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Research Article

QUALITATIVE AND QUANTITATIVE ANALYSIS OF FENVALERATE, AND METHYL PARATHION PESTICIDES IN MANGO AND GRAPES COLLECTED BY HPLC METHOD

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ABSTRACT

A new HPLC method was developed for Analysis of FENVALERATE, AND METHYL PARATHION PESTICIDES IN MANGO AND GRAPES. The method has maximum recoveryi.e. 99.0-100.0 %. The method was applied for analysis of FENVALERATE, AND METHYL PARATHION in fruit samples.

Key words:FENVALERATE, METHYL PARATHION, HPLC Method, Pesticide, Recovery, Mango,Grapes.

INTRODUCTION

After green revolution the use of pesticides increased very rapidly for all crops i.e. food grains, vegetables, fruits, cotton, tobacco. In Andhra Pradesh, Nuziveedu is famous for Mango exporting and Hyderabad is famous for exporting of Grapes. In this area formers are using pesticides in huge quantity to prevent pest. We analyzed few Mangos and Grapes fruit covers identification of Parathion-methyl, Fenvalerate.

Parathion-methyl:

Parathion-methyl [1-6] "Parathion-methyl", also known as methyl parathion or dimethyl parathion, was also developed and is marketed for similar uses. It is a distinct compound with diminished toxicity. Some trade names of parathion-methyl include Bladan M, Metaphos. As a pesticide, parathion is generally applied by spraying. It is often applied to cotton, rice and fruit trees. The usual concentrations of ready-to-use solutions are 0.05 to 0.1%. The chemical is banned for use on many food crops. Parathion acts on the enzyme acetylcholinesterase, but indirectly. After being ingested by the parathion becomes oxidized by oxidizes to give paraoxon, replacing the double bonded sulfur with oxygen.[2]

The phosphate ester is more reactive in organisms than the phosphorothiolate ester, as the phosphorus atoms become much more electronegative Parathion is a cholinesterase inhibitor. It generally disrupts the nervous system by inhibiting the acetylcholinesterase. It is absorbed via skin, mucous membranes, and orally. Absorbed parathion is rapidly metabolized to paraoxon, as described above. Paraoxon exposure can result in headaches, convulsions, poor vision, vomiting, abdominal pain, severe diarrhea, unconsciousness, tremor, dyspnea, and finally lung-edema as well as respiratory arrest. Symptoms of poisoning are known to last for extended periods of time, sometimes months. The most common and very specific antidote is atropine in doses of up to 100 mg daily. Because atropine may also be toxic, it is recommended that small frequently repeated doses be used in treatment. If human poisoning is detected early and the treatment is prompt fatalities are infrequent. Insufficient oxygen will lead to cerebral hypoxia and permanent brain damage. Peripheral neuropathy including paralysis is noticed as late sequelae after recovery from acute intoxication. Parathion has been used for committing suicide and deliberately poisoning other persons. It is known as "Schwiegermuttergift" in Germany. For this reason most formulations contain a blue dye providing warning.

Parathion has been used as a chemical weapon, most notably by the Selous Scouts during the Rhodesian Bush War.[3] Based on animal studies, parathion is considered by the U.S. Environmental Protection Agency to be a possible human carcinogen.[4] Studies show that parathion is toxic to fetuses, but does not cause birth defects.[5] It is classified as a UNEP Persistent Organic Pollutant and WHO Toxicity Class, "Ia, Extremely Hazardous".Parathion is very toxic to bees, fish, birds, and other forms of wildlife.[5] Parathion can be replaced by many safer and less toxic alternatives.

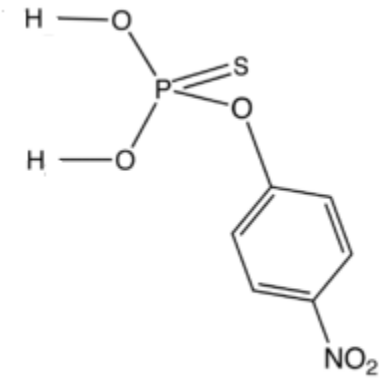

Figure 8.A:StructureofMethylParathion

Fenvalerate [7-16]:

Fenvalerate is an insecticide. It is a mixture of four optical isomers which have different insecticidal activities. The 2-S alpha configuration is the most insecticidally active isomer. Fenvalerate consists of about

