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Effect of Pesticide Residues on Soil Arthropods in Okra Crop

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Abstract:

The field experiment was conducted at Tando Allahyar district, and soil samples from experimental field were collected, they were analyzed at NCEAC, University of Sindh Jamshoro. The study was conducted in two parts. In first part, the effects of pesticide applications on soil arthropods. Application of four insecticides namely bifenthrin, chlorpyrifos, endosulfan and imidacloprid at recommended doses severely reduced soil-arthropod populations. Earthworms were not found in okra field. Population of spiders, black spiders, ants, field cricket, snow bug, insect larvae, and silverfish drastically reduced

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during at 1 day post-treatment interval. There after population of these arthropods slowly recovered but did not reach at the control level up to 14 days post-treatment interval. In second part, physical characteristics of soil and residues of above mentioned pesticides in soil were determined. The pH of soil was slightly alkaline (7.79) with high salinity and low organic matter (1.144 %). Pesticide residue was not detected in okra soil at pre-treatment. However, maximum residue was found at 1 day post-treatment interval for all the four pesticides used and the residue levels were 3.21, 7.94, 5.89 and 3.01ppm for bifenthrin, chlorpyrifos, endosulfan and imidacloprid respectively. The minimum residue was found at 14 day post-treatment interval for all the four pesticides used. At this interval bifenthrin residues were minimum (0.57 ppm) with 82.24% reduction, and endosulfan residues were maximum (359 ppm) representing 39.05% reduction.

Key words: Okra, Pesticides residues, Soil PH, Arthropods.

INTRODUCTION

Pakistan is an agricultural country. Agriculture is an important sector in providing food to the fast growing population of the country. It is the backbone of the economy of Pakistan, as it contributes 24 percent of gross domestic product (GDP) and 60 percent in total export earnings. It employs 47 Percent of the labor force of the country. Approximately 65 percent people are directly or indirectly associated with this field (source: http://www.pakistanhotline.com/2012/03/agriculture-of pakistan.html).

In Pakistan, Sindh province is the second largest contributor of agricultural production, particularly the vegetables. Production of vegetables like okra, bitter gourd, tomato and cauliflower in Sindh during 2005 was 22.1, 37.1, 60.5 and 13.3 thousand tones respectively (Government of Pakistan, 2005b). Okra is severely attacked by insect pests. Moreover, cotton and okra belong to same family Malvacease and share the same pest complex. Because cotton is a main cash crop, agriculture department and farmers have focused on cotton IPM along with the sowing of bollworm resistant cotton varieties. It is evident that Sind a poly-culture cropping system which mean sowing many crop adjacent to each other. Clive Pankhurst (2006) were reported during the latter half of the 20th century the development and use of pesticides to control weeds and animal pests and diseases has increased steadily and pesticide use has now become an integral component of agricultural farming systems in most developed countries. The principal forms of pesticides used in Australia can be categorized into herbicides, insecticides, nematicides and fungicides. Early pesticides were generally broad spectrum in their action and generally more toxic or "hard" compared to those that have been developed in the last 20 years which target pests more specifically, are more efficacious and generally less toxic or "soft" (AATSE report, 2002). Also associated with the early use of pesticides was little appreciation of their potential impact on non-target biota or their fate in the environment. These perceptions have since changed and now all pesticides are subjected to a rigorous environment and health risk assessment before they are registered for use. In the last 20 years; Australian farmers have become increasingly concerned about potential impacts of pesticides on soil organisms and the functions they carry out. They have also become more aware of the importance of maintaining soil health as a prerequisite to sustaining crop productivity. Consequently, farmers are seeking more detailed information about the environmental impact of the pesticides they use, including their persistence in the soil, their propensity to move through the soil and into groundwater and their effect on soil organisms and biological processes associated with soil health. Whilst there has been a world-wide concerted research effort (particularly during the period from

1970-1990), into the environmental impact of many of the pesticides in current use, much of this information is contained in scientific publications or reports and is not readily accessible to farmers and land users. In addition there have been few studies carried out in Australia where the information is readily accessible, and there has been no in depth study of the effect of pesticides on soil health in sugarcane farming systems.
Since no work has been conducted on effect of pesticides residues on soil arthropods in Sindh, Pakistan and keeping in view the importance of okra and the frequency of pesticide applications this study on the effect of pesticides residues on soil arthropods in okra crop is planned.

MATERIALS AND METHODS

2.1 Pesticide Spray on Okra

Okra was grown on the experimental field ofTando Allahyar district, (area about half acre) through organic farming with plain water spray to serve as control. Pesticide application experiment was conducted on commercial okra farm in Tando Allahyar district. Experiment was laid in CRBD with four replications and four insecticides namely endosulfan, bifenthrin, chlorpyrifos and imidacloprid. There were 16 plots and plot size was ¼ acre. Insecticides were sprayed at recommended dose [Buriro 2006] with Knapsack sprayer (Table-1).

Table-1 Recommended dose of pesticides per acre

Pesticide Common name	Pesticide Brand name	Group	Dose/acre
Bifenthrin	Talstar 100 CE	Pyrithroid	250 ml/acre
Chlorpyrifos	Lorsbon 500 EC	Organophosphate	400 ml/acre
Endosulfan	Thiodan 35 EC	Organochlorine	800 ml/acre
Imidacloprid	Confidor 200 SL	Nictinamide	200 ml/acre

2.2 Effect of pesticides on soil arthropod population

Soil sampling for pesticide residue analysis as well as for arthropod population determination were done at pre-determined intervals, that is, one pretreatment and post-treatment at 1 day, 3days, 7 days, 14 days).

