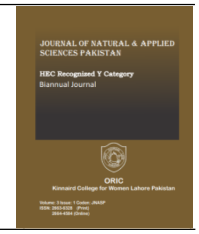
Contents list available http://www.kinnaird.edu.pk/

Journal of Natural and Applied Sciences Pakistan

Journal homepage: http://jnasp.kinnaird.edu.pk/



DETECTION OF PESTICIDE RESIDUES IN RICE GRAINS OF KOT HUSSAIN, SHEIKHUPURA

Fajar Fatima[1*], Amber Fatima[1]
[1]Department of environmental science, Kinnaird College for Women, Lahore

Article Info
*Corresponding Author
Email: Fajarfatima290@gmail.com

Abstract

Pakistan is an agricultural country and its backbone depends on the export of crops like rice, wheat, etc. Gross Domestic Product from agriculture in Pakistan has increased to 25 million in 2020 and is expected to rise. Rice is the source of livelihood for many farmers in Pakistan. Excessive use of pesticides during the pre- harvest and post-harvest period of rice causes pesticide residues in the grains. To determine the pesticide residue in rice grains, different types of rice were collected from the village of Kot Hussain located in Sheikhupura, Pakistan. Imidacloprid, Emectin, Timer, Glyphosate, Bifenthrin, and Lufenuron were the most commonly used pesticides on the rice crops of 386 safaid, super fine stem, 386 laal, C-9, 1121 Kainat, and super karnal rice. Results indicated that glyphosate, emectin and timer were below the maximum residue level (MRL). On the other hand, imidacloprid, bifenthrin, and lufenuron were present in higher concentrations exceeding the MRL values. The concentration of bifenthrin was between 0.122ppm-0.141ppm, the concentration of imidacloprid was between 0.093 ppm - 0.111 ppm and the concentration of lufenuron was between 0.175 ppm-0.207 ppm. Pesticide residues of imidacloprid, lufenuron and bifenthrin were higher in super karnal, 386 safaid, super fine stem, 386 laal, C-9 and 1121 kainat in rice grains of Kot Hussain in Sheikhupura than the standard limits. To control the pesticide residues in rice crops post-harvest spray of pesticides should be avoided as well as situation based and tactical use of pesticides should be adopted instead of routine-based spray of pesticides.

Keywords
Pesticides Imidacloprid, Rice Grains

1. Introduction

Pakistan is an agricultural country and its backbone depends on the export of crops like rice, wheat, etc. Gross Domestic Product from agriculture in Pakistan has increased to 25 million in 2020 and is expected to rise. Rice crops are a source of livelihood for many farmers in Pakistan. Rice is also the major source of foreign exchange earnings and is the most consumed domestic staple food among cereals, wheat, etc. About 30 million Pakistanis depend on rice for livelihood, income, and employment. Basmati rice is the most supreme variety of rice among the other rice varieties because of its aroma, long slender grains, and delicious taste. Basmati rice is Pakistan's premium export because it can only be grown under favorable environmental conditions present in some districts of Punjab province (Andreazza & Scola, 2017.) Pest outbreaks cause heavy decline in yield of rice production, cause human health problems and many other detrimental impacts on the environment. Preventive measures such as use of different pesticides help in controlling the pest attacks. Pesticides are regularly sprayed on rice paddies and remain in use till the ripening of the crops. Pesticides are chemicals that are used to safeguard crops from harmful insects and weeds. The use of pesticides is not new and has been practiced for many years (4500 years), different farmers used different types of pesticides according to their preferences and needs. The formula of the mixture that makes up the pesticides has been changed over the past years and has been made more effective. Since the population has increased the demand for food has also increased thus the excessive use of pesticides to double the crop yield which in turn led to pesticide residue in rice (Stamati *et al*., 2015). Pesticide residue in rice crops can cause harmful impacts such as deterioration of the nervous system in humans, reproductive issues, respiratory problems and increased risk of cancer in case of acute and chronic exposure. Pesticide residues in rice cannot be completely eliminated by washing or cooking and thus a small concentration of pesticides remain in the ingested food (Upadhayay *et al., 2020).* Pakistan is the world's 10th largest producer of rice and makes up 8% of the world's total rice trade. Pakistan is the major producer of basmati rice, rich in its flavor and aroma famous all around the globe. In Pakistan rice is the 3rd largest crop grown in respect of area after wheat and cotton. Rice is divided into three broad categories known as Basmati (Basmati 370, Basmati 198, Basmati Pak, Basmati 385, Super Basmati, Basmati 2000, Shaheen Basmati, Basmati 515 Punjab Basmati, Chenab Basmati, Kissan Basmati) Non-basmati varieties (PK 386, PK 1121 Aromatic) Medium grain varieties (IRRI 6, KS 282, KSK 133, KSK 434) and Hybrid rice (Pukhraj, *et al*). During World War 2 (1939-1945) pesticides helped the crops because there was dire need of enhancing food production. Taking a look at the history of these pesticides, it is known that DDT (Dichloro-Diphenyl-Trichloroethane), a pesticide that was used on a large scale and proved to be very effective was banned in eighty-six countries including Pakistan from being used twenty years later due to the harmful effects it had left on the plants and humans as well. Tzanetou EN & Karasali Diverse groups of pesticides such as insecticides, herbicides, rodenticides, bactericides, fungicides and larvicides are used in agriculture to control diseases and pests. Insecticides are used to kill insects, herbicides are used to kill unwanted plants, rodenticides are used to kill rats and mice, bactericides are used to kill bacteria, fungicides are used to kill fungi and larvicides are used to kill larvae. Pesticide residues' effect on environment, wildlife and humans in a long-term manner should be recognized as it is not just a matter of one country or region but it is the problem for the whole globe. Thus, the community should take action as a whole. The toxicological effects e.g., carcinogenic, mutagenic pesticides are harmful to humans and animals acutely and chronically (Munshi*et al.,* 2011) Over the years (1970 onwards) researchers have tried to produce pesticides that are less harmful but to date, no such pesticide has been produced that has proven to be safe for both plants and humans. These farmers do not realize how much of a threat they are causing to human health that can even get serious with time. Toxic substances are used in the manufacturing of these pesticides that contaminate the environment. These cannot be used extremely precisely and reach other parts of the environment too where they are not needed, this way they are also found to be in the air and water instead of reaching their targeted pest by which they end up being in the food humans and animals consume NSW EPA . In most rice-growing countries insecticides are the dominant class of pesticides used in fields, for example in the Philippines 50-60 percent of pesticides applied to rice fields before 1980 were insecticides, 5-16% herbicides, and 20-25% fungicides. In some countries herbicide usage in fields has increased due to a rise in labor wages. Recommended pesticide application depends on the area of land e.g., a few hundred grams to a few kilograms (kg) of active ingredient per hectare (ai/ha) with a median of about 2kg. The Median is relatively higher for herbicides (2.5 kg ai/ha) than fungicides (1.7 kg ai/ha) and insecticides (1.1 kg ai/ha). Teng P However, in developing countries like Pakistan recommended application levels are not strictly followed and farmers sometimes ignore the rules and either use an excessive amount of pesticide or reduced amount in some cases has also been reported. In this regard, during field trials, high variability of concentrations was observed in the same field. For example, determination of propanil in 4 soil samples from the same field after 40 days of application ranged between (0.001-0.2ppm).(Pingali *et al*., 2010). Studies of fungicide and insecticide residues in different parts of the rice plant indicate that pesticide residues were mostly found in aerial parts of the plant and the rice. A study in India involved the application of thiamethoxam and lambda-cyhalothrin insecticides at the recommended level and twice the recommended rate to the aerial parts of the rice plant, results indicated no pesticide residue in grains (Teló et *al.,* 2017*)*. Another similar study in China indicated the presence of chlorantraniliprole insecticide and some fungicide residues in brown rice thus the residue determination may be different in different countries and can also depend on the part of the plant or grain examined e.g., hull, bran, or polished grain. Although the external structure of rice may accumulate higher pesticide residues as it is the physical barrier between the environment and the rice. Similarly, some articles suggest that residue detection or pesticide persistence could be affected by the temperature as high temperatures can reduce 38% difenoconazole concentration in rice hulls. Thus, highest concentrations of pesticides are most commonly found in the grain of rice (Zhang,.*et al*.2012). Different types of pesticides are used in the agricultural fields of Pakistan to control pest production. Pesticides were first introduced in 1954 and by 2003 consumption of pesticides increased to 78,132 tonnes per annum. By 2012 thousands of tonnes of pesticides had been imported from Europe and other western countries in Pakistan to control pest infections (Sharma A *et al.,* 2019*).*