23% of this isomer. Fenvalerate is an insecticide of moderate mammalian toxicity. In laboratory animals, central nervous system toxicity is observed following acute or short-term exposure. Fenvalerate has applications against a wide range of pests. Residue levels are minimized by low application rates. Fenvalerate is most toxic to bees and fish. It is found in some emulsifiable concentrates, ULV, wettable powders, slow release formulations, insecticidal fogs, and granules. It is most commonly used to control insects in food, feed, and cotton products, and for the control of flies and ticks in barns and stables. Fenvalerate does not affect plants, but is active for an extended period of time. Fenvalerate may irritate the skin and eyes on contact, and is also harmful if swallowed.

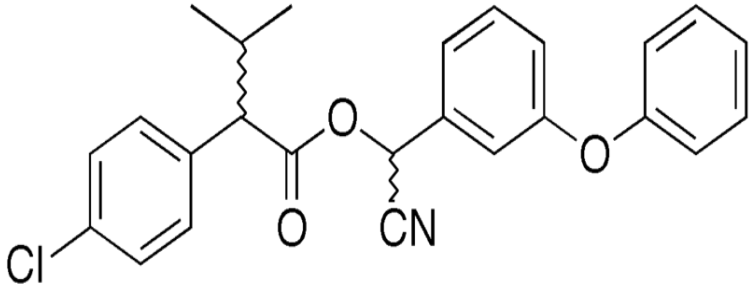

Figure 8.B: **StructureofFenvalerate**

MATERIALS AND METHODS

2.1. Instrumentation:

For quantitative estimation of Fenvalerate, Methyl Parathion in Fruit an isocratic peak HPLC instrument with chromosil c18, c8 column, (100 mm x 4.6 mm, 5µ) (250 mm x 4.6 mm, 5µ), (150 mm x 4.6 mm, 5µ) was used. Theinstrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable UV-Visible detector, SPD-10AVP. A 20µL Hamilton syringe was used for injecting the samples. Data was analyzed by using PEAK software. Techcomp UV 2301UV-Visible spectrophotometer (Hitach software) was used for spectral studies. Degassing of the mobile phase was done by using a Loba ultrasonic bath sonicator. A Denverbalance was used for weighing of the materials.

2.2. Chemicals and Solvents:

The reference standard of Fenvalerate was obtained from Aimco Pesticides Ltd, Mumbai. The reference standard of Fenvalerate was obtained from Bhaskar Agro Chemicals Limited, Hyderabad.The fruit samples were collected from the local fruit markets fruit tree forms, Acetonitrile, Methanol, Water used is HPLC grade are purchased from Merck Specialties Private Limited, Mumbai, India. T.E.A of AR grade purchased from local market.

2.3 Sample collection:

Fruit samples are collected from local market of Vijayawada, directly from fruit tree forms, Hypermarkets. The samples are collected randomly from different shops, from each shop we are collected average 6 fruits. In garden frits are collected from different trees randomly and different gardens.

2.4. The Mobile Phase:

Two different suitable mobile phases are prepared individually for analysis of target Pesticides in Fruits. The prepared mobile phases are sonicated up to 30 min, and filtered through 0.45 µ nylon filter paper.

2.5. Standard Solution of the Drug:

For analysis of 1000 ppm stock solutions are prepared with reference standards of Fenvalerate, Methyl Parathion pesticides with their mobile phases. From the stock solution calibration curves prepared to estimate target pesticides.

2.6. Extraction of pesticides from fruits covers [17]:

20 g of fruit cover was collected from the sample. .The covers were kept into a cone flask and thoroughlymixed with dichloromethane (30 ml) and sodium carbonate (15 g). Then the mixture was to standing 12 hin the well-sealed cone flask. After that the mixture was filtered through filter paper and then the tundishwas washed with dichloromethane. The filtered liquid phase was contained in an open watch glass. Whendichloromethane was dried out, methanol (5 ml) wasadded to extract the DDVP. The extraction was repeated twice with methanol (2 ml). These extractionswere mixed and diluted by methanol to 10 ml thenfiltered for analysis.

3. Optimization of HPLC methods from Standard Methods:

During HPLC method optimization, a systematic study on effect of various factors was performed by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wavelength and chromatographic conditions like stationary and mobile phase. The following studies were conducted for this purpose.