Soil arthropods population was assessed in the field by in situ counting and values were expressed as the number of arthropods per square foot. `Arthropod population reduction was calculated by Abbott's formula (Abbott 1925) given below:

$$\text{Corrected \%} = \left(1 - \frac{\text{N in T after treatment}}{\text{n in Co after treatment}}\right) * 100$$

Where : n = Insect population , T = treated , Co = control

2.3 Pesticide residue analysis

Soil samples were brought at NCEAC. For pesticide residue analysis soil shall be air-dried followed by grinding and sieving. The sieved soil samples shall be stored in freezer at -20^{0}C until further analysis. Pesticide residue extraction, cleanup and instrumental analysis shall be done as per procedures laid down in Pesticide Analysis Manual (1999). Residue values shall be expressed as ppm.

2.3.1. Chemicals and Reagents

High purity (99.4%) endosulfan and Deltamethrin standards were gifted from Bayer Crop Science. Commercial Endosulfan was purchased from local market Hyderabad-Sindh, Pakistan (35% EC). Other chemicals, reagents and solvents used in the present work were purchased from Sharlau (Barcelona, Spain). Stock solutions (1000ppm) these two pesticides were prepared in ethyl acetate and stored in a freezer at -10°C. Working standard solutions were prepared by appropriate dilutions with ethyl acetate and stored under refrigeration (4°C). All the glassware were cleaned with soap water, rinsed with distilled water followed by acetone and dried at 110 ◦C for 2 h.

2.3.2. Soil Sampling

Soil samples were collected from experimental field of National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Sindh-Pakistan. This field did not receive pesticide applications, samples were collected from the 0 to 15cm soil depth for leaching and adsorption studies, air-dried, and ground to pass through a 2-mm sieve. Processed soil samples were stored in glass bottles at room temperature till further analysis. Physical and chemical properties of the soil were measured with the methods described in *Methods of Soil Analysis* (Sparks 1996).

2.3.4 Extraction and cleanup of soil samples

50 g air dried soil was transferred to 250ml stoppered conical flasks and 50ml ethyl acetate was added. The samples were equilibrated on a rotary shaker for 2h, the extract was filtered. Ethyl acetate extract was washed with 100ml distilled water in separatory funnel. Next the water layer containing impurities was discarded; ethyl acetate layer containing residues was dried with anhydrous sodium sulphate and concentrated to 5ml gently with stream of nitrogen. Now the soil samples were ready for instrumental analysis.

2.3.5 Extraction of water samples

Each 100ml water portion eluted from column was mixed with 50ml ethyl acetate in a 250ml separating funnel. The separating funnel was shaken vigorously for 5 minutes to ensure the complete mixing of two layers. Then separating funnel was placed on ring-stand to allow separation of layers. Lower aqueous layer was discarded and ethyl acetate layer containing residues was dried through anhydrous sodium sulphate and concentrated to 5ml as described above.

2.3.6 Instrumental analysis

Endosulfan residues were analyzed GC-μECD and confirmed with GC-MS. An Agilent Technologies 7890A GC system equipped with μECD was used. The GC column used was HP-5 (30m × 0.320mm × 0.25μm). The GC is fitted with 7683 series autosampler and 7683B series injector. The operating conditions were as follows: injector port temperature 250 °C, injection volume 2μl in a split mode(50:1), nitrogen as carrier gas at a flow rate of 60mL/min; oven temperature program, initial 70°C, increased at 30°C/min to 210°C(2 min), then increased to 250°C at 25°C/min(2 min) and finally increased at 30°C/min to 290°C and held for 5 min. The total analysis time is 16.6 min. The temperature of μECD was 310^{0}C. All analyses were carried out by autosampler injection.

GC-MS analysis was performed with an Agilent Technologies 6890N network GC system equipped with a 5975 inert MSD operated in Electron Impact ionization mode (EI), and automatic split-splitless injector model Agilent 7683. HP-5MS 5% Phenyl Methyl Siloxane capillary column (30m × 0.25mm × i.d., 0.25μm film thickness) supplied by Agilent Technologies, was employed. The carrier gas used was Helium (with purity up to 99.9993%). The operating conditions were as follows: injector port temperature 280°C, pressure 12.75psi, injection volume 2μl in splitless mode, helium as carrier gas at a flow rate of 17.1mL/min; oven temperature program, 80°C (2 min), increased at 30°C/min to 180°C, then increased to 200°C at 1.5 °C/min and finally increased at 20°C/min to 280°C and held for 8 min, solvent delay, 4.00 min. The total analysis time is 30.67min. The mass spectrometer was operated in electron impact ionization mode (ionizing energy = 70 eV) scanning from m/z 80 to 550. The ion source (EI) and quadrupole temperatures were 230 and 150°C, respectively.

