). The presence of pesticide

[1]Joint Director Research (e mail: rs_c@rediffmail.com), Directorate of Research, [2]Joint Director (Trainings) (e mail: idsharmaainp@gmail.com), Directorate of Extension Education, [3]Scientist (e mail: rajanishentom@rediffmail.com), Department of Entomology, [4]Scientist (Fruit Science) (e mail: pk09sharma@rediffmail.com), RHRTS, Dhaulakuan 173031 Sirmour, Himachal Pradesh, [5]Research Scholar (e mail: dbakshi30@gmail.com), Department of Entomology.

residues further confirms its presence in apple ecosystem in temperate horticultural produce of India (Anonymous 2013). Keeping this in view, the present work was planned with the objective of environmental risk assessment focussing the residue contamination of soil and surface water through frequent pesticides usage in the apple ecosystem under dry temperate and cold desert region of Himachal Pradesh.

MATERIALS AND METHODS

The present investigation was carried out on full bearing Royal Delicious apple trees of uniform age group (20 years), planted at 5×5 m apart. Two districts, Kinnaur and Lahaul and Spiti of Himachal Pradesh were selected purposely according to sampling scheme, and that they were major apple growing areas of the dry temperate and cold desert region. Twelve apple orchards at each of the two locations namely, Kaza (Lahaul and Spiti, 32º 30' 00" North latitude and 77º 50' 00" East longitude) and Reckong Peo (Kinnaur 31°32'20" North latitude and 78°16'03" East longitude) in agro-climatic Zone-IV (dry temperate, high hills and cold desert) were selected. The sampling locations were in the geographic proximity to villagers that have apple orchards with intensive crop production system (with regular pesticide usage) vis-a-vis none of the cultivation fields at each location. At crop harvest, for composite soil sampling, two identical sites with apple orchards, the intercropped orchards and uncultivated soils within each of the location were selected. The baseline soil samples, weighed up to 1 kg were collected on a transect at 30 cm soil depths using an auger of 5 cm diameter in the rhizosphere of common vetch. These samples were analyzed for various physico-chemical characteristics including textural class, pH and organic matter content using standard methods. The soil samples were collected from dominant farming systems, i.e. apple+ rajmash/pea after having pesticide usage history from farmers. The samples were also collected from the surrounding uncultivated areas. The air dried and sieved (20 mm brass) soil samples were analysed in the Pesticide Residues Laboratory. From each sample, three sub-samples (15 g each) were drawn for further use. Out of these sub-samples, one sample was processed for the analysis of organochlorines, organophosphates and synthetic pyrethroids, while, the remaining two were analyzed for mancozeb and carbendazim fungicides, separately.

Subsequently, 20 water samples (5 litre each) from surface water (potable water) sources in and around the study sites were collected. Thereafter, a sub-sample of 500 ml was taken up for pesticide residues analysis. Pesticide grade solvents and analytical reagents purchased from M/s Merck (India) were used for extraction and clean-up. Pesticide standards (purity: 97-99%) were purchased from Dr Ehrenstorfer GmbH (Germany). Spiking solutions for measuring method efficacy (% recovery) were prepared from the stock solutions. Calibration curves of working standards were used to evaluate the linearity of the gas chromatograph response on each day of the analysis, and the quantification of the pesticide residues were based on these external standards (Sharma *et al*. 2016).

Soil samples were extracted using a soil-packed sintered column. Each sub-sample (15 g) was blended with 0.3 g Florisil and 0.3 mg charcoal in mortar until free flowing. The sample mixture was then transferred to 250 ml sintered column. The mortar was triple rinsed with hexane (5 ml) and transferred to the column contained 1 g anhydrous Na_2SO_4. After slight tapping, the packed material was eluted with 100 ml of acetone: hexane in the ratio of 1:9 v/v. The eluate was then evaporated to dryness at 40-50 ^{0}C using a rotary evaporator (Heidolph). The residues were re-dissolved in 5 ml hexane and subjected to an additional cleanup steps. The n-hexane containing pesticide residues and soil extract was loaded on 1 g activated silica gel column. The column was eluted with 15 ml of acetone: hexane in the ratio of 1:9 v/v. Soil eluate was evaporated to dryness at 40-50 ^{0}C using a rotary evaporator. The sides of the flask were rinsed with hexane (20 ml) and then evaporated to 5 ml as final volume in hexane prior to GC analysis (Sharma 2010).

