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DETERMINATION OF THE ORGANOPHOSPHORUS PESTICIDE IN OKRA CROP, ABELMOSCHUS ESCULENTUS (L.) BY GAS-LIQUID CHROMATOGRAPHY IN MEERUT REGION

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ABSTRACT
A multi pesticide residues analysis was carried out for farmgate samples of okra fruits from four markets places of Meerut region. The okra fruits samples were analyzed for monocrotophos, chlorpyriphos, dimethoate. Okra vegetable was analyzed for pesticide residues using multiresidue analysis by GLC. It was observed that 39.0% of the samples were contaminated residues below MRL three pesticides. The results showed that 42% of the samples tested contained residues higher than the MRLs and 19 % BDL. Based on observations made in these studies; it is proposed that more extensive monitoring investigations covering Okra from different farmgate and market Meerut regions of the city be carried out to find the exact position of pesticide residues.

KEYWORDS: HPLC Organophosphate pesticides Farmgate sample okra Pesticide Residues

INTRODUCTION
Agriculture play an important role in economic structure in India. Okra, *Abelmoschus esculentus* (L). MOENCH, often known as bhindi, lady's finger is valued for its edible green fruit. In India, it is grown over 3.58 lakh ha area with production of 35.25 lakh tones and productivity of 9.84 tones/ha. (Anonymous, 2005). The avoidable losses in yield and fruit damage due to this pest have been estimated as 36-90% (Misra *et al.,* 2002). The jassid is causing damage throughout the growing period of the crop and reduces the plant vigour and fruit yield (Mahal *et al.*, 1994). Insecticides play vital role in management of these pests. Still pest control is largely dependent on the use of many pesticides of different groups as organophosphate, which causes ill effect on the human health. Synthetic pyrethroid being effective at very low dosage, having low mammalian toxicity and involving less risk of contamination have been recommended to control fruit borers (Gajbhiye *et al*., 1985 and Rai *et al*., 1980). Pesticide, a critical modern input, has become an established global practice. Some people are more vulnerable than others to pesticide impacts. For example, infants and young children are known to be more susceptible than adults to the toxic effects of pesticides. People with asthma may have very severe reactions to some pesticides, particularly with pyrethrin / pyrethroid, organophosphate and carbamate pesticides.These chemicals may leave toxic residue in the harvested produce which is consumed by human beings (Babu *et al*., 1996). Pesticide residues come from four sources: on farm pesticide use, post harvest pesticide use, pesticide used on imported food and discarded pesticides that persist in the environment. Above all post harvest pesticide accounts for the largest share of residue detection (Kuchler *et al*., 1996). Organophosphorus pesticides It is selectively toxic to insects compared to mammals due to different metabolism of the propylthiol group Li, W., *et al*., 2008. Chlorpyrifos is an organophosphate pesticide used throughout the world around the world and Chlorpyrifos is currently registered as insecticide with the used in a wide variety of crops and vegetables. The primary degradant of chloropyrifos is 3, 5, 6-trichloro-2-pyridinol, a base hydrolysis product Gotoh, M., *et al*., 2001. As compare to other organophosphate pesticide, Chlorpyrifos is fairly stable and persistent member of organophosphate pesticides.

On an average 13-14% of total pesticides used in the country are applied in vegetable crops. Since the produce is harvested at short intervals and consume fresh in many cases, the surveys of market samples show high level of pesticide residues in vegetables (Arora and Gopal, 2002, Agnihotri, 1999, Awasthi and Ahuja, 1997).

Selection of Pesticide: However, nowadays organochlorine (OC) pesticides were not used primarily due to their persistence in the environment Jain, A.V. *et al*., 2006. At this time, the use of OPs and CMs glided due to their availability and quick degradation in the environment Organophosphorus pesticides have been widely manufactured and used in the world. And they replaced the chlorinated pesticides.

Monocrotophos: Monocrotophos is an organophosphate insecticide. It is acutely toxic to birds and humans. Widespread bird kills, especially of Swainson's Hawks, have resulted from the use of monocrotophos. It is mainly applied against cotton pests. Further it is used on citrus, olives, rice, maize, sorghum, sugar cane, sugar beet, peanuts, potatoes, soya beans, vegetables, ornamentals and tobacco crops for the management of different pests.