2.3.7 Percent recovery

For percent recovery study, 50g blank soil was spiked with endosulfan standard solution prepared in ethyl acetate so as to make final soil concentration of 0.1 and 1ng/µl. Soil was mixed thoroughly for even distribution of endosulfan and was left for 24 hours so that the pesticide can be adsorbed completely. After that endosulfan was extracted by placing soil in 250mL stoppered conical flasks and 50ml ethyl acetate was added. The sample was equilibrated on a rotary shaker for 2h, filtered and filtrate was concentrated by evaporating ethyl acetate up to 5ml. This final volume of sample was analyzed by GC-µECD. For calibration the endosulfan standards from 1 to 5ng/µl were run on GC-µECD.

2.3.8 Limits of detection & quantification

Limit of detection (LOD) of endosulfan was calculated at a signal-to-signal ratio of 3, while the limit of quantification (LOQ) was obtained at a signal-to-signal ratio of 10.

RESULTS

Effect of pesticides on soil arthropod population

3.1.1 Spiders in okra field

Effect of pesticide treatments on spider population in okra field is shown in table 2. The population was 4.0, 4.22, 3.80, 3.1 and 4.33 spiders per square foot and was stable (ranged between 3.5 to 4.75) in untreated control plot up to 14 days after treatment. Pesticides caused severe mortality and at 1 day after treatment no spiders were found in endosulfan treated plots. However, 0.25 spiders were observed in other treatments. On 14 day post-treatment interval spider population recovered very slowly to about 25% of control in Imidacloprid, Chlorpyrifos and Endosulfan treated plots, and barely up to half of the control in Bifenthrin treated plots. In terms of reduction percentages endosulfan wiped out entire spider population whereas in Bifenthrin, Imidacloprid and Chlorpyrifos treated plots 92.86% reduction was observed. Even, at 14 days post-treatment interval more than 73% spider reduction was noted in case of Imidacloprid, Chlorpyrifos and Endosulfan. However, residual toxicity was reduced at 14 days interval for bifenthrin and 47.37% reduction was noted.

Table 2 Effect of pesticide treatments on spiders population in okra field

Treatments	Population					Percent Reduction			
	Pre-treat	1	3	7	14	1	3	7	14
Control	4.00	3.50	4.00	4.00	4.75				
Bifenthrin	4.22	0.25	0.50	1.25	2.50	92.86	87.50	68.75	47.37
Imidacloprid	3.80	0.25	0.25	1.00	1.25	92.86	93.75	75.00	73.68
Chlorpyrifos	3.1	0.25	0.25	0.75	1.25	92.86	93.75	81.25	73.83
Endosulfan	4.33	0.00	0.50	0.75	1.00	100	87.50	81.25	78.95

3.1.2 Black Spiders in okra field

In the table 3 depicts the effect of pesticide treatments on black spider population in okra field. The population was 0.25, 1.25, 0.80, 1.80, 1.10 and 0.75 black spiders per square foot and was stable (ranged between 0.75 to 1.75) in untreated control plot up to study periods of 14 days after treatment. Pesticides caused severe mortality and at 1 day after treatment no black spiders were found in these pesticides Bifenthrin, Imidacloprid, Chlorpyrifos and endosulfan treated plots. However, 0.25 black spiders were observed in pre-treatments. The black Spider populations were not recovered to about 100% control in bifenthrin, Imidacloprid, Chlorpyrifos and Endosulfan. On 14 day post-treatment interval black spider population recovered to about 57% of control in bifenthrin and Imidacloprid treated plots and barely up to 28.57% of the control in endosulfan treated plots. In Chlorpyrifos treated plots 71.43% population recovery was observed. Even, at 14 days post-treatment interval ~ 57% black spider population recovery was noted in case of Bifenthrin and Imidacloprid.

Table 3 Effect of pesticide treatments on black spiders population in okra field

Treatments	Population					Percent Reduction			
	Pre-treat	1	3	7	14	1	3	7	14
Control	0.25	0.75	1.25	1.25	1.75				
Bifenthrin	1.25	0.00	0.25	0.75	1.00	100	80.00	40.00	42.86
Imidacloprid	0.80	0.00	0.50	1.00	1.00	100	60.00	20.00	42.86
Chlorpyrifos	1.10	0.00	0.50	1.00	1.25	100	6.00	20.00	28.57
Endosulfan	0.75	0.00	0.25	0.25	0.50	100	8.00	80.00	71.43

3.1.3 Ants in okra field

Table 4 indicates the effect of pesticide treatments on ants population in okra field. The population was 10.0, 9.25, 7.75, 8.75 and 8.80 ants per square foot and was stable (ranged between 12 to 12.75) in untreated control plot up to study period of 14 days after treatment. Pesticides caused severe mortality and at 1 day after treatment 4.50 and 2.00 ants were found in Bifenthrin and imidacloprid treatment. However, ants were not found in Chlorpyrifos and endosulfan treated plots at 1 day post-treatment interval. On 14 day post-treatment interval ants population recovered to about 94.12% of control in bifenthrin treated plots and barely up to 86.97% of the control in endosulfan treated plots. In Chlorpyrifos treated plots 70.59% population recovery was observed. Ant population fully recovered at 14 day post-treatment interval of imidacloprid.