Water samples were extracted using liquid-liquid extraction. Each sample (500 ml) was poured through a folded filter paper and measured in a graduated cylinder (1 litre) before transfer to a separator funnel (1 litre). Sodium chloride (10 g) was added to the separator funnel and shaked well. Water-sodium chloride mixture was extracted with dichloromethane (3×100 ml). After each separation, the organic layer was filtered through granular Na_2SO_4. The combined dichloromethane layers were evaporated on a rotary evaporator (Heidolph) to dryness, thereafter, hexane (15-20 ml) was added, and the evaporation continued till it reduced to about 2 ml.

Soil and water extracts were analyzed using Agilent 6890N Gas Chromatograph equipped with dual detectors ^{63}Ni electron capture detector (ECD), nitrogen-phosphorus detector (NPD), capillary column, and Hewlett Packard Chemstation software. The instrument conditions were kept as per the method standardized by Sharma *et al.* (2016). The mancozeb residues were analyzed according to dithiocarbamate fungicides estimation method (Schwack and Nyanzi 1995). Subsequently, carbendazim residues were determined using the method suggested by Nath *et al*. (1993).

Soil samples from non-cultivable areas, and deionized water with pesticide-free matrix were used as control. The analytical method employed to estimate residues was validated by spiking the control soil and water samples at three different concentrations, viz. 0.001, 0.01, 0.1 mg/kg for organochlorines and chlorpyrifos, and 0.005, 0.05, 0.1 mg/kg for organophosphates, synthetic pyrethroids, hexaconazole, carbendazim and mancozeb. The average recoveries obtained were in the range of 79.74- 95.42 per cent for different estimated pesticides (Table 1). Though the limit of determination was 0.01 mg/kg for organochlorines and chlorpyrifos, and 0.05 mg/kg each for organophosphate and synthetic pyrethroids yet the low values mentioned in the study are due to concentration of the sample to the

Table 1 Pesticides recovery from soil and water samples at different fortification levels

Name of Pesticide	Fortification level (mg/kg)	Recovery (%)
∑-HCH (α, β, γ, δ HCH)	0.001, 0.01, 0.1	88.25 (82.56-93.80)
∑-DDT (p-p DDE, o-p DDD, p-p DDD, p-p DDT)	0.001, 0.01, 0.1	86.70 (84.10-94.28)
∑-Endosulfan (α-endosulfan, β-endosulfan, endo-sulphate)	0.001, 0.01, 0.1	83.40 (81.00-87.46)
Chlorpyriphos	0.001, 0.01, 0.1	88.50 (82.35-94.00)
Dicofol	0.001, 0.01, 0.1	88.80 (80.00-94.53)
∑-Pyrethroids (λ-cyhalothrin, α-methrin, deltamethrin, permethrin, fenvalerate)	0.005, 0.05, 0.1	82.60 (80.62-91.60)
Malathion	0.005, 0.05, 0.1	85.28 (82.10-92.50)
Methyl demeton	0.005, 0.05, 0.1	87.00 (80.52-89.85)
Carbendazim	0.005, 0.05, 0.1	82.40 (79.74-87.32)
Mancozeb	0.005, 0.05, 0.1	84.94 (82.08-95.42)
Hexaconazole	0.005, 0.05, 0.1	82.45 (81.78-85.20)

Figures in parenthesis are the values for range.

determination level and further extrapolation of the data accordingly.

The information were gathered from 12 farmers in each of the study location on farmers knowledge with respect to the adoption of recommended package of practices for the crops grown, use of other non-recommended pesticides, frequency of pesticide use and source of advisory services during the past 15 years. The soil samples collected from different farming systems of Kinnaur and Lahaul and Spiti districts were also analyzed for total microbial counts. From each composite sample, one gram soil was taken in 9 ml of sterilized water blank and the soil suspension was diluted 100 times. From this suspension, 0.1 ml was taken and added to a test tube containing 9.9 ml water. For fungi, the dilution was done at 1×10^{-3} while for bacteria and actinomycetes, the dilutions were made at 1×10^{-5} and 1×10^{-6}, respectively. The microbial counts were determined by standard pour plate technique on different media, namely Peptone-Dextrose Rose Bengal Agar media, Soil Extract Agar media and Starch Ammonium Agar for fungi, bacteria and actinomycetes, respectively (Subba Rao 1999). The population was expressed as colony forming units (cfu/g soil).

RESULTS AND DISCUSSION

Soil physico-chemical characteristics

The samples drawn from orchards rhizosphere of two locations were analyzed for the physico-chemical characteristics using standard methods. In Zone-IV, soil type of 'Kaza' and 'Reckong Peo' were recorded as sandy-loam (Sand 59-62%, silt 14-25.8%, clay 7.2-15.4%) with pH 6.9-8.4 and 0.74-0.90% organic carbon. These studies corroborate with those of Sharma and Kanwar (2010) who reported the soils of dry temperate zone of Himachal Pradesh as neutral to alkaline (6.2-10.3) in reaction and sandy loam to sandy clay loam in texture. The reported presence of most of the pesticides at lower concentration in the present study was due to the rapid degradation of these pesticides under neutral to alkaline conditions of the soil.

Pesticide usage pattern

In Himachal Pradesh, apple orchardists generally spray different pesticides as many as six times in a cropping season. It was observed that 8 pesticides in Kaza block of Lahaul and Spiti and 17 pesticides in Reckong Peo (Kalpa block) of Kinnaur were being used frequently (Table 2). In Kaza, the less consumption of pesticides was observed as compared to Reckong Peo. This difference in consumption may be attributed to the climatic variations in these areas. The congenial climatic condition in Reckong Peo areas, which receives comparatively higher rainfall, favours the infection of some diseases in addition to the attack of insect-pests there by invited frequent application of pesticides for crop pest management. Partap and Partap (2002**)** have reported that almost 100% of farmers apply 6-7 sprays in Kullu district and 9-10 sprays in Shimla district of Himachal Pradesh during whole of the cropping period in apple.

Table 2 Pesticide usage pattern in different farming systems of Lahaul and Spiti and Kinnaur

District/location	No. of samples	Pesticide usage pattern
Lahaul and Spiti		
Kaza	10	Chlorpyrifos 20 EC, Thiacloprid 240 SC, Fenpropathrin 10 EC, Oxydemeton methyl 25 EC, Dimethoate 30 EC, Fenzaquin 10 EC, Mancozeb 75 WP, Carbendazim 50 WP
Kinnaur		
Reckong Peo	10	Copper oxychloride 50 WP, Copper hydroxide 77 WP, Tebuconazole 25.9 EC, Hexaconazole 5 SC, Propineb 70WP, Chlorpyrifos 20 EC, Cypermethrin 25 EC, Thiacloprid 240 SC, Mancozeb 75 WP, Carbendazim 50 WP, Fenpropathrin 10 EC, Oxydemeton methyl 25 EC, Dimethoate 30 EC, Spiromesifen 22.9 SC, Fenazaquin 10 EC, Propergite 57 EC, Nativo 75WG

Table 3 Status of pesticide residues in apple farming systems

Pesticide reporting status	Pesticide	Pesticide/ isomer/ metabolite detected	Apple orchard soil		Apple intercropped soil	
			Samples (%)	Range concentration (mg/kg)	Sample (%)	Range concentration (mg/kg)
	Chlorpyrifos	Chlorpyrifos	2 (8.33)	0.005-0.006	0	BDL
	Dicofol	Dicofol	1 (4.16)	0.005	0	BDL
	Endosulfan	α-endosulfan	0.00	BDL	0	BDL
		β-endosulfan	2 (8.33)	0.005-0.008	1 (4.16)	0.008
		Endo-sulphate	4 (16.67)	0.005-0.015	2 (8.33)	0.005–0.007
Not in use since 15 years, however, detected	HCHs	α-HCH	2 (8.33)	0.005–0.006	2 (8.33)	0.005–0.006
		β-HCH	2 (8.33)	0.005–0.007	1 (4.16)	0.006
		γ-HCH	0	BDL	0	BDL
		p,p'-DDE	2 (8.33)	0.005–0.008	2 (8.33)	0.005–0.007
		p,p'-DDD	0	BDL	0	BDL

Sample size, n-24; BDL- below detection limit

Residue estimation

Orchards and intercropped soils: The data presented in Table 3 describes the estimation of 10 pesticides in soils of apple orchards of Zone-IV. Among these 'endosulfan sulphate' was the predominant contaminant detected in 4 samples (16.67%) followed by 'β-endosulfan', 'chlorpyrifos', 'α-HCH', 'β-HCH'and 'p,p'-DDE' each in all 2 samples (8.33%) with a maximum concentration of 0.015, 0.008, 0.006, 0.006, 0.007 and 0.008 mg/kg, respectively. Besides, out of total 24 samples analysed, the other contaminants viz. 'α-HCH', 'α-endosulfan', 'β-HCH', 'p,p'-DDT' and 'p,p'-DDD' were also detected in 4, 2, 2, 2 and 1 number of samples, respectively. However in intercropped apple orchards soils, the metabolite endosulfan sulphate (0.005-0.007 mg/kg), α-HCH (0.005–0.006 mg/kg) and p,p'- DDE (0.005-0.007 mg/kg) were detected in all 8.33% samples, respectively. The residues of 'β-endosulfan' and 'β-HCH' were detected in 1 (4.16%) with maximum concentration 0.0008 and 0.005 mg/kg, respectively in 24 samples monitored.

Uncultivated soil: Organochlorine pesticides were found in the uncultivated soil in and around apple growing areas. The data shown in Table 4 indicated the detection of maximum contamination of dicofol, α-HCH and p,p'-DDE (8.33% each) in Zone-IV. All the samples showed the concentration of insecticides in the range of 0.005-0.006 mg/kg.

Pesticide residues in water: The residue estimation of 20 water samples collected from each study location of Lahaul and Spiti and Kinnaur districts confirmed that the water available in natural resources were free from pesticide contaminants. The outcome of the farmers' survey and the recommended integrated spray schedule for the management of apple pests and diseases revealed the use of 17 pesticides for the last 15 years in apple orchards. Out of these, the endosulfan, chlorpyrifos, dicofol, carbendazim and mancozeb were the important constituents of the integrated spray schedule programme recommended against different pests. In both districts under study, endosulfan was estimated as the more prevalent soil contaminant followed by chlorpyrifos, p,p'-DDE and α and β isomers of HCH (8.33% each). This indicated the tendency of endosulfan to persist for several years after its application (Mullins *et al*. 1971). Furthermore, the soil application of chlorpyrifos at higher dose of 0.1% against apple root borer supports its presence in apple soils. Similar observations on chlorpyrifos soil contamination have also been reported from northern India (Kumari *et al*. 2008, Sharma *et al.* 2016). The presence of dicofol residues in soils might have been appeared due to its earlier recommended use against mite pests on apple and also one of the reasons for the presence of DDT metabolites (Liu *et al*. 2006). The isomers/metabolites of HCH, DDT and endosulfan were also reported in soil of Haryana and north Indo-Gangetic alluvial plains (Kumari *et al*. 2008, Singh *et al*. 2007). The observations on no contamination of water from apple ecosystem of Himachal Pradesh has earlier been recorded by Sharma *et al*. (2016). Some other studies, conducted in the Indo-Gangetic alluvial plains and parts of Haryana, reported the occurrence of HCHs (α, β, γ and δ) and DDT analogues in surface and underground water (Kumari *et al*. 2008, Singh *et al*. 2007).

Table 4 Pesticide residues detected in uncultivated soils

Pesticide reporting status	Pesticide	Pesticide/ isomer/ metabolite detected	Sample (%)	Range concentration (mg/kg)
	Dicofol	Dicofol	1 (8.33)	0.006
Not in use since 15 years, however, detected	HCHs	α-HCH	1 (8.33)	0.005
		β-HCH	0	BDL
		p,p'- DDE	1 (8.33)	0.005
		p,p'-DDD	0	BDL

Sample size, n-12; BDL, below detection limit.

Estimation of microbial population

The count for fungi, bacteria and actinomycetes in different locations of the study area were estimated. The fungi count ranged from 0-3.66 (× 10^3) and 1.33-7.66 (× 10^3), the bacteria from 33.33-100 (× 10^5) and 68.00- 121.60 (× 10^5) and actinomycetes from 0.33-6.00 (× 10^6) and 1.33-6.66 (× 10^6) cfu/g in respective Lahaul and Spiti and Kinnaur districts. The presence of respective microorganisms in the soils of apple farming systems was higher in Kinnaur district than in Lahaul and Spiti. Kinnaur has sandy clay loam to sandy loam soil texture, with comparative more soil organic carbon, thus promoted more population of microbes (Sharma and Kanwar 2010).

The estimation of pesticide residue contamination in soils and water from apple growing dry temperate and cold desert region revealed Σ-endosulfan as the major soil contaminant. Endosulfan sulphate was detected in 16.67 % samples followed by chlorpyrifos, β-endosulfan, HCH (α and β), p,p'-DDE (each 8.33%) and dicofol (4.16%) soil samples from alone apple orchards as well as intercropped orchards soils. All pesticide contaminants were estimated in the range of 0.005-0.015 mg/kg. Whereas, dicofol, α-HCH and p,p'-DDE (each in 8.33%) were detected in the range of 0.005- 0.006 mg/kg, in uncultivated soils around the apple orchards. The monitoring of surface water did not reveal the presence of any pesticides.

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