3.1. Detection Wavelength:

The proper wavelength was needed to determine maximum detector response. The first step was to run a UV-VIS spectrum (from 190-320 nm) using an HPLC system equipped with the Photo Diode Array Detector.

3.2. Choice of Stationary Phase:

In general, develop all methods with HPLC columns from the same vendor. The preferred brand of HPLC column should be selected primarily based on the long term stability and lot-to-lot reproducibility. Preliminary development trials have performed with octadecyl columns form different manufacturers with different configurations.

3.3. Selection of the Mobile Phase:

Liquid chromatography method development began with the optimizing mobile phase composition and column type. The feasibility of several mixtures of solvent such as acetonitrile, water and methanol using different buffers such as ammonium acetate, ammonium formate, acetic acid and formic acid with variable pH range 3–6 was tested for complete chromatographic resolution.

In order to get sharp peak and base line separation of the components, a number of experiments were carried out by varying the composition of various solvents and its flow rate. Under isocratic conditions, mixtures of solvents like methanol, water and Acetonitrile with and without different buffers indifferent combinations were tested as mobile phase on a C18 stationary phase.

3.4. Flow Rate:

Flow rate of the mobile phase was changed from 0.5 – 1.5 mL/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents.

3.5. HPLC Conditions Optimization for Analysis of Methyl Parathion [18]:

For analysis of Methyl Parathion .in tissue samples, HPLC with UV-detector set at 225 nm was used, with low sensitivity and specificity. So, HPLC with PDA detector is used to analysis ofMethyl Parathion. In this study C18 reversed phase CHROMOSIL column was employed at 25C temperature Water and methanol P^H (5.3) as the mobile phase in 65:35 v/v ratio. The isocratic elution under the condition employed allows the separation of Methyl Parathion, Good separation and peak shape was obtained at flow rate of 1.0 ml/min.

S.No	Condition	Parameter
1	Mobile Phase	Water and Methanol 65:35 (v/v)
2	Column	Chromosil, C18 (4.6 mm, 100 mm) column
3	Wave length	225 nm
4	Flow rate	1.0 ml/Min
5	Column temperature	25 c
6	Run time	10 min
7	Sample volume	20 µL
8	P^H	5.3

Table 8.1: Chromatographic conditions of Methyl Parathion

3.5 HPLC Conditions Optimization for Analysis of Fenvalerate [19]:

For analysis of Fenvalerate, in tissue samples, HPLC with UV-detector set at 239 nm was used, with low sensitivity and specificity. So, HPLC with U.V detector is used to analysis of Fenvalerate, In this study C18 reversed phase GEMINI column was employed at 30c temperature, Acetonitrile: Methanol, KH_2PO_4 (50:40:10 V/V/V)P^H (6.8) as the mobile phase. The isocratic elution under the condition employed allows the separation of Fenvalerate, Good separation and peak shape was obtained at flow rate of 1.0 ml/min.

S.No	Condition	Parameter
1	Mobile Phase	Acetonitrile: methanol-potassium dehydrogenate phosphate (50:40:10)
2	Column	GEMINI C18, 250 mm×4.6 µm
3	Wave length	239 nm
4	Flow rate	1.0 Ml/Min
5	Column temperature	30c
6	Run time	10 min
7	Sample volume	20 µL
8	P^H	6.8