Chlorpyriphos; Chlorpyriphos is a toxic crystalline organophosphate insecticide that inhibit acetylcholinesterase and is used to control insect pests. Trade names include Dursban (home and garden uses) and Lorsban (agricultural uses). Dursban is highly toxic and has been linked to neurological effects, birth defects, ADHD, palsey and other disorders associated with asthma, reproductive and developmental toxicity and acute toxicity. The chlorpyriphos, while in the womb have an increased risk of delays in mental and motor development at age 3 and an increased occurrence of pervasive developmental disorders such as ADHD, An earlier study demonstrated a correlation between pre natal chlorpyriphos exposure and lower weight and smaller head circumference at birth.

Dimethoate: Dimethoate is a widely used organophosphate insecticide used to kill insects on contact. It was patented and introduced in the 1950s by American Cyanamid. Like other organophosphates, dimethoate is an anticholinesterase, which disables cholinesterase, an enzyme essential for central nervous system function.

MATERIAL AND METHOD

Chemicals: An accurately weighed 0.0105g amount of an individual analytical grade pesticide in a weighing bottle, was transferred to a volumetric flask (25ml) and dissolving it in distilled acetone and washing the weighing bottle simultaneously with acetone, the volume was made up to the mark of volumetric flask to give 420 ppm stock solution. 6ml of this stock solution was diluted with hexane in 25ml volumetric flask to obtain 100ppm solution.

Serial dilutions were made in a similar manner so as to get 10ppm and 1ppm solution of each compatible (monocrotophos, chlorpyriphos, dimethoate,) pesticide in a 10 ml volumetric flask and making the volume up to the mark with n-hexane. Serial dilutions were made in a similar manner so as to get 10ppm and 1ppm solution for residues analysis. They were stored in a refrigetor at 5^0c.

Samples Collection: Farmgate vegetable samples of okra fruits each (20 in numbers) were collected at random from four different markets *viz*. Daurala, Lawer, Kesharganj and Partapur, located in Meerut region (U.P.), during the month of August, 2009. About one kg. Sample each with 20 baskets from all four locations of markets was collected for pesticides residues analysis. Samples were analyzed for the organophosphate group of insecticides based on the information provided by the vegetable growers. Each sample was sub-divided into three replicates from each basket; a sub sample of 50g was withdrawn for processing for residue analysis.

Extraction and clean up: The collected sub samples of okra fruits were chopped and processed for pesticides residues analysis. The samples were homogenized in a blender (Remei mixie).The extract was treated with 100ml solvent mixture (1:1 hexane: acetone) and was filtered through a Buchner funnel fitted with a What man No. 1 filter paper. The residual pieces of okra fruits on the filter paper were again transferred to the blender. This process was repeated two more times with 25ml acetone. After extraction, the blender jar was rinsed with 25ml acetone and reinstate was also filtered the same way and the final volume of the extract, around 100ml was collected.

Solvent from the extract was evaporated off with the help of rotary vacuum evaporator to around 10ml and then transferred to a 250ml separating funnel. 100ml of sodium chloride solution (18%, w/v) followed by 50ml of distilled

Dichloromethane were added to it. Separating funnel was thoroughly shaken for 1 minute by slowly releasing pressure through stop cork and allowed to stand (for about 5 minutes) until the two layers were separated. The bottom layer was collected in a conical flask after passing through anhydrous sodium sulphate (5 g) layer. The aqueous solution of the separating funnel was shaken two more times with 50ml DCM. The anhydrous sodium sulphate layer was given additional washing with 10ml DCM and the filtrate was collected in the same flask. Glass column (50cm × 1.5cm) was plugged with cotton at the bottom, then dry packed with 5g of anhydrous sodium sulphate followed by 5g of Florisil and finally again with 5g of anhydrous sodium sulphate. It was prewashed with 20ml hexane. The extract from DCM was taken in 10 ml hexane and was added to the adsorbent column. The column was sequentially eluted with 100ml hexane: acetone (7:3 v/v) and the eluate were collected in a conical flask for final estimation of pesticides using GLC. The samples were analyzed using GLC for determination of organophosphates insecticide. GLC (HP 5890 series II) equipped with Ni^{63}, mega bore column (10m, 0.53mm id, 2.65 mm film thickness) and ECD detector. The GLC working conditions were as flows: Nitrogen gas flow rate 12 ml/min Detector, 260°C: and Injector 250°C. The column temperature 150°C maintained for 10 minutes raised @ 5°C/min to 220°C and held for 5 minutes. The retention time (Rt) of monocrotophos 3.0 min, chlorpyriphos 15.4 min, Dimethoate 18.8 min.