1.1 Harmful effects of pesticides

Most of the pesticides are non-biodegradable in nature, especially organochlorines that are resistant to microbial degradation and tend to accumulate in the human body fats and the environment. Pesticides residues through their persistence and lipophilicity accumulate in the adipose tissues and other body parts. Among pesticides, insecticides are considered the most toxic while herbicide and fungicides are second and third on the list of toxicity. Some people are more vulnerable to pesticide poisoning than others such as infants and children, as the majority of the population gets affected by the ingestion of pesticide contaminated food. Pesticide toxicity may be immediate or it may take years or months to manifest. Acute or immediate effects of the pesticides include headache, dizziness, nausea, skin or eye irritation, abdominal discomfort and rarely death. However, long term effects of the pesticide exposure include loss of vision, memory, motor signaling, damaged immune system, cancer etc. (Mahmood *et al* 2016) Rice farmers in the village of Kot Hussain use lufenuron, timer, bifenthrin, emectin, glyphosate and Imidacloprid to protect their crops from different kinds of pests, weeds, insect infestation and diseases. Without the use of pesticides, the crops would be destroyed and lost to different insects such as locusts etc., thus pesticides are used to increase the productivity per hectare. Synthetic and natural pesticides are both harmful to the environment and cause moderate to high level of toxicity in the ecosystem. Importance & Benefits of Pesticides. Imidacloprid is an insecticide used in rice crops and various other food crops. Imidacloprid is lethal to sucking insects, termites, fleas and some other insects. Imidacloprid when exposed to soil can last for months and years and its residues can be tightly bound to the soil particles with time which means they become part of the ecosystem. When humans are exposed to higher quantities of Imidacloprid it can cause dizziness, skin irritation, dizziness, develop tremors, vomiting etc Importance & Benefits of Pesticides Bifenthrin is an insecticide primarily used in agricultural crops of different types. Bifenthrin is generally less toxic to humans than insects as insects have lower body temperature and smaller body structure. The U.S. EPA has classified bifenthrin as a possible human carcinogen. Some studies conducted on mice and other laboratory animals concluded that it could cause cancer. Bifenthrin is slowly absorbed by the body tissues after being ingested, some of which is released by the body Singh VK Glyphosate is an herbicide used in the crops to increase or regulate the plant growth and mature rice crops. It is also used to kill specific types of herbs and unwanted plants. Rice higher levels of glyphosate residues can cause burns in the mouth, eye irritation, skin irritation, throat, nausea, diarrhea and vomiting. Glyphosate general fact sheet. However, glyphosate is highly unlikely to cause cancer according to WHO but some studies indicate that it could possibly be carcinogenic. Glyphosate herbicide has also been linked to causing liver, kidney damage and it is a risk for pregnant women and children .Lufenuron is an insecticide used in rice crops to control the infestation of fleas and other insects that could harm the growth of crops. Lufenuron is also described as an effective antifungal in agricultural crops. Residual level lufenuron exposure or sub lethal doses of lufenuron can be toxic to non-specific targets but pregnant women and fetuses are at higher risk than the general population. Site selected for this study of pesticide residue in rice is a village named Kot Hussain near Sheikhupura in Punjab province of Pakistan. Different types of rice such as 386 safaid, super fine stem, 386 laal, C-9, 1121 Kainat and super karnal rice are cultivated in this area yearly.