Table 8.2: Chromatographic conditions of Fenvalerate

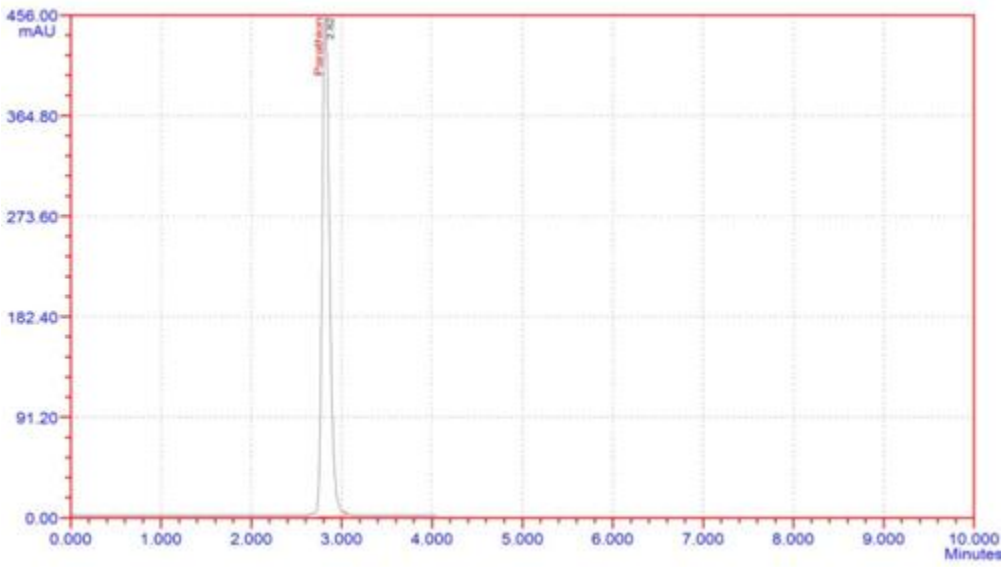

Figure 8.C:HPLC Chromatogram forMethylParathion

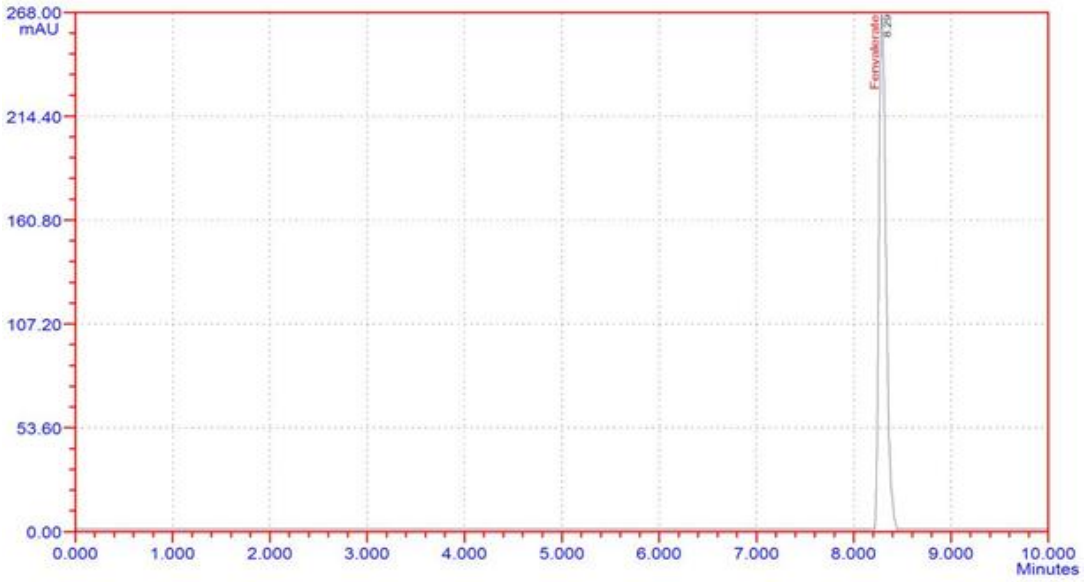

Figure 8.D:HPLC Chromatogram forFenvalerate

RESULTS

1. Calibration curve with standard:

From the stock solutions different concentrations METHYL PARATHION (0.2ppm-1.2ppm) FENVALERATE, (0.5ppm-3ppm) of standard pesticide solutions are injected in to HPLC at system suitable condition what are optimized from standard procedures. the calibration curves are plotted between area of peak and pesticide concentrations.

S.NO	Standard concentration (ppm)	Peak Area
1	0.2	2352
2	0.4	4398
3	0.6	6347
4	0.8	8309
5	1.0	10396
6	1.2	12539
7	Slope = 10288.57	Intercept = 161.2857