Insecticides residues formula:

$$\text{Residue (in ppm)} = \frac{A \times B \times C}{D \times E}$$

Where, A = Area of sample peak,B =Concentration of standard ,C =Volume of aliquot,D =Area of standard peak, E=Weight of sample

RESULT

The farmgate samples of okra fruits were collected from four markets of Meerut region for carrying out pesticides residues analysis. The samples of okra fruits were observed to be contaminated with monocrotophos and chlorpyriphos, dimethoate. Out of twenty samples of okra fruits for multi-residues analysis of pesticides, for Daurala market, Eight samples were observed to have residues of monocrotophos above MRL (0.20), seven samples below MRL and six samples were BDL (Below Detectable Limits). Nine samples were observed to have residues of chlorpyriphos above MRL (0.20), eight samples below MRL and three samples were BDL. Six samples were observed to have residues of dimethoate above MRL (2.00), seven samples below MRL and seven samples were BDL. (Table-1).

For Lawer market, thirteen samples were observed to have residues of monocrotophos above MRL (0.20), five samples below MRL and two samples were BDL. Eleven samples were observed to have residues of chlorpyriphos above MRL (0.20), six samples below MRL and three samples were BDL. Nine samples were observed to have residues of dimethoate above MRL (2.00), eight samples below MRL and three samples were BDL. (Table-1). For Kesarganj market, seven samples were observed to have residues of monocrotophos above MRL (0.20), ten samples below MRL and three samples were BDL. Six samples were observed to have residues of chlorpyriphos above MRL (0.20), nine samples below MRL and five samples were BDL. Eight samples were observed to have residues of dimethoate above MRL (2.00), nine samples below MRL and three samples were BDL. (Table-1). For Partapur market, nine samples were observed to have residues of monocrotophos above MRL (0.20), eight samples below MRL and three samples were BDL. Eight samples were observed to have residues of chlorpyriphos above MRL (0.20), eight samples below MRL and four samples were BDL. Six samples were observed to have residues of dimethoate above MRL (2.00), ninesamples below MRL and five samples were BDL. (Table-1).

For Daurala market 23 replicates were found to be pesticide residues exceeding MRL. For Lawer market 33 replicates were found to pesticide residues exceeding MRL. For Kesarganj market 21 replicates were found to be pesticide residues exceeding MRL. For Partapur market 23 replicates were found to be pesticide residues exceeding MRL (Fig. 1).

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Table –1. MRL value of insecticides for okra fruit

S.N.	Insecticides	MRL (ppm)
1.	Monocrotophos	0.20
2.	Chlorpyriphos	0.20
3.	Dimethoate	2.00

(Arora, 2008)

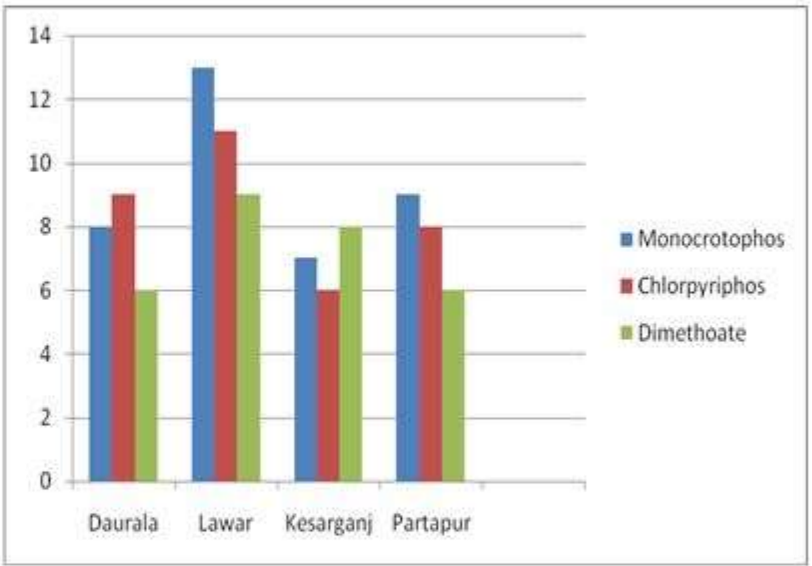

Fig. 1- Samples with pesticides residues exceeding MRL in different locations of Meerut Region