Table 4 Effect of pesticide treatments on ants population in okra field

Treatments	Population					Percent Reduction			
	Pre-treat	1	3	7	14	1	3	7	14
control	**10.00**	12.00	12.50	13.00	12.75				
Bifenthrin	9.25	4.50	8.00	11.00	12.00	62.50	36.00	15.38	5.88
Imidacloprid	7.75	2.00	7.00	10.00	13.00	83.33	44.00	23.08	-1.96 *
Chlorpyrifos	8.75	0.00	1.00	7.00	9.00	100	92.00	46.15	29.41
Endosulfan	8.80	0.00	6.00	8.00	11.00	100	52.00	38.46	13.73

***Net increase in ant population**.

3.1.4 Field Cricket in okra field

Table 5 has shown the effect of pesticide treatments on field cricket population in okra field. The population was 2.25, 1.30, 2.50, 1.80 and 2.30 field cricket per square foot and was stable (ranged between 2.00 to 2.25) in untreated control plot up to study periods up to 14 days after treatment. Pesticides cause severe mortality and at 1 day after treatment however, ants were not found in Bifenthrin, imidacloprid, Chlorpyrifos and endosulfan at 1 day post-treatment interval. On 14 day post-treatment interval ants population recovered to about 50% of control in bifenthrin and imidacloprid treated plots and barely up to 41.67% of the control in endosulfan treated plots. In Chlorpyrifos treated plots 33.33% population recovery was observed.

Table 5 Effect of pesticide treatments on field cricket population in okra field

Treatments	Population					Percent Reduction			
	Pre-treat	1	3	7	14	1	3	7	14
control	2.25	2.00	2.00	3.00	2.25				
Bifenthrin	1.30	0.00	1.25	1.50	2.00	100	37.50	50.00	11.00
Imidacloprid	2.50	0.00	0.75	2.25	2.25	100	62.50	25.00	0.00
Chlorpyrifos	1.88	0.00	1.25	3.00	2.75	100	37.50	0.00	-22.22
Endosulfan	2.30	0.00	1.50	2.25	2.25	100	25.00	25.00	0.00

3.1.5 Snow Bugs in okra field

Effect of pesticide treatments on snow bug population in okra field is shown in table 6. The population was 2.00, 2.80, 1.90, 2.33 and 2.75 snow bugs per square foot and was stable (ranged between 3.00 to 6.00) in untreated control plot up to study periods up to 14 days after treatment. Pesticides caused severe mortality and at 1 day after treatment 1.50 and 0.75 were found in bifenthrin and Imidacloprid treatment. On 14 day post-treatment interval snow bug population recovered to about 50% of control in bifenthrin and imidacloprid treated plots and barely up to 33.33% of the control in Chlorpyrifos in treated plots. In endosulfan treated plots 41.67% population recovery was observed.

Table 6 Effect of pesticide treatments on snow bugs population in okra field

Treatments	Population					Percent Reduction			
	Pre-treatment	1	3	7	14	1	3	7	14
Control	2.00	3.00	4.00	4.75	6.00				
Bifenthrin	2.80	1.50	2.00	2.25	3.00	50.00	50.00	52.63	50.00
Imidacloprid	1.90	0.75	1.50	2.00	3.00	75.00	62.50	57.89	50.00
Chlorpyrifos	2.33	0.00	1.25	2.00	2.00	100.00	68.75	57.89	66.67
Endosulfan	2.75	0.00	1.25	2.00	2.50	100.00	68.75	57.89	58.33

3.1.6 Insect larvae in okra field

Table 7 reveals the effect of pesticide treatments on insect larvae population in okra field. The population was 0.80, 0.75, 1.10, 1.40 and 0.90 larvae per square foot and was stable (ranged between 0.50 to1.25) in untreated control plot up to study periods up to 14 days after treatment. Pesticides caused severe mortality and at 1 day after treatment no insect larvae were found these pesticides Bifenthrin, Chlorpyrifos and endosulfan post-treatment interval. However, 0.25 insect larvae observed in pre-treatments. On 14 day post-treatment interval insect larvae population recovered very slowly to about 20% of control in imidacloprid treated plots and rapidly up to the 80% in bifenthrin, imidacloprid 40% and endosulfan 40% treated plots population recovery percentage was observed.

Table 7 Effect of pesticide treatments on insect larvae population in okra

Treatments	Population					Percent Reduction			
	Pre-treatment	1	3	7	14	1	3	7	14
Control	0.80	0.50	1.00	1.50	1.25				
Bifenthrin	0.75	0.00	0.75	0.50	1.00	100.00	25.00	66.67	20.00
Imidacloprid	1.10	0.25	0.25	0.50	0.25	50.00	75.00	66.67	80.00
Chlorpyrifos	1.40	0.00	0.00	0.25	0.50	100.00	100.00	83.33	60.00
Endosulfan	0.90	0.00	0.25	0.25	0.50	100.00	75.00	83.33	60.00

3.1.7 Silverfish in okra field

Table 8 shows the effect of pesticide treatments on silverfish population in okra field. The population was 1.11, 0.75, 2.00, 1.75 and 2.11 silverfish per square foot and was stable (ranged between 1.00 to 2.50) in untreated control plot up to study periods up to 14 days after treatment. Pesticides caused severe mortality and at 1 day after treatment 0.25 were found in imidacloprid post-treatment interval. However, silverfish are not found in other treatments. On 14 day silverfish population recovered very slowly to about 10% of control in Chlorpyrifos post-treatment interval, whereas better recovery in silverfish was noted in Bifenthrin 60%, imidacloprid 40% and endosulfan 40%.