Table8.3: Calibration table for MethylParathion

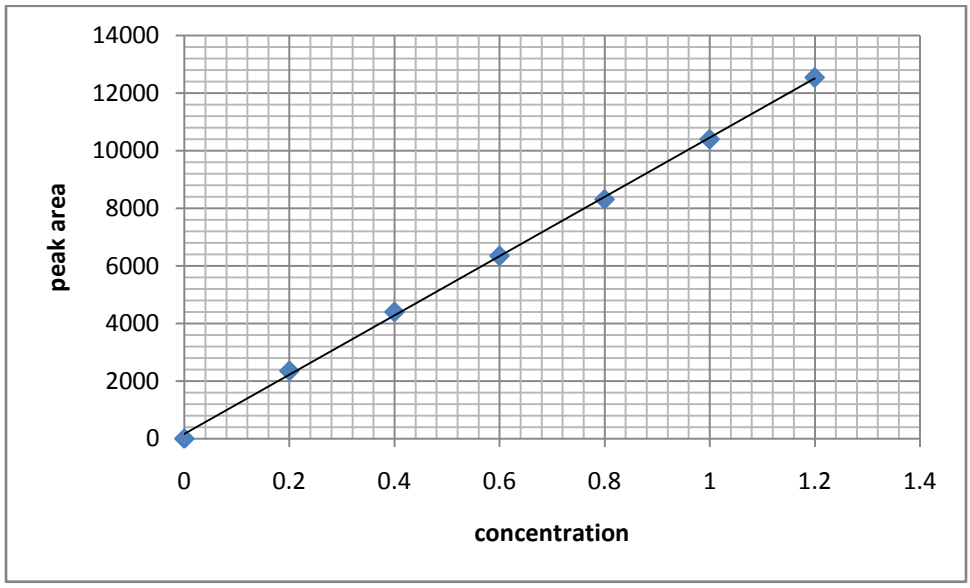

Figure 8.E: Calibration curve Methyl Parathion

S.NO	% OF RECOVERY	Fixed conc in ppm1	Spiked conc in ppm1	Total sample concentration	Amount of recovery	% of recovery	% of Average recovery
1	50%	0.4	0.2	0.6	0.599	99.83	99.55
2	100%	0.4	0.4	0.8	0.797	99.62	
3	150%	0.4	0.6	1	0.992	99.2	

Table 8.4: Recovery studies of Methyl Parathion

S.NO	Parameter	Concentration in ppm
1	L.O.Q	0.01
2	L.O.D	0.005

Table8.5:L.O.Q and L.O.D studies of Methyl Parathion

S.NO	Standard concentration (ppm)	Peak Area
1	0.5	1927
2	1.0	3496
3	1.5	5221
4	2.0	6798
5	2.5	8506
6	3.0	10024
7	Slope = 3323.714	Intercept = -153.2857

Table8.6: Calibration table for Fenvalerate

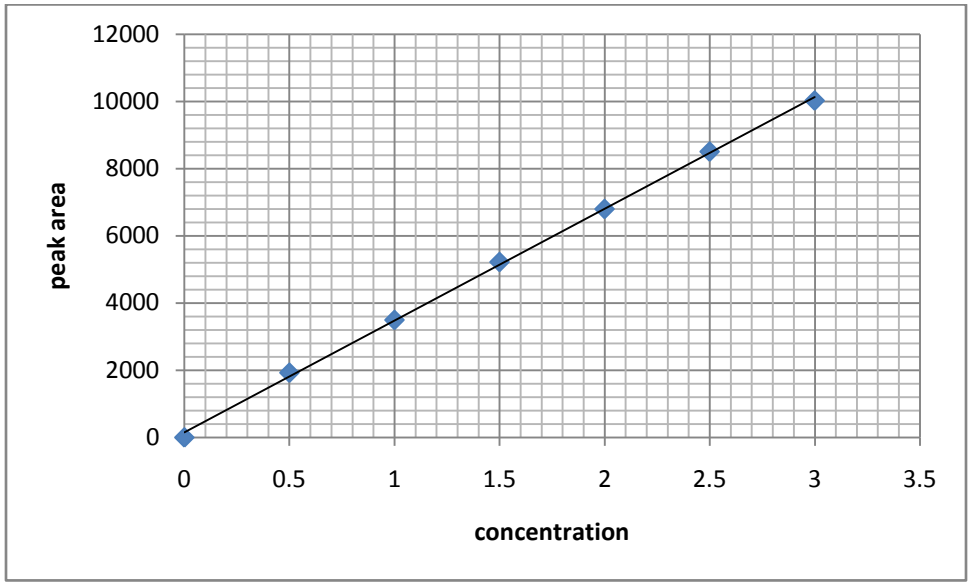

Figure 8.F: Calibration curve Fenvalerate

S.NO	% OF RECOVERY	Fixed conc in ppm1	Spiked conc in ppm1	Total sample concetration	Amount of recovery	% of recovery	% of Average recovery
1	50%	1	0.5	1.5	1.496	99.73	99.27
2	100%	1	1	2	1.97	98.5	
3	150%	1	1.5	2.5	2.499	99.6	