DISCUSSION

The sample of okra fruits collected for multi-residual analysis from for markets of Meerut district, viz. Daurala, Lawer, Kesarganj and Partapur, monitoring of pesticides (monocrotophos, chlorpyriphos and dimethoate) residues in farmgate samples (80) to above markets, the farmgate vegetable samples have been done by several workers in different parts of India, however many of them recorded pesticide residues above and below MRL.

The residues in Daurala market was detected to the range of 0.02-5.34ppm, out of twenty contaminated okra fruit samples were 8, 9, and 6 exceeded the MRL and 6, 8 and 7samples were below MRL values of monocrotophos (0.20), chlorpyriphos (0.20), dimethoate (2.00), respectively. The present observations are in agreement with those of Kole *et al*. (2002) who reported the range of 0.01-1.26ppm. Out of 12 contaminated okra fruit samples, four were exceeded the MRL values of cypermethrin and monocrotophos (0.20ppm), whereas two samples were found to contain cypermethrin (0.5ppm), the minimum waiting period allowed for cypermethrin and monocrotophos treated crops is only 1 day. According to Kumawat *et al*. (2000) initial deposit of 0.42ppm of monocrotophos was obtain in/on okra fruits from 0.036% sprays, the residues were translocated to the extent of 0.19ppm at three days intervals, which were well below the MRL of 0.20ppm, and the residues reached 0.11ppm at six days of spray which remain undetected after nine days of spray.

The residues in okra fruit samples collected from Lawer markets was detected to the range of 0.01-6.13ppm, out of twenty contaminated okra fruit samples 13, 11 and 9 were exceeded the MRL and 5, 6, and 8 samples were below MRL values of monocrotophos (0.20), chlorpyriphos (0.20), and dimethoate (2.00), respectively. Shah *et al*. (2000) reported out of 15 okra fruit samples were contaminated with cypermethrin (1) dimethoate (2) and monocrotophos

(4), to have residues 0.040, 0.027-0.052 and 0.033-0.851 mg/kg below MRL 0.20, 2.00 and 0.20ppm respectively, also reported by Khan *et al*. (1999) initial deposit of 1.31 mg/kg of cypermethrin, okra fruit dissipated to 0.78, 0.50, 0.17 and 0.05 mg/kg at 1, 3, 7 and 10 days respectively, corresponding dissipation was 40-46, 61.83-87.02 and 96.18 per cent, reported safe waiting period of 5.81 days when cypermethrin was sprayed on okra crop.

The residues in collected samples from Kesarganj market was detected to have residues in the range of 0.01-6.04ppm, out of twenty contaminated samples 7, 6, and 8 were exceeded MRL and 10, 9 and 9, samples were below MRL values of monocrotophos (0.20), chlorpyriphos (0.20) and dimethoate (2.00) respectively. Among the 30 okra fruit samples, one each with cypermethrin (2.3) and endosulfan (2.21) exceeded the MRL of 0.20 and 2.00ppm respectively, the residues of cypermethrin was to the tune of 0.09 and 1.8 mg/kg (Chandrasekaran *et al*., 1997). These findings also presented by Deka *et al*. (2005) five samples of okra fruit contaminated with cypermethrin (3) and dimethoate (2), they have residues 0.009-0.012 and 0.007-0.013 mg/kg below MRL 0.20 and 2.00ppm, respectively.

Reported that to initial deposit of dimethoate @ 0.06%, 2.93 mg/kg were detected, this degraded to 0.31 mg/kg by 10 days after five spraying on okra crop. The residues of 2.45, 1.90, 0.83 and 0.31 mg/kg were recorded after 1, 3, 7 and 10 days, respectively, the residues dissipated to 16.38, 35.16, 71.67 and 89.42% on 1, 3, 7 and 10 days respectively, to required waiting period of two days for safe consumption at harvest (Khan *et al*., 1999).