2. Literature Review

Pakistan is an agricultural country thus the consumption of pesticides is like other South Asian countries and the pesticide residues have increased over the last 10 years in soil, water and crops. The intensive and regular cultivation of agricultural lands and increased usage of fertilizers to fulfill the requirements of minerals has led to the recurring and persistent pest problems in fields and the availability of pesticides in markets has made the unprofessional use of pesticides normal and easy which alternatively led to excessive use of pesticides. In the last 10 years' pesticide use in grain production has doubled, especially in rice crops (Jabbar *et al.,* 1994*)*. In July 2021, Lahore chamber of commerce industry pointed out that the European Union has issued an alert to Pakistan and also 161 consignments have been rejected by Europe and the US because of high levels of pesticides residue in rice exported. They claimed that the farmers had done post-harvest spray of pesticides and thus the high levels of residue in rice. If the situation continues and no strict action is taken Pakistan will lose its share in the international market, as experts warn of a ban on rice shipments to the EU and that will be the darkest time for Pakistan's economy. Pakistan export promotion Bureau has made WTO standards for the import and export of rice crops in which the pesticides residues should not exceed permissible levels. Farmers are not educated about the harmful and extremely dangerous impacts of excessive pesticides usage. 30% of the food grown is lost annually because of weeds, pests and diseases; loss of food would double if we stop using pesticides. Nowadays, many countries have given attention to pesticide residues in food, especially staple foods such as rice. Buyers and consumers of rice pay extra attention to the quality of rice and amount of pesticide residues within. Presence of pesticide above the permissible limits are main sources of health problems (*Munshi et al.,* 2011). A study conducted in Thailand to determine the harmful effects of organophosphorus and organochlorine pesticides on agricultural activities concluded that they are used widely in rice paddies production but they are highly lipophilic in nature and have high tendency to surpass and accumulate in the tissues of living organisms and can cause health issues. (Chaiyarat *et al.,* 2015). In a review of pesticide residue on rice, researchers investigated the negative and positive impacts of pesticides in agricultural practices. It has come to light those positive impacts such as protection of crops against vector diseases, prolonged shelf life and increased productivity of crops are the most important functions of pesticides. As pesticides are the most common and advanced tool for modern agricultural practices the harmful chemical effects of pesticides should not be neglected and thus rational pesticide use (RPU), selection of pesticide which have least impacts on non-target organisms can help in optimizing pesticide dosage and minimizing the environmental and health costs(Lozowicka *et al.,* 2015*)*.Furthermore, a study conducted in Kazakhstan to determine the pesticide residue in different cereals such as rice, barley, oat, rye and wheat concluded that 77.5 % samples were free of pesticide but 13.75 % of samples had pesticides above the MRL values. Acute risks from the daily intake of cereals contaminated with pesticides rise on a daily basis. India is one of the largest cultivators of rice and for the evaluation of pesticide residues in rice, wheat and pulses an investigation was conducted in Bidar district, Karnataka. Samples were analyzed with the help of HPLC and gas chromatography (GC) and the results indicated that out of 250 samples, 80 samples were contaminated with pesticides and the residue level was above the maximum residue limit (MRL) in 22 samples. Similarly, a study was conducted along the two tributaries of river Chenab in Pakistan to assess the pesticide contamination and their harmful effects on human health. Estimated daily intake of organochlorine pesticides through wheat and rice was calculated to be 39-40 ng kg-1 day-1. Results from this study suggested that the human population is at severe risk due to consumption of contaminated cereal food such as rice and wheat Mahmood A *et al* 2019 conducted a study in Nigeria to determine herbicides concentrations in foreign and local rice brands. All samples obtained were influenced by concentration of different types of pesticides however all pesticide residues exceeded the maximum residue limit set by the European Union for cereals. Pesticide residues accumulate in the body over a prolonged time period and cause serious health concerns.(Adah *et al.,*2017). In 2014, pesticide residue in soil, water and grain of IPM basmati rice was analyzed in India. This study was focused on the harvest samples of basmati rice grains and the results indicated that residues of imidacloprid, DDVP, carbendazim and hexaconazole were present below detectable limit (BDL) in some samples. (Arora *et al., 2014).* Pest resistance to pesticides and excessive use of pesticides is an issue all over Asia which has led to the environmental degradation and contamination of natural resources and agricultural products. The use of integrated pest management (IPM) is the best solution as it offers individual pest control methods, and complimentary crop and region-specific crop protection. 51% of food in India is contaminated with residues out of which 20% were above MRL (Maximum residue limit). Developing nations cannot discard contaminated food due to their financial situation and ignore the safety of consumers. Integrated pest management (IPM) in rice is extremely useful in controlling and preventing long term effects of pests. Pest management techniques should be taken into account before the application of pesticides to crops, such techniques help in keeping the pest population below the economically adverse levels. Many cultural, mechanical, biological and chemical control measures help to minimize the yield losses with a lesser impact on the environment. Pests happen to occur in rice fields twice a week and if pests reach or exceed economic threshold level (ETL) then farmers should apply pesticides. Economic threshold level means when pest population has increased and could be economically detrimental (Sharma *et al.*, 2015).

3. Materials and Methods

Site Selection the site selected for the study of pesticide residues in rice crops is a village in Sheikhupura named Kot Hussain. The agricultural lands of this village are used for the growth of rice, wheat and potatoes. 386 safaid, super fine stem, 386 laal, C-9, 1121 Kainat and super karnal rice are cultivated by the end of the year.

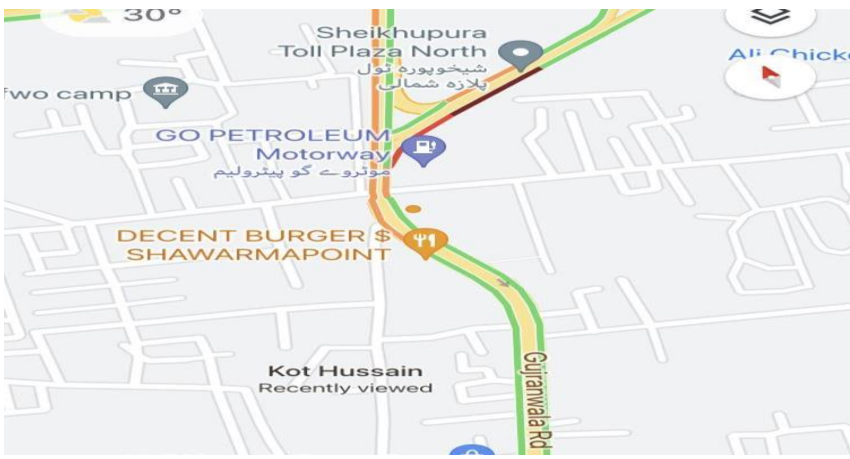


Figure 1: Location of Kot Hussain on map

Data Collection Data was collected from the agricultural lands of village Kot Hussain, Sheikhupura through interview-based surveys to gather information regarding varieties of rice grown, irrigation of crops through groundwater, type and number of pesticides applied, and reason for the application of different pesticides on rice crops. On-field, samples and information regarding the overall usage of pesticides in the fields were gathered. The interviewees were the farmers of the respective rice crops as they were experts in their field. The interview took place before and during the harvest season of rice crops. Sampling A total of 18 rice samples were collected for this study to inspect the insecticide and herbicide residues in the crops. Rice was collected from the fields during the harvesting season of November-December. Extracted rice was stored in polythene sampling bags after proper labeling. Next, the labeled plastic bags were stored in an ice bag to be transported to the lab. Lab analysis Rice samples were collected from the fields of Kot Hussain and the samples were analyzed in the environmental laboratory to determine pesticide residue. Sample preparation to remove moisture from the rice samples firstly all rice grains were sundried for 5-6 days and then crushed to make a fine powder so that the digestion process can begin. During the digestion process, 10g of rice sample, 5g of NaCl, 20 ml of dichloromethane, and 20 ml of ethyl acetate were added into a beaker. Next, a magnet was added into the beaker and then stirred for accurately 20 minutes on a magnetic stirrer. After the stirring, the solution was allowed to stand for 5 minutes and then filtered with the help of filter paper. 20 ml of dichloromethane and 20 ml of ethyl acetate were added to the filtered solution then it was poured into a separating funnel. In the separating funnel two layers were formed; one of aqueous content and one of organic content. We separated the organic layer from the aqueous layer and for evaporation; we moved the organic layer into the rotary evaporator. Before adding the organic layer into the rotary flask, the rotary evaporator was preheated for 20 minutes. After complete evaporation of the organic layer, 10 ml of methanol was added into the flask. Next, the solution was stored in a vial for HPLC analysis. Standard solution preparation for the preparation of standard solution 20 ml of methanol was added into10 ml of pesticide. The solution was stirred for about twenty minutes and after that it was filtered. After filtration the solution was stored in a vial. Pesticide detection analysis High performance liquid chromatography method was used to detect the pesticides in the different rice samples e.g., 1121 Kainat, super karnal, C-9 etc.

Table 1: Chemical formulas for different pesticides Analysis

Sr.no	Pesticide name	Chemical formula
1	Imidacloprid	C9H10CIN5O2
2	Emectin	C49H75NO13
3	Timer	C10H11ClN4
4	Glyphosate	C3H8NO5P
5	Bifenthrin	C23H22ClF3O2
6	Lufenuron	C17H8Cl2F8N2O3

Table 1: Chemical formulas for different pesticides Analysis We analyzed the sample in the Agilent 1260 high-performance liquid chromatography (HPLC) which has a UV/ visible detector. HPLC

equipment consisted of pumps, samplers, detectors, and degasser. First standards of Lufenuron, Imidacloprid, Glyphosate, Bifenthrin, Timer, and Emectin were run in the HPLC after that the samples were run to determine pesticide residue. During HPLC analysis of the mixture, the components were separated based on their retention times and a chromatogram was produced. Quantitative Analysis Quantitative analysis is defined as measurement of the quantities of particular constituents in a solution. Pesticide concentrations in different samples are determined by observing peaks of standards and samples. Calculation of Response Factor: RF= response factor = (peak area)/(standard amount) Peak area = peak area of the standard Standard amount = standard amount used in the solvent. Calculation of concentration of pesticide in sample: Concentration of pesticide =(peak area of sample)/(RF of standard) 3.9- Apparatus Beakers, Rotary evaporator, weighing balance, Tripod stand, Filter paper, Spatula Funnel, separating funnel, Measuring cylinder, Conical flask, Magnets Magnetic Stirrer Vials High-performance liquid chromatography (HPLC) Ethyl acetate Methanol NaCl Dichloromethane.

4. Results

High-performance liquid chromatography (HPLC) was used to analyze the amount of pesticide residue in rice grains. All the experiments were conducted in the environmental science laboratory of Kinnaird College for Women to collect the data. The samples were tested against the following pesticide standards i.e., Imidacloprid, Glyphosate, Bifenthrin, Timer and Lufenuron. 4.1-Bifenthrin: The chromatogram of the bifenthrin standard was produced. In the spectrum it was shown that the highest peak was with the retention time of 3.678 min and thus it was the strongest peak.

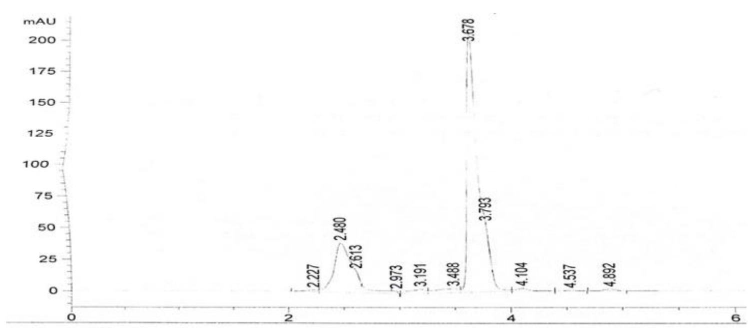


Figure 2: Chromatogram of Bifenthrin

Table 2: Peak areas of Bifenthrin (standard)

Peak	Ret Time (minutes)	Type	Width (minutes)	Area (mAU*s)	Height (mAU)	Area %
1	3.191	BV	0.1208	30.44791	3.26521	1.4525
2	3.488	VV	0.1930	44.95836	3.06813	2.1447
3	3.678	VV	0.1042	1476.19849	209.55391	70.4211
4	3.793	VV b	0.0436	16.28714	6.42955	0.7770

5	4.104	VB	0.1194	19.07904	2.28468	0.9102
6	4.537	BB	0.1338	2.27909	2.989729e-1	0.1087
7	4.892	BB	0.1031	8.59070	1.30076	0.4098
8	9.495	BB	0.1875	14.54407	1.18229	0.6938
Total				2096.24311	269.67305	

Timer A chromatogram of Timer standard was produced. In this spectrum the highest peak was with the retention time of 3.673 min and thus it was the strongest peak in respect of height.

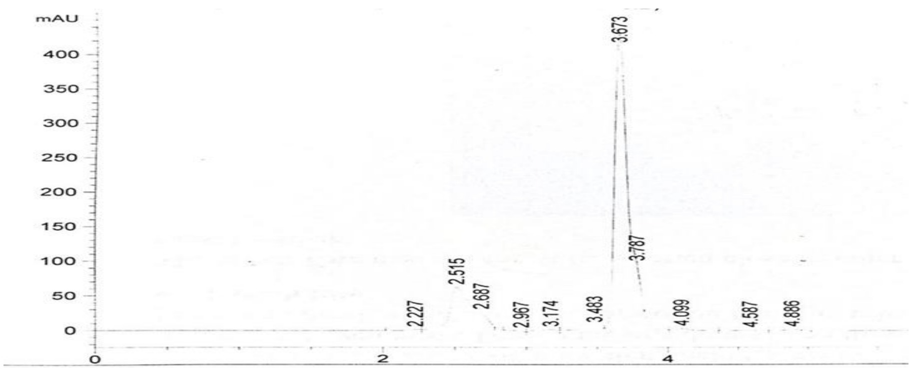


Figure 3: Chromatograph of Timer

Table 3: Peak areas of Timer (standard)

Peak	Ret Time (minutes)	Type	Width (minutes)	Area (mAU*s)	Height (mAU)	Area %
1	3.174	BV	0.1194	30.66599	3.33236	0.7532
2	3.483	VV	0.14113	88.72247	8.82299	2.1792
3	3.673	VV	0.1038	3006.34033	439.45541	73.8413
4	3.787	VV b	0.0392	20.18661	8.67341	0.4598
5	4.099	VB	0.1151	24.54228	3.07457	0.6028
6	4.587	BV	0.1560	4.60630	4.79983e-1	0.1131
7	4.886	VB	0.1008	10.64548	1.611771	0.2615
8	9.418	BB	0.1877	61.16430	5.10691	1.5023
Total				4071.35345	540.99470	

Lufenuron In the following figure 4.3 chromatogram of Lufenuron standard was shown. In this spectrum the highest peak was with the retention time of 3.487 min and thus it was the strongest peak in respect of height. Figure 4 Chromatogram of Lufenuron.

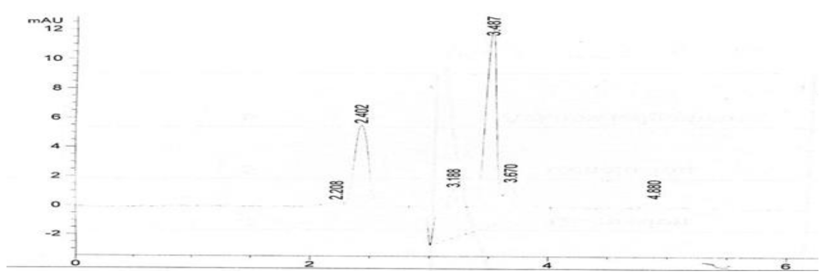


Figure 4: Chromatogram of Lufenuron

Table 4: Peak areas of Lufenuron (standard)

Peak	Ret Time (minutes)	Type	Width (minutes)	Area (mAU*s)	Height (mAU)	Area %
1	3.670	VB	0.1377	24.15188	2.43623	7.4470
2	4.880	BB	0.1022	3.10087	4.87583e-1	0.9561
3	9.366	BB	0.1854	109.21257	9.14041	33.6745
Total				324.31846	34.82634	

Glyphosate in the following figure 4.4 chromatogram of Glyphosate standard was shown. In this spectrum, the highest peak was with the retention time of 3.679 min and thus it was the strongest peak in respect of height.

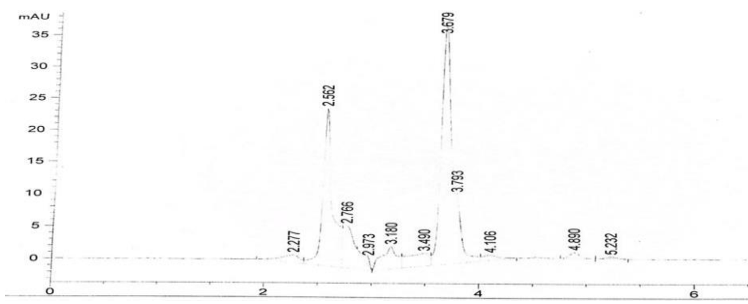


Figure 5: Chromatogram of Glyphosate

Table 5: Peak areas of Glyphosate (standard)

Peak	Ret Time (minutes)	Type	Width (minutes)	Area (mAU*s)	Height (mAU)	Area %
1	3.180	BV	0.1264	32.92029	3.41840	5.5704
2	3.490	VV	0.2005	29.41480	1.94427	4.9773
3	3.679	VV	0.1088	278.02219	37.35426	47.0440
4	3.793	VV b	0.0445	4.05083	1.54996	0.6854
5	4.106	VB	0.1438	8.36848	8.01421e-1	1.4160
6	4.890	BB	0.1027	5.70951	8.91398e-1	0.9661
7	5.232	BB	0.1131	2.11811	2.83795e-1	0.3584
8	9.479	BB	0.1659	3.36676	2.80713e-1	0.5697
Total				590.98361	79.73885	

In the following figure, chromatograms of Imidacloprid standard were shown. In this spectrum, the highest peak was with a retention time of 3.670 min and thus it was the strongest peak in respect of height.

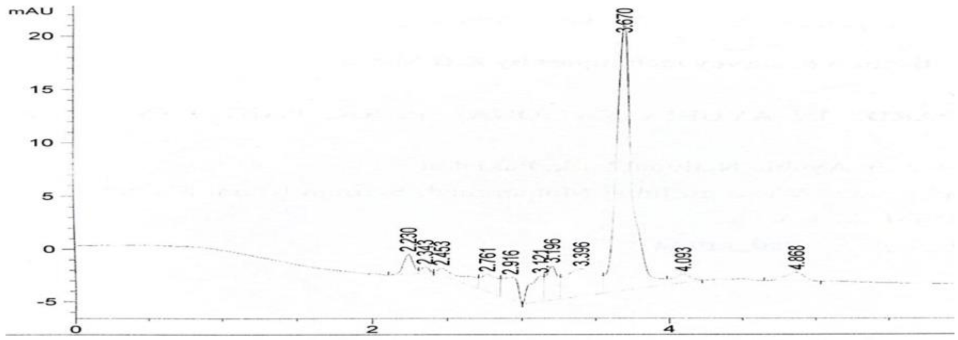


Figure 6: Chromatogram of Imidacloprid

Table 6: Peak areas of Imidacloprid (standard)

Peak	Ret Time (minutes)	Type	Width (minutes)	Area (mAU*s)	Height (mAU)	Area %
1	3.196	VV	0.0739	17.37619	3.27613	5.3587
2	3.396	VV	0.2084	39.11514	2.58763	12.0628
3	3.670	VV	0.1081	188.61952	25.55705	58.1690
4	4.093	VB	0.1216	6.87139	8.05006e-1	2..1191
5	4.868	BB	0.1311	7.43780	8.25859e-1	2.2938
Total				324.26124	42.30031	

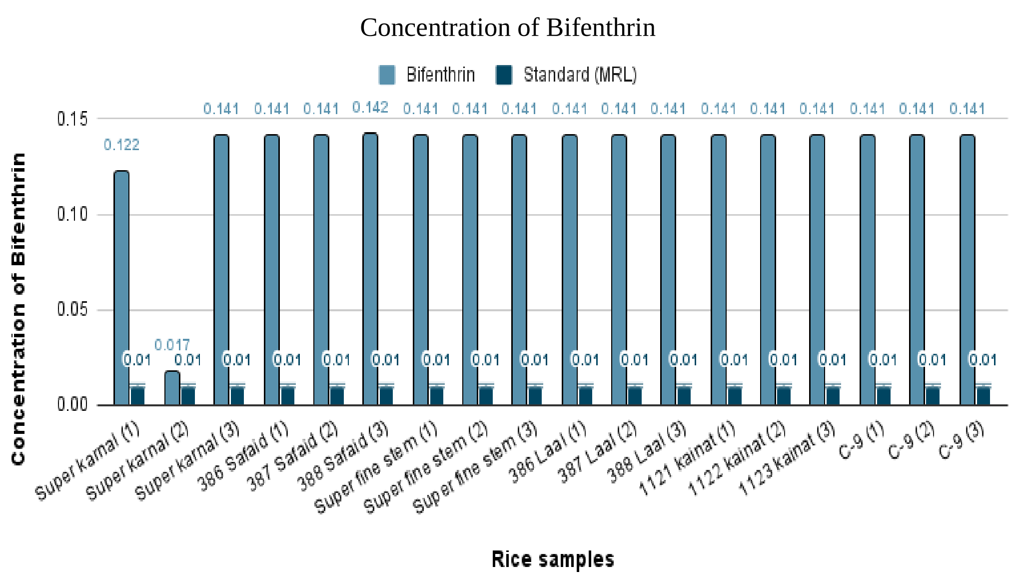


Figure 7: Concentration of Bifenthrin in rice samples

Figure 7 and table 7 showed the concentration of bifenthrin in different rice samples. The above figure showed that bifenthrin was present in the rice samples in high concentrations i.e., 0.122ppm, 0.017 ppm, 0.141ppm compared to the MRL of 0.01ppm.

Table 7: Concentration of Bifenthrin in different rice samples

Sr.no	Rice Samples	Bifenthrin Concentration (ppm)
1	Super karnal (1)	0.122
2	Super karnal (2)	0.017
3	Super karnal (3)	0.141
4	386 Safaid (1)	0.141
5	387 Safaid (2)	0.141
6	388 Safaid (3)	0.142
7	Super fine stem (1)	0.141
8	Super fine stem (2)	0.141
9	Super fine stem (3)	0.141
10	386 Laal (1)	0.141
11	387 Laal (2)	0.141
12	388 Laal (3)	0.141
13	1121 kainat (1)	0.141
14	1122 kainat (2)	0.141
15	1123 kainat (3)	0.141
16	C-9 (1)	0.141
17	C-9 (2)	0.141
18	C-9 (3)	0.141

Concentration of Timer

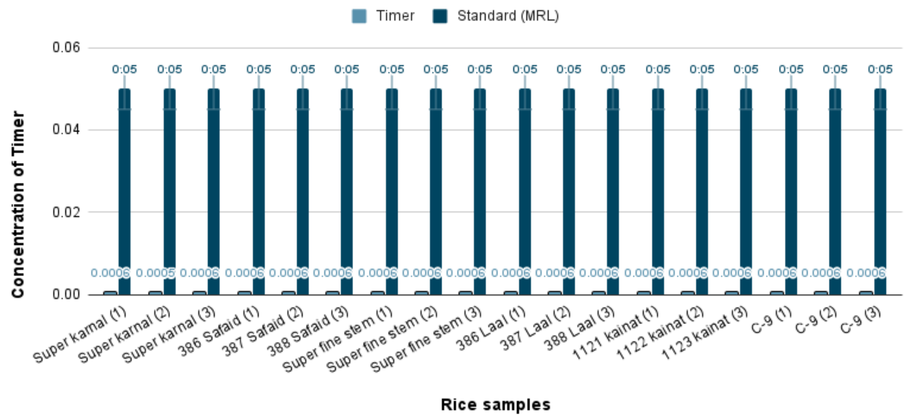


Figure 8: Concentration of Timer in rice samples Figure

The above figure showed that the timer was present in the rice samples in almost unidentifiable and extremely low concentrations and below the standard limit of 0.05ppm i.e., 0.0006ppm and 0.0005 ppm.

Table 8: Concentration of Timer in different rice samples

Sr.no	Rice Samples	Timer Concentration (ppm)
1	Super karnal (1)	0.0006
2	Super karnal (2)	0.0005
3	Super karnal (3)	0.0006
4	386 Safaid (1)	0.0006
5	387 Safaid (2)	0.0006
6	388 Safaid (3)	0.0006
7	Super fine stem (1)	0.0006
8	Super fine stem (2)	0.0006
9	Super fine stem (3)	0.0006
10	386 Laal (1)	0.0006
11	387 Laal (2)	0.0006
12	388 Laal (3)	0.0006
13	1121 kainat (1)	0.0006
14	1122 kainat (2)	0.0006

15	1123 kainat (3)	0.0006
16	C-9 (1)	0.0006
17	C-9 (2)	0.0006
18	C-9 (3)	0.0006

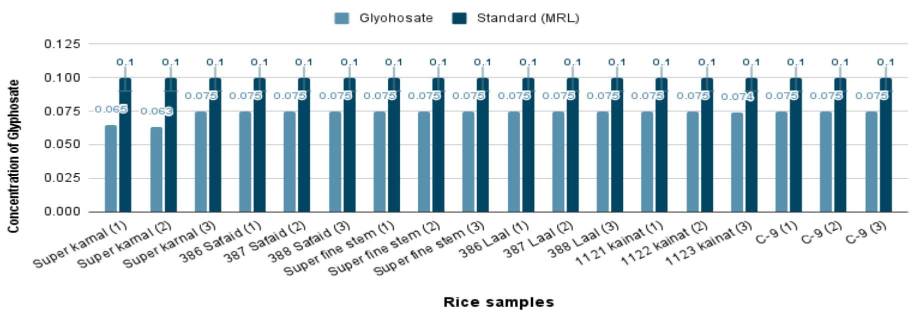


Figure 9: Concentration of Glyphosate in rice samples

Figure 9 and table 9 showed the concentration of glyphosate in different rice samples. Above figure showed glyphosate was present in the rice samples in low concentrations i.e., 0.065 ppm, 0.063, 0.075 ppm compared to the MRL value of 0.1 pp.

Table 9: concentration of Glyphosate in different rice samples

Sr.no	Rice Samples	Glyphosate Concentration (ppm)
1	Super karnal (1)	0.065
2	Super karnal (2)	0.063
3	Super karnal (3)	0.075
4	386 Safaid (1)	0.075
5	387 Safaid (2)	0.075
6	388 Safaid (3)	0.075
7	Super fine stem (1)	0.075
8	Super fine stem (2)	0.075
9	Super fine stem (3)	0.075
10	386 Laal (1)	0.075

11	387 Laal (2)	0.075
12	388 Laal (3)	0.075
13	1121 kainat (1)	0.075
14	1122 kainat (2)	0.075
15	1123 kainat (3)	0.074
16	C-9 (1)	0.075
17	C-9 (2)	0.075
18	C-9 (3)	0.075

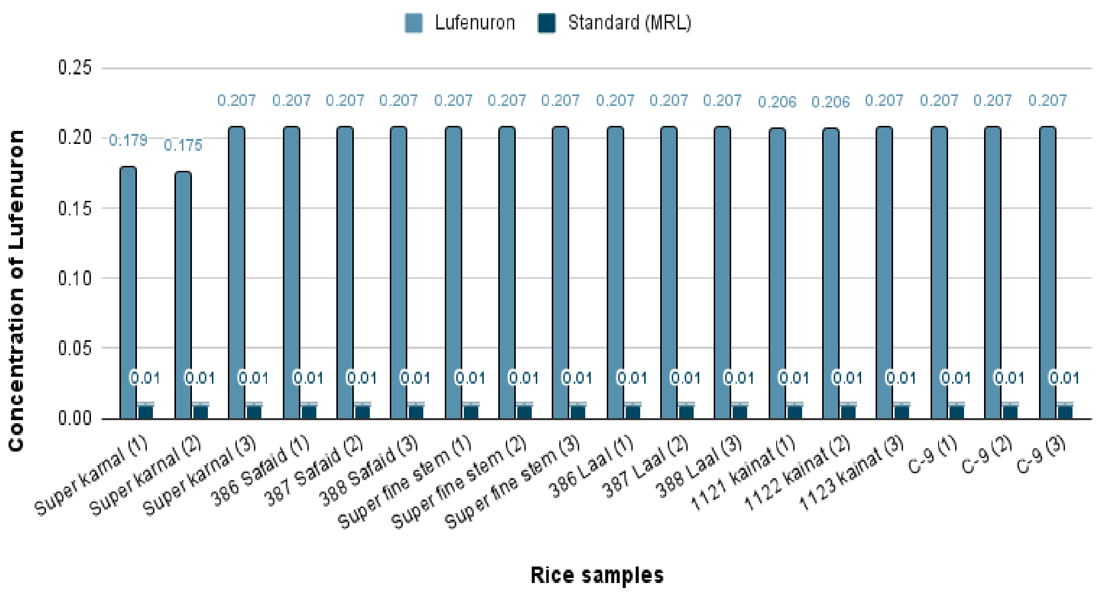


Figure 10: Concentration of Lufenuron in rice samples

Concentration of Lufenuron in rice samples Figure and Table showed the concentration of Lufenuron in different rice samples. The above figure showed that Lufenuron was present in the rice samples in higher concentrations i.e., 0.179 ppm, 0.175 ppm, 0.207 ppm compared to the MRL value of 0.01ppm.

Table 10: Concentration of Lufenuron in different rice samples

Sr.no	Rice Samples	Lufenuron (ppm)	Concentration
1	Super karnal (1)	0.179	
2	Super karnal (2)	0.175	
3	Super karnal (3)	0.207	
4	386 Safaid (1)	0.207	
5	387 Safaid (2)	0.207	

6	388 Safaid (3)	0.207
7	Super fine stem (1)	0.207
8	Super fine stem (2)	0.207
9	Super fine stem (3)	0.207
10	386 Laal (1)	0.207
11	387 Laal (2)	0.207
12	388 Laal (3)	0.207
13	1121 kainat (1)	0.206
14	1122 kainat (2)	0.206
15	1123 kainat (3)	0.207
16	C-9 (1)	0.207
17	C-9 (2)	0.207
18	C-9 (3)	0.207

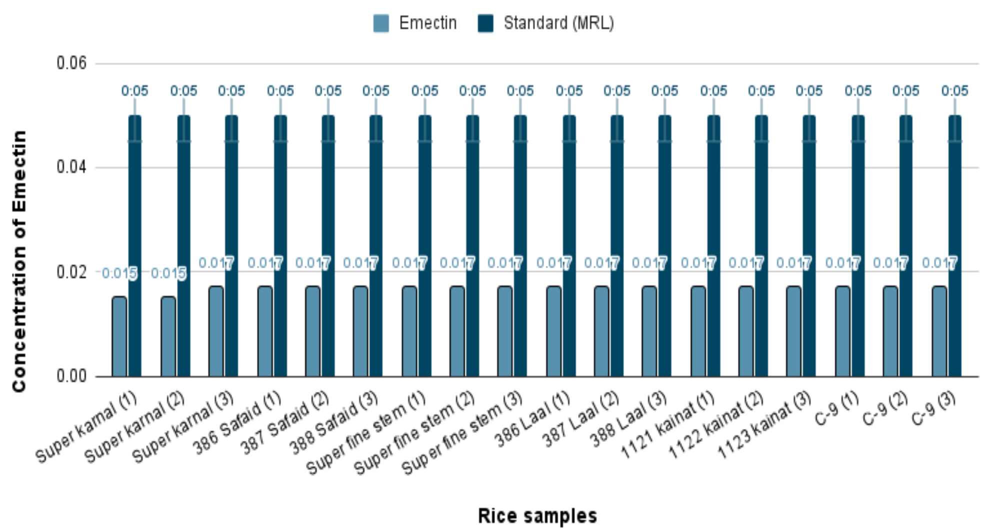


Figure 11: Concentration of Emectin in rice samples

Figure 10 and table 10 showed the concentration of Emectin in different rice samples. Above figure showed that Emectin was present in the rice samples in lower concentrations i.e., 0.015 ppm, 0.017 ppm compared to the MRL value of 0.05 ppm.

Table 11: Concentration of Emectin in different rice samples

Sr.no	Rice Samples	Emectin Concentration (ppm)
1	Super karnal (1)	0.015
2	Super karnal (2)	0.015
3	Super karnal (3)	0.017
4	386 Safaid (1)	0.017
5	387 Safaid (2)	0.017
6	388 Safaid (3)	0.017

7	Super fine stem (1)	0.017
8	Super fine stem (2)	0.017
9	Super fine stem (3)	0.017
10	386 Laal (1)	0.017
11	387 Laal (2)	0.017
12	388 Laal (3)	0.017
13	1121 kainat (1)	0.017
14	1122 kainat (2)	0.017
15	1123 kainat (3)	0.017
16	C-9 (1)	0.017
17	C-9 (2)	0.017
18	C-9 (3)	0.017

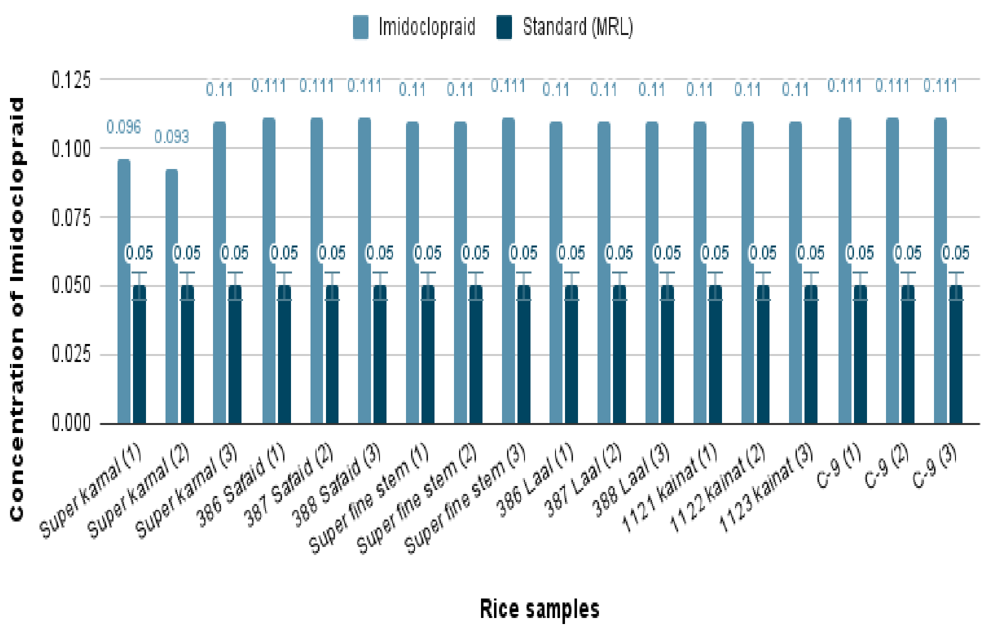


Figure 12: Concentration of Imidacloprid

Figure Concentration of Imidacloprid in rice samples Figure 12 and table 12 showed the concentration of Imidacloprid in different rice samples. The above figure showed that Imidacloprid was present in the rice samples in higher concentrations i.e., 0.096 ppm, 0.093 ppm, 0.11 ppm, 0.111 ppm compared to the MRL value of 0.05 ppm.

Table 12: Concentration of Imidacloprid in different rice samples

Sr.no	Rice Samples	Imidacloprid Concentration (ppm)
1	Super karnal (1)	0.096
2	Super karnal (2)	0.093
3	Super karnal (3)	0.11
4	386 Safaid (1)	0.111
5	387 Safaid (2)	0.111
6	388 Safaid (3)	0.111
7	Super fine stem (1)	0.11
8	Super fine stem (2)	0.11
9	Super fine stem (3)	0.111
10	386 Laal (1)	0.11
11	387 Laal (2)	0.11
12	388 Laal (3)	0.11
13	1121 kainat (1)	0.11
14	1122 kainat (2)	0.11
15	1123 kainat (3)	0.11
16	C-9 (1)	0.111
17	C-9 (2)	0.111
18	C-9 (3)	0.111

Comparative graph Following graph shows that lufenuron, bifenthrin and Imidacloprid exceeds the MRL in samples of rice while timer, emectin and glyphosate are below the MRL in all the rice samples of super karnal, 386 safaid, super fine, 386 Laal, 1121 kainat and C-9.

Table 13: Presence of pesticides in given samples

Sr.no	Rice samples	Lufenuron	Glyphosate	Timer	Emectin	Bifenthrin	Imidacloprid
1	Super karnal (1)	✓	×	×	×	✓	✓
2	Super karnal (2)	✓	×	×	×	✓	✓
3	Super karnal (3)	✓	×	×	×	✓	✓
4	386 Safaid (1)	✓	×	×	×	✓	✓
5	387 Safaid (2)	✓	×	×	×	✓	✓
6	388 Safaid (3)	✓	×	×	×	✓	✓
7	Super fine stem (1)	✓	×	×	×	✓	✓
8	Super fine stem (2)	✓	×	×	×	✓	✓
9	Super fine stem (3)	✓	×	×	×	✓	✓
10	386 Laal (1)	✓	×	×	×	✓	✓
11	387 Laal (2)	✓	×	×	×	✓	✓
12	388 Laal (3)	✓	×	×	×	✓	✓
13	1121 kainat (1)	✓	×	×	×	✓	✓
14	1122 kainat (2)	✓	×	×	×	✓	✓
15	1123 kainat (3)	✓	×	×	×	✓	✓
16	C-9 (1)	✓	×	×	×	✓	✓
17	C-9 (2)	✓	×	×	×	✓	✓
18	C-9 (3)	✓	×	×	×	✓	✓

5. Discussion

The research was focused on evaluating the pesticide concentration in the rice grains of Kot Hussain village near Sheikhupura which is an agricultural land. Many cereal crops are grown in this particular area and the pesticides are regularly used in these fields to protect against diseases and increase productivity. All rice samples were tested to identify which pesticide was present above the maximum residue limit. Quantitative and qualitative analysis were carried out in this study to examine 18 rice samples against 6 pesticides of imidacloprid, Lufenuron, timer, emectin, Glyphosate and Bifenthrin. Pesticide residue in food items is a major concern all over the world. Pesticides are harmful chemicals which lead to health disorders if consumed in higher concentrations. As pesticides are vital for the growth of crops its sudden elimination from agriculture is impossible but certain measures should be taken to prevent the pesticide residue in rice. Rice samples were taken from the village of Kot Hussain near Sheikhupura. Kot Hussain has a wide variety of agricultural practices and rice is their most popular food staple. After sample collection, they were analyzed in the laboratory and 3 pesticides were found in higher concentration than the maximum residue limit in all 18 rice samples. Pesticide residue in rice samples were verified with help of HPLC and comparison with the maximum residue limit. Bifenthrin, lufenuron and imidacloprid were detected above the MRL while timer, emectin and glyphosate were below the detectable limit in all the rice samples. Figure 4.6 and table 4.6 showed the concentration of bifenthrin in different rice samples. The figure 4.6 showed that bifenthrin was present in the rice samples in high concentrations i.e., 0.122ppm, 0.017 ppm, 0.141ppm compared to the MRL of 0.01ppm. Bifenthrin is an insecticide used in many forms such as granules and sprays in the agricultural fields of rice. Higher residue in rice grains suggest excessive use of bifenthrin. Due to untimed and unprofessional sprinkling of bifenthrin it gets into the food chain and can cause probable health issues in humans. On the other hand, figure 4.7 showed that the timer was present in the rice samples in very low concentrations and below the MRL of 0.05ppm i.e., 0.0006ppm and 0.0005 ppm. The concentration of glyphosate in different rice samples was shown in figure 4.8. The figure showed glyphosate was present in the rice samples in lower concentrations i.e., 0.065 ppm, 0.063, 0.075 ppm compared to the MRL value of 0.1 pp. Figure 4.10 and table 4.10 showed the concentration of Emectin in different rice samples. Figure 4.10 showed that Emectin was present in the rice samples in lower concentrations i.e., 0.015 ppm, 0.017 ppm compared to the MRL value of 0.05 ppm. Glyphosate, emectin and timer are herbicides and are used for plant growth and to kill broadleaf plants, these pesticides were found in concentrations below the MRL. Which indicates that glyphosate, emectin and timer were used in lesser concentration compared to other pesticides and thus lower pesticide residue in rice grains. The figure 4.9 and table 4.9 showed that Lufenuron was present in the rice samples in higher concentrations i.e., 0.179 ppm, 0.175 ppm, 0.207 ppm compared to the MRL value of 0.01ppm. Lufenuron is used to control the insects and other animals in agricultural crops. Due to unnecessary and excessive use of lufenuron on the rice crops the pesticide residue increased in all 18 samples. A study was conducted to determine residual contents of pesticides in different regions of Punjab, Pakistan and results indicated the presence of L-Cyhalothrin, Malathion, Monocrotophos and Cartap. Mean levels of all pesticides were below the lethal dose and thus no risk of acute poisoning however in the long run pesticide exposure could be harmful to health [33]. Imidacloprid is an insecticide used vastly in crops to control the infestation of insects. Figure 4.11 showed that Imidacloprid was present in the rice samples in higher concentrations i.e., 0.096 ppm, 0.093 ppm, 0.11 ppm, 0.111 ppm compared to the MRL value of 0.05 ppm. Due to its persistent nature, Imidacloprid was detected in higher levels in rice grains. As the concentration of imidacloprid exceeds the MRL in rice grains there is probability of health effects. A similar study was carried out in China to study the residue behavior and dietary risk assessment of 6 pesticides namely lufenuron, imidacloprid, dimethomorph, methoxyfenozide, pyridaben and spinetoram with help of QuEChERS method and UPLC-MS/MS. The analysis revealed that many factors such as rainfall, sunlight and other environmental factors influence the degradation rate of pesticides [34]. The higher concentration of pesticide residues of imidacloprid, bifenthrin and lufenuron suggest non-professional use of pesticides. In order to reduce the use of pesticides farmers should use organic methods such as crop rotation, use of organic manure and intercropping. To protect the environment and human health it is important to take proper steps regarding pesticide practice. Pesticides should be only applied when absolutely necessary instead of routine application.

References

Adah CA, Asemave K, Ado O. Determination of some herbicides in foreign and local rice brands obtained from Makurdi, Benue, Nigeria. *Thematics Journal of Chemistry. 2017;(1)*

Andreazza AC, Scola G, editors. Toxicology studies cells, drugs and environment. InTech; 2015.

Arora S, Mukherji I, Kumar A, Tanwar RK. Pesticide residue analysis of soil, water, and grain of IPM basmati rice. *Environ Monit Assess* [Internet]. 2014;186(12):8765–72. Availablefrom:http://dx.doi.org/10.1007/s10661-014-4042-9

Asiah N, David W, Ardiansyah, Madonna S. Review on pesticide residue on rice. *IOP Conf Ser Earth Environ Sci* [Internet]. 2019;379(1):012008. Available from: http://dx.doi.org/10.1088/1755-1315/379/1/012008

Basal, W. T., Ahmed, A. R. T., Mahmoud, A. A., & Omar, A. R. (2020). Lufenuron induces reproductive toxicity and genotoxic effects in pregnant albino rats and their fetuses. Scientific Reports, 10(1), 1-24.

Bifenthrin general fact sheet, NPIChttp://npic.orst.edu/factsheets/bifgen.html#body[Retrieved on September 8, 2021]Glyphosate general fact sheet, NPIChttp://npic.orst.edu/factsheets/glyphogen.html#whatis[Retrieved on September 8, 2021]

Chaiyarat, R., Sookjam, C., Eiam-Ampai, K. *et al*. Organochlorine pesticide levels in the food web in rice paddies of BuengBoraphet wetland, Thailand. *Environ Monit Assess* 187, 230 (2015).

Gupta PK. Pesticide exposure--Indian scene. *Toxicology*. 2004 May 20;198(1-3):83-90. Doi: 10.1016/j.tox.2004.01.021. PMID: 15138033.

Herbicides and Your Health, WebMDhttps://www.webmd.com/cancer/herbicide-glyphosate-cancer[Retrieved on September 8, 2021]

High residue levels in rice may hurt exports, DAWNNEWShttps://www.dawn.com/news/1633830/high-residue-levels-in-rice-may-hurt-exports[Retrieved on September 8, 2021]

Importance & Benefits of Pesticides, PESTICIDE FACTShttps://pesticidefacts.org/topics/necessity-of-pesticides/[Retrieved on September 8, 2021]Imidaclopridgeneralfactsheet,NPIhttp://npic.orst.edu