Table 8.7: Recovery studies of Fenvalerate

S.NO	Parameter	Concentration in ppm
1	L.O.Q	0.07
2	L.O.D	0.03

Table8.8:L.O.Q and L.O.D studies of Fenvalerate

S.NO	Location of Sample collection	Fruit sample	Concentration of Methyl Parathion µg/Kg	Concentration Fenvalerate, µg/Kg
1	Garden	Mango	13.64± 0.43	7.38± 0.34
2	Local market	Mango	10.65± 0.28	6.39± 0.67
3	Ready for exporting	Mango	NDL	NDL
4	Reliance fresh	Mango	8.54± 0.55	5.35± 0.28
5	Spancer	Mango	7.63± 0.47	4.99± 0.83
6	Garden	Grape	14.85± 0.34	9.37± 0.09
7	Local market	Grape	11.27± 0.50	8.81± 0.64
8	Ready for exporting	Grape	NDL	NDL
9	Reliance fresh	Grape	8.63± 0.24	7.67± 0.45
10	Spancer	Grape	8.14± 0.12	6.89± 0.68

Table8.9: Concentrations ofMethyl Parathion and Fenvalerate, in fruit cover samples

DISCUSSION

Mango and Grapes are famous and very high nourishing fruits for all age people. Especially mangos are famous in India. In Andhra Pradesh state Grapes are cultivating in around surroundings of Hyderabad. The mango fruits are highly exporting from the city Nuzeveedu, Krishna district. But the main issue is the presence of pesticide residues in fruit cover of these two fruits. Due to this problem the price of fruits is decreasing in international market. And the fruits importing from India also banned in Singapore, Australia, U.S, U.K. in order to prove this concept we analyzed fruit samples by H.P.L.C technique. The results are given in Table.8.5.

For analysis of Methyl Parathion we are developed a HPLC method with UV-detector at 225 nm, with C18 reversed phase CHROMOSIL column, at 25_C temperature, Water and methanol P^H (5.3) as the mobile phase in 65:35 v/v ratio, at flow rate of 1.0 ml/min. The recovery of this developed method is 99.55. We can estimate the Methyl Parathion at very low level concentrations up to 0.07 ppm, we may detect the of Methyl Parathion up to 0.03 ppm

For analysis of Fenvalerate, in fruit samples, we developed a HPLC method with UV-detector at 239 nm, with C18 reversed phase GEMINI column, at 30c temperature and Acetonitrile: Methanol, KH_2PO_4 (50:40:10 V/V/V)P^H (6.8) as the mobile phase, flow rate of 1.0 ml/min. With our method we recovered 99.27 Fenvalerate by standard addition method. L.OQ and L.OD values are 0.07 ppm, 0.03 ppm respectively.

From above results we conformed that the sample has Methyl Parathion, Fenvalerate, in high

concentration in samples which are collected directly from fruit forms. In Mangos Methyl Parathion is 13.64 µg/Kg, Fenvalerat is 7.38 µg/Kg, In Grapes Methyl Parathion is 14.85 µg/Kg, Fenvalerat is 9.37 µg/Kg But in the exporting quality fruits there are no presence of our target pesticides. May be exporters taken care, or followed any process to remove pesticide residues in fruit cover. In local fruit market we found Methyl Parathion, Fenvalerate in high concentrations. i.e. Methyl Parathion is 10.65 µg/Kg, Fenvalerat is 6.39 µg/Kg (In Mango) Methyl Parathion is 11.27 µg/Kg, Fenvalerat is 9.37 µg/Kg (In Grapes). But in high per markets we found target pesticids in less amount compare to local markets. In Reliance fresh i.e. Methyl Parathion is 8.54 µg/Kg, Fenvalerat is 5.35 µg/Kg (In Mango), Methyl Parathion is 8.63 µg/Kg, Fenvalerat is 7.67 µg/Kg (In Grapes). in Spencer i.e. Methyl Parathion is 7.63 µg/Kg, Fenvalerat is 4.49 µg/Kg(In Grapes), Methyl Parathion is 8.14 µg/Kg, Fenvalerat is 6.89 µg/Kg (In Grapes).

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