The residues in okra fruit samples at Partapur market was detected to have residues in the range of 0.01-5.20ppm, out of twenty contaminated samples 9, 8 and 6 were exceeded MRL and 8, 8 and 9, samples were below MRL values of monocrotophos (0.20), chlorpyriphos (0.20), and dimethoate (2.00) respectively. Singh et *al.* (2004) who reported deposit of cypermethrin on okra fruit were 0.274 and 0.382 mg/kg when the application of insecticides used @ 50 and 100 gram ai ha^{1}, the safe waiting period was worked out as 2 days. Deen *et al*. (2009) reported dose of cypermethrin dissipated by 39.4, 56.4, 72.5 and 85% at 2, 4, 6 and 9 days after treatment, respectively. Half life and safe waiting period were 3.3 and 4.7 days respectively in case of endosulfan deposit of 5.33 µg/g while dissipated by 29.5, 42.5, 66.0 and 81.0% at 2, 4, 6 and 9 days of treatment, respectively. Residues reached below MRL values for 2.0 µg/g in 4 days, half life and safe waiting period were 3.8 and 8.3 days, respectively. The reduction of residues was observed in the range of 16-37% and 16-24% for cypermethrin and endosulfan, respectively.

Goswami *et al.* (2002) recorded 0.057 and 0.081ppm residues in lower and higher dosage, respectively at 20 days after the insecticidal treatment. According to Chahal *et al*. (2006) recorded monocrotophos level on brinjal fruits were 1.93 and 2.59 mg/kg at single and double recommended rate respectively, dissipated to non-detectable level and 0.24 mg/kg respectively in four days.

Collected farmgate samples (20) from Daurala market 40, 45, and 30%, samples were exceeded MRL and 30, 40, and 35%, samples were recorded below their respective MRL values of monocrotophos (0.20), chlorpyriphos (0.20), and dimethoate (2.00) respectively. The residues were found in 79.17% samples of okra, 43.75% samples of brinjal, 73.68% samples of tomato and in 91.30% samples of cauliflower (0.35-2.10ppm), the residues of monocrotophos were found in 75% samples of okra, 78.12% samples of brinjal, 68.42% samples of tomato and 78.76% samples of cauliflower (Shukla *et al.*, 1996). According to Chahal *et al.* (1997) who reported that farmgate samples of vegetables for insecticide residues, revealed that 67% of samples were contaminated with insecticides last sprayed and about 7% of these had residues above their respective MRL values.

From lower market, 65, 55, and 45%, samples contained exceeded MRL and 25, 30, and 40%, samples had residues below MRL values of monocrotophos (0.20), chlorpyriphos (0.20), and dimethoate (2.00) respectively. Anonymous,(2001) reported out of 40 farmgate samples of vegetables including brinjal, okra, tomato, chilli, cabbage, pea, reddish, spinach and singhara, collected from different locations of Kanpur city, on an average 65% vegetable samples were found contaminated with either insecticides viz. lindane, endosulfan and cypermethrin, out of contaminated samples, 12.5% vegetable samples contained pesticides residues more than their respective MRL values. The reported residues of 0.58 mg/kg deltamethrin in okra fruit declined by 56.89% in 3 days, 82.75%in 5 days and reached to non-detectable limit after 7 days, okra fruits were safe for consumption after 5 days of insecticidal application (Dixit *et al.*, 2005).

In the samples collected from Kesarganj and Partapur markets, each (20), 35, 30, and 40%, in Kesarganj market and 45, 40, and 30%, samples in Partapur market, residues exceeded MRL and 50, 45 and 45% and 40, 40 and 45 samples, respectively contained residues below MRL values of monocrotophos (0.20), chlorpyriphos (0.20), and dimethoate (2.00) respectively. Similar findings were also reported, when 84 farmgate samples of vegetable were analyzed, revealed that 26% samples contained residues above MRL values. The contamination was mainly with organophosphate followed by synthetic pyrethroid and organochlorines. Among organophosphate, residues of monocrotophos, quinalphos and chlorpyriphos exceeded the MRL values in 23% samples. Residues of monocrotophos were higher than MRL values in three samples of brinjal and one sample of okra and quinalphos one sample in okra. Among synthetic pyrethroid, cypermethrin was the major contaminant and its residues exceeded MRL values in one sample each of brinjal and okra (Kumari *et al.*, 2004).