Table 8 Effect of pesticide treatments on Silverfish population in okra field

Treatments	Population					Percent Reduction			
	Pre-treatment	1	3	7	14	1	3	7	14
control	1.11	1.00	2.00	3.00	2.50				
Bifenthrin	0.75	0.00	0.75	1.00	1.50	100.00	62.50	66.67	40.00
Imidacloprid	2.00	0.25	0.50	1.25	1.00	75.00	75.00	58.33	60.00
Chlorpyrifos	1.75	0.00	0.00	0.25	0.25	100.00	100.00	91.67	90.00
Endosulfan	2.11	0.00	0.50	1.00	1.00	100.00	75.00	66.67	60.00

3.2 Pesticide residues in soil of okra field

3.2.1 Physicochemical properties of the soil

The physicochemical properties of the soil are shown in table 9. The pH of soil is approximately neutral (7.79), however, the slight tendency to be acidic in the presence of a salt may favour the stronger cation exchange mechanisms of chemisorption. The EC measurement of soil ($808\mu S.cm^{-1}$) indicates saline conditions, when considered in relation to the effect on plants. The electrical conductivity between 0 and $200\mu S.cm^{-1}$ is considered to have negligible effects. The higher EC value of soil enhances water retention by increasing the osmotic potential of the soil, thereby increasing the contact-time for adsorption. As our soil is sandy loam in texture (sand 76.75 %), therefore adsorption may thus be limited because sand particles have less vacant sites as compared to silt or clay. Soil adsorption, presence of organic matter, soil texture, electrical conductivity, pH, bulk density all directly influences the degradation of endosulfan in soil. As Sindh soil has low organic matter (1.144 %), and this is expected to reduce the soil's ability to immobilize endosulfan in the soil profile. (Ashraf *et al.*, 2012).

Table 9 Selected physicochemical properties of soil samples (mean + standard deviation; n = 3)

Parameter	Value
Ph	7.79 ± 0.01
Electrical Conductivity (µS/cm)	808 ± 0.32
Total Dissolved Solids (mg/l)	395 ± 0.45
Cation exchange capacity (meq/100g)	12 ± 0.01
Organic matter content (%)	1.44 ± 0.01
Total Organic Carbon	0.664 ± 0.001%
Specific Gravity	2.912 ± 0.01
Bulk Density (g/ml)	1.117 ± 0.01
Sand (%)	76.75 ± 0.68
Slit (%)	18.15 ± 0.24
Clay (%)	7.1 ± 0.17
Classification	Sandy Loam

Percent recovery of Endosulfan pesticide

For percent recovery five standard endosulfan solutions from 1 to 5ng/µl were run on GC-µECD. These all standard endosulfan concentrations were in linear range, giving R^2 value 0.998 and 0.995 for α- and β-endosulfan respectively. The percent recovery of α-endosulfan and β-endosulfan in soil was 96.19± 1.2% and 96.16± 1.2 %, while in water 93.47 % and 93.07 % respectively.

Percent recovery of Deltamethrin pesticide

Blank soil of 50g weight was spiked with 0.1, 0.5 and 1 ng/µl deltamethrin standard solutions. The soil was mixed thoroughly for even distribution of pesticide and left for overnight so that the pesticide can be adsorbed completely. Then deltamethrin was extracted and analyzed as above. For calibration the deltamethrin standards from 0.01 to 2 ng/µl concentration were run on GC-µECD. The percent recoveries of deltamethrin found in soil and water were 93.43 ± 1.2% and 87.15 ± 1.09% respectively.

Limits of detection & quantification for Endosulfan pesticide

The limits of detectionfor α- and β-endosulfan from soil samples were 0.00026mg/kg and 0.00013mg/kg respectively while limits of quantification these samples for α- and β-endosulfan were 0.00089mg/kg and 0.00073mg/kg respectively.

Limits of detection & quantification for Deltamethrin pesticide

Limit of detection (LOD) of deltamethrin was calculated at a signal-to-signal ratio of 3, while the limit of quantification (LOQ) was obtained at a signal-to-signal ratio of 10. The LOD and LOQ for deltamethrin were 0.00134± 0.002mg/kg and 0.004481± 0.006 mg/kg mg/kg respectively.

Figure-1 Whereas, HPLC chromatogram of imidacloprid residues shown in depicts retention time of 11.54 minutes.

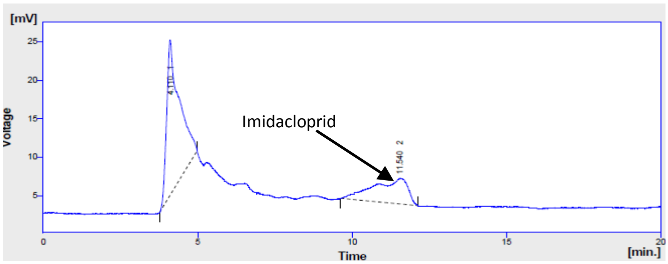


Fig-1 Imidacloprid HPLC chromatogram residues in okra soil samples.

Imidacloprid HPLC chromatogram residues inokra soil samples.

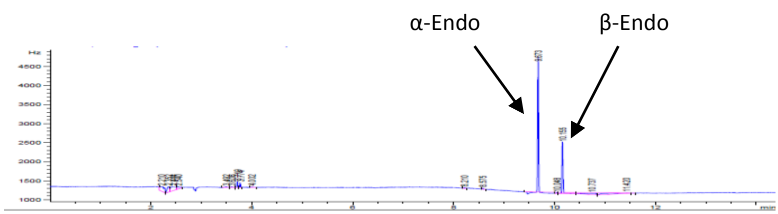
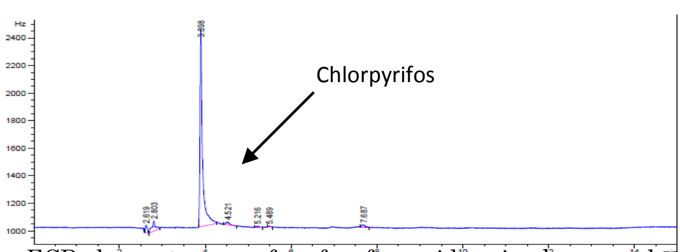


Figure-2 GC-µ ECD chromatogram of endosulfan residues in okra soil samples

The endosulfan gave two peaks one at 9.67 minutes for alpha endosulfan and second peak at 10.15 minutes for beta endosulfan.

Figure-3 The chlorpyrifosin okra samples had retention time of 3.698 minutes as shown in



GC-µ ECD chromatogram of profenofos residues in okra samples

Figure-4 Thebifenthrin pesticide showed the retention time of 10.133 minutes as shown in

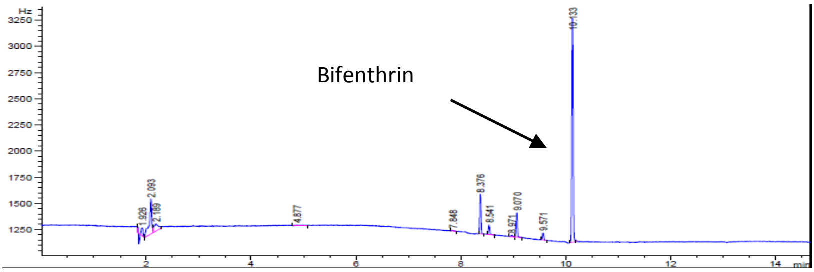


Fig-4 GC-µ ECD chromatogram of bifenthrin residues in okra samples.

GC-µ ECD chromatogram of bifenthrin residues in okra samples

Table-10 pesticide residues in okra field

Pesticides	Pre-treatment	Post-treatment (Residues in ppm)*			Residue reduction %	
		1 day	7 days	14 days	7 days	14 days
Bifenthrin	**ND**	**3.21**±0.21	**2.05**±0.73	**0.57**±0.08	36.14	82.24
Chlorpyrifos	**ND**	**7.94**±0.16	**6.03**±0.44	**3.14**±0.31	24.05	60.45
Endosulfan	**ND**	**5.89**±0.09	**4.82**±0.39	**3.59**±.28	18.17	39.05
Imidacloprid	**ND**	**3.01**±0.01	**2.13**±0.59	**1.53**±0.19	29.23	49.17

ND = none detectable

*Values are expressed in mean+standard deviation.

Data on pesticide residues indicates that no residue was detected in okra soil at pre-treatment. However, maximum residue was found at 1 day post-treatment interval for all the four pesticides used and the residue levels were 3.21, 7.94, 5.89 and 3.01ppm for Bifenthrin, Chlorpyrifos, Endosulfan and Imidacloprid respectively. The minimum residue was found at 14 day post-treatment interval for all the four pesticides used. At this interval bifenthrin residues were minimum (0.57 ppm) with 82.24% reduction. And endosulfan residues were maximum (359 ppm) representing 39.05% reduction.

DISCUSSION

Effect of pesticides on soil arthropod population

The population was 4.0, 4.22, 3.80, 3.1 and 4.33 spiders per square foot and was stable (ranged between 3.5 and 4.75) in untreated control plot up to 14 days after treatment. Pesticides caused severe mortality and at 1 day after treatment no spiders were found in endosulfan treated plots. However, 0.25 spiders were observed in other treatments. On 14 day post-treatment interval spider population recovered very slowly to about 25% of control in Imidacloprid, Chlorpyrifos and Endosulfan treated plots, and barely up to half of the control in Bifenthrin treated plots. In terms of reduction percentages endosulfan wiped out entire spider population whereas in Bifenthrin, Imidacloprid and Chlorpyrifos treated plots 92.86% reduction was observed. Even, at 14 days post-treatment interval more than 73% spider reduction was noted in case of Imidacloprid, Chlorpyrifos and Endosulfan. However, residual toxicity was reduced at 14 days interval for bifenthrin and 47.37% reduction was noted.

The population was 0.25, 1.25, 0.80, 1.80, 1.10 and 0.75 black spiders per square foot and was stable (ranged between 0.75 to 1.75) in untreated control plot up to study periods of 14 days after treatment. Pesticides caused severe mortality and at 1 day after treatment no black spiders were found in these pesticides Bifenthrin, Imidacloprid, Chlorpyrifos and endosulfan treated plots. However, 0.25 black spiders were observed in pre-treatments. The black Spider populations were not recovered to about 100% control in bifenthrin, Imidacloprid, Chlorpyrifos and Endosulfan. On 14 day post-treatment interval black spider population recovered to about 57% of control in bifenthrin and Imidacloprid treated plots and barely up to 28.57% of the control in endosulfan treated plots. In Chlorpyrifos treated plots 71.43% population recovery was observed. Even, at 14 days post-treatment interval ~ 57% black spider population recovery was noted in case of Bifenthrin and Imidacloprid.

The population was 10.0, 9.25, 7.75, 8.75 and 8.80 ants per square foot and was stable (ranged between 12 and 12.75) in untreated control plot up to study period of 14 days after treatment. Pesticides caused severe mortality and at 1 day after treatment 4.50 and 2.00 ants were found in Bifenthrin and imidacloprid treatment. However, ants were not found in Chlorpyrifos and endosulfan treated plots at 1 day post-treatment interval. On 14 day post-treatment interval ants population recovered to about 94.12% of control in bifenthrin treated plots and barely up to 86.97% of the control in endosulfan treated plots. In Chlorpyrifos treated plots 70.59% population recovery was observed. Ant population fully recovered at 14 day post-treatment interval of imidacloprid.

The population was 2.25, 1.30, 2.50, 1.80 and 2.30 field cricket per square foot and was stable (ranged between 2.00 to 2.25) in untreated control plot up to study periods up to 14 days after treatment. Pesticides cause severe mortality and at 1 day after treatment however, ants were not found in Bifenthrin, imidacloprid, Chlorpyrifos and endosulfan at 1 day post-treatment interval. On 14 day post-treatment interval ants population recovered to about 50% of control in bifenthrin and imidacloprid treated plots and barely up to 41.67% of the control in endosulfan treated plots. In Chlorpyrifos treated plots 33.33% population recovery was observed.

The population was 2.00, 2.80, 1.90, 2.33 and 2.75 snow bug per square foot and was stable (ranged between 3.00 to 6.00) in untreated control plot up to study periods up to 14 days after treatment. Pesticides caused severe mortality and at 1 day after treatment 1.50 and 0.75 were found in bifenthrin and Imidacloprid treatment. On 14 day post-treatment interval snow bug population recovered to about 50% of control in bifenthrin and imidacloprid treated plots and barely up to

33.33% of the control in Chlorpyrifos in treated plots. In endosulfan treated plots 41.67% population recovery was observed.

The population was 0.80, 0.75, 1.10, 1.40 and 0.90 larvae per square foot and was stable (ranged between 0.50 to1.25) in untreated control plot up to study periods up to 14 days after treatment. Pesticides caused severe mortality and at 1 day after treatment no insect larvae were found these pesticides Bifenthrin, Chlorpyrifos and endosulfan post-treatment interval. However, 0.25 insect larvae observed in pre-treatments. On 14 day post-treatment interval insect larvae population recovered very slowly to about 20% of control in imidacloprid treated plots and rapidly up to the 80% in bifenthrin, imidacloprid 40% and endosulfan 40% treated plots population recovery percentage was observed.

The population was 1.11, 0.75, 2.00, 1.75 and 2.11 silverfish per square foot and was stable (ranged between 1.00 to 2.50) in untreated control plot up to study periods up to 14 days after treatment. Pesticides caused severe mortality and at 1 day after treatment 0.25 were found in imidacloprid post-treatment interval. However, silverfish are not found in other treatments. On 14 day silverfish population recovered very slowly to about 10% of control in Chlorpyrifos post-treatment interval, whereas better recovery in silverfish was noted in Bifenthrin 60%, imidacloprid 40% and endosulfan 40%.

Similar results have been reported by previous workers on the effect of pesticides on soils arthropod populations. Rajagopal *et al.* (1990) reported that the insecticides such as Chloropyrifos, Carbosulfan, Phorate and Isofenphos showed significant effect on the non-target soil fauna, which included mainly mites and collembolans. These authors further reported that soil fauna that included mainly mites and collembolans were affected by insecticide application in the field. The effect of pesticides on the soil mesofauna is complex because of their action on both predacious and non-predacious groups. Therefore, several workers have reported increased population of collembolan and mites after the use of pesticides, mainly because of mortality of predacious mites, which exercise a great check on other mites and collembolan, (Veeresh and Rajagopal, 1989). Epstein *et al.* (2000) reported the effect of broad-spectrum insecticides on Epigeal Arthropod biodiversity in Pacific Northwest apple orchards. They reported that the highly mobile invertebrates were strongly susceptible to application of broad spectrum neural active insecticides.

Deleterious effects of pesticides greatly modify some biological functions, such as soil organic matters decomposition and nutrient availability in the soil by reduction of the diversity of soil biota, (Ferraro and Pimentel, 2000). AL-Haifi *et al.* (2006) screened the effect of overuse of the insecticide Dimethoate and its residues within 0 -15 cm soil layer on the soil micro-arthropods population, which play a very important role in maintenance of the fertility of the soil in Zendan, Yemen. The results revealed that the detected levels ranged from 0.914 to 5.180 mg/kg air-dried soil for Dimethoate residues. Mites and collembolans were the most affected micro-arthropods by Dimethoate. The reduction in the soil microarthropods population varied from 59% to 69% as compared to its population in control substations.

Pesticide residues in soil of okra field

The pH of soil is approximately neutral (7.79), however, the slight tendency to be acidic in the presence of a salt may favour the stronger cation exchange mechanisms of chemisorption. The EC measurement of soil (808µS.cm^{-1}) indicates saline conditions, when considered in relation to the effect on plants. The electrical conductivity between 0 and 200µS.cm^{-1} is considered to have negligible effects. The higher EC value of soil enhances water retention by increasing the osmotic potential of the soil, thereby increasing the contact-time for adsorption. As our soil is sandy loam in texture (sand 76.75 %), therefore adsorption may thus be limited because sand particles have less vacant sites as compared to silt or clay. Soil adsorption, presence of organic matter, soil texture, electrical conductivity, pH, bulk density all directly influences the degradation of endosulfan in soil. As Sindh soil has low organic matter (1.144 %), and this is expected to reduce the soil's ability to immobilize endosulfan in the soil profile (Tang *et al.* 2009).

Similar results have been witness by other researchers elsewhere. Soil is a sink for the greater part of the pesticides applied in agriculture. Residues can be transported through the soil, and leaching is the main transportation process which leads to pollution of the ground water with pesticides. This is a global problem since ground water is used as drinking water and for irrigation in many countries. Degradation of imidacloprid in soil is decreased if organic amendment, organic material used to improve soil quality, is added (Rouchaud *et al.*, 1996). The lack of leaching that some authors have described, and this decrease in degradation upon addition of organic amendment, may well be due to the sorption-desorption characteristics of the chemicals. Sorption of imidacloprid, as well as its metabolites, increases when soil organic carbon content increases (Cox *et al.*, 1997).

Pesticide leaching studies are important for determining a pesticide's capacity to pollute ground water, especially if the pesticide in question is highly soluble in water (Gupta *et al.*, 2002), like imidacloprid. Data on the persistence of imidacloprid in soil are rather inconsistent, however. Some authors have reported that it is relatively immobile in soil and that leaching below the topmost layer and into the groundwater is not likely to occur, while other authors have claimed the exact opposite (Tišler *et al.*, 2009).

Jia and Sun (2009) screened the Endosulfan is one of the organochlorine pesticides (OCPs) and also a candidate to be included in a group of new persistent organic pollutants (UNEP 2007). The first national endosulfan usage inventories in China with 1/4 degrees longitude by 1/6 degrees latitude resolution has been reported in an accompanying paper. In the second part of the paper, we compiled the gridded historical emissions and soil residues of endosulfan in China from the usage inventories. Based on the residue/emission data, gridded concentrations of endosulfan in Chinese soil and air have been calculated. These inventories will provide valuable data for the further study of endosulfan.

Peterson *et al.* (2007) has reported that after an initial rapid decline in imidacloprid in the test plot soil, after two months a lagging phase occurs, with about 10% of initial imidacloprid still remaining in the soil after six months. The concentration was not influenced by the presence of vegetation, although mobility into lower soil layers was lower if vegetation was present, which was probably because of the effect of vegetation on soil moisture.

Fang and Feng (2010) has reported a method was developed for the determination of organochlorine pesticide (OCP) residues in soil/sediment using high resolution gas chromatography coupled with low resolution mass spectrometry. The analytical procedures consisted of Soxhlet extraction, sulfur removal with copper powder, clean-up with gel permeation chromatography (GPC) and a florisil column of solid phase extraction (SPE). The analytes were separated on an HP-5MS capillary column, detected in selected ion monitoring (SIM) mode and quantified using internal standard calibration curves of isotope dilution technique. The linear correlations of calibration standard solutions were good for all the OCPs. The recoveries and relative standard deviations of labeled compound solutions ranged from 60% to 110% and from

1.5% to 18%, respectively. The limits of detection ranged from 0.20 to10.3 micro g /kg were established for the 23 OCPs. The method showed satisfactory clean-up effect and precision quantification. It is suitable for the determination and confirmation of pesticides in complex matrices such as soil, sediment.

Pin Zhang and Xiaojun Hu (2008) in this work, a reliable method for simultaneous determination 15 organochlorine pesticide (OCP) residues (alpha-HCH, beta-HCH, gamma-HCH, delta-HCH, hexachlorobenzene, heptachlor, aldrin, alpha-chlordane, gamma-chlordane, dieldrin, endrin , O,P'-DDE, O,P'-DDT ,P,P'-DDT and P,P'-DDD) in soil was reported. The method was based on sonicated extraction with a mixture of acetone and petroleum ether (50:50,v/v) and a cleanup with liquid-liquid-extraction (LLE), followed by gas chromatographic separation and a GC-ion trap-MS-MS detection.The determination of 15 compounds was achieved in about 20 min. Pesticide residues could be determined in low level range.Limits of detection (LOD) were from 1 mug/kg for alpha-chlordane to 0.025 mug/kg for O,P'-DDE(S/Nges3). Limits of quantification (LOQ) were all lower than 1 mug/kg (S/Nges10), which indicated the method established was more sensitive than the traditional ways (GC-ECD, GC/MS/SIM).Average recoveries ranged from 75 to 106% and relative standard deviations (RSDs) were in the range 2.4-12.7% for all analytes across three spiked soil levels.The results demonstrated the suitability of the GC/MS/MS approach for the analysis of multi-residue OCPs in soil.

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