According to Dethe *et al.* (1995) endosulfan, cypermethrin dimethoate, monocrotophos and mancozeb, detectable level of residues were observed in 33.3% of tomato (endosulfan, dimethoate and monocrotophos), 73.3% of brinjal (endosulfan, cypermethrin, fenvalerate, quinalphos, dimethoate and monocrotophos), 14.3% of okra (endosulfan), however, the level of pesticides residues were lower than recommended maximum residue limit.

The present pattern of insecticides detected in the vegetables samples collected from the four sampled areas does not seem to contribute toward excessive residues. However, the use of insecticides should be need based only and recommended insecticides should be applied as and when required.
Further to safeguard the consumer's interest, proper waiting period must be practiced by the producer before marketing vegetables.

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Table – 2. Insecticides residues of okra farmgate samples from Daural, Lawar, Kesarganj and Partapur

S.N.	Insecticides Residues (ppm)											
	Monocrotophos(0.20)*				Chlorpyriphos(0.20)*				Dimethoate(2.00)*			
	Daurala	Lawar	Kesharganj	Partapur	Daurala	Lawar	Kesharganj	Partapur	Daurala	Lawar	Kesharganj	Partapur
1	0.19	BDL	0.18	0.13	BDL	1.04	BDL	1.04	1.34	1.45	2.45	1.45
2	BDL	4.11	BDL	3.00	0.03	3.08	0.08	0.18	0.36	1.98	1.98	2.08
3	0.15	2.99	0.99	2.11	3.04	0.14	3.04	BDL	BDL	4.24	0.24	BDL
4	1.13	0.10	0.13	0.19	0.08	0.91	0.01	0.11	BDL	0.11	BDL	1.11
5	BDL	3.76	2.76	2.52	2.10	BDL	BDL	2.13	3.06	3.09	4.06	4.09
6	4.43	0.14	BDL	1.14	0.15	0.15	0.15	1.15	BDL	BDL	BDL	0.22
7	1.02	1.02	1.02	0.12	1.12	3.12	3.12	0.12	0.88	1.10	0.10	2.10
8	1.15	2.02	3.02	BDL	0.02	5.02	0.02	BDL	1.10	5.10	1.10	1.12
9	0.13	0.01	0.19	0.19	2.22	BDL	BDL	0.08	5.03	4.03	3.03	2.63
10	4.32	BDL	2.03	0.01	5.01	0.98	2.98	0.98	3.79	2.59	2.59	BDL
11	4.02	1.02	1.02	BDL	BDL	1.87	BDL	1.87	0.82	BDL	0.82	BDL
12	0.18	0.88	0.18	0.88	0.09	0.19	0.09	BDL	2.22	1.10	1.22	4.13
13	BDL	4.11	0.10	3.11	0.13	0.01	0.04	0.01	BDL	2.77	2.77	0.10
14	2.00	2.03	0.13	2.13	0.11	4.18	0.18	2.18	2.98	1.98	3.98	1.98
15	BDL	0.12	0.12	0.11	5.20	0.09	0.09	0.09	0.18	BDL	BDL	BDL
16	BDL	1.00	BDL	0.16	2.43	3.43	2.43	3.43	4.00	0.12	0.12	0.12
17	0.17	5.10	0.18	2.10	4.21	2.21	3.21	2.21	BDL	2.77	2.77	2.77
18	0.19	0.10	0.10	5.10	BDL	BDL	BDL	0.17	1.98	3.98	1.98	BDL
19	BDL	3.16	BDL	BDL	2.75	0.07	0.07	0.07	BDL	BDL	4.98	1.98
20	2.94	1.00	0.13	0.19	0.17	1.88	1.17	BDL	BDL	2.55	0.55	1.05
Number of samples												
>MRL	8	13	7	9	9	11	6	8	6	9	8	6
<MRL	6	5	10	8	8	6	9	8	7	8	9	9
BDL	6	2	3	3	3	3	5	4	7	3	3	5

BDL (Below Detectable Limits). * MRL (Maximum Residual Limits)

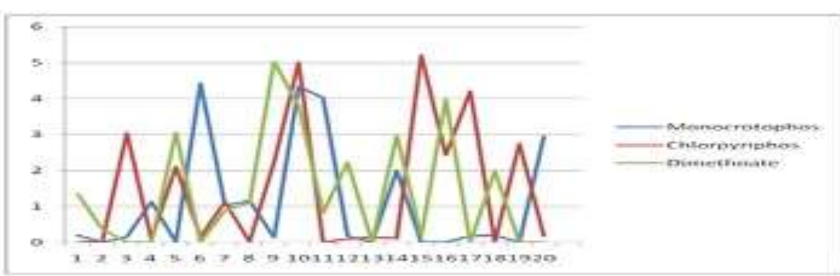

Fig. 2. Insecticides residues of okra farmgate samples from Daural

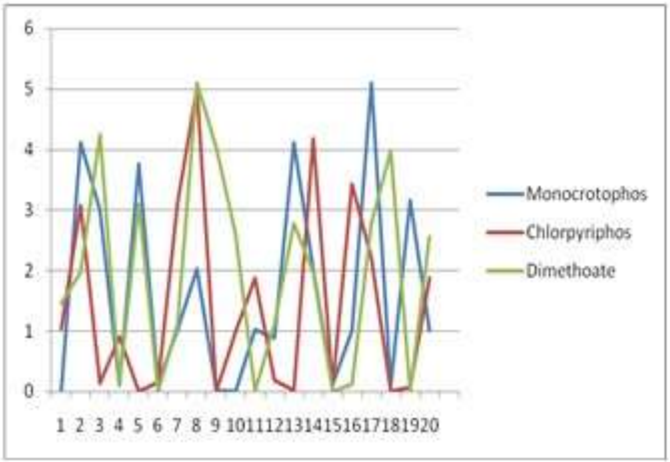

Fig. 3.Insecticides residues of okra farmgate samples from Lawer

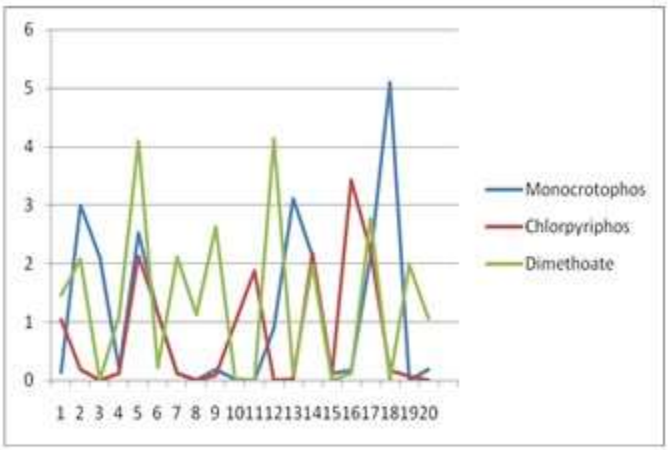

Table – 4. Insecticides residues of okra farmgate samples from Kesarganj

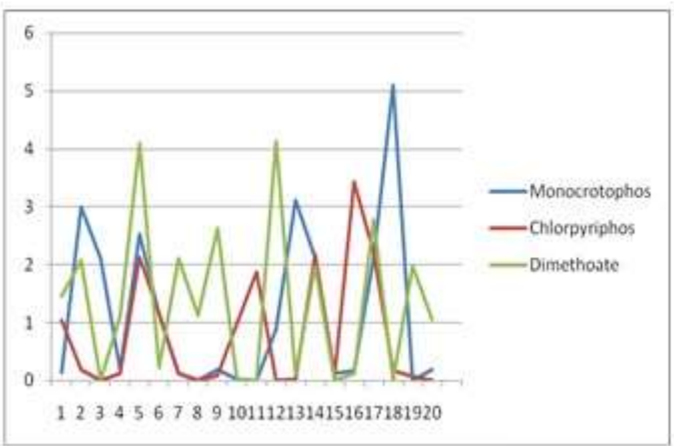

Fig. 5. Insecticides residues of okra farmgate samples from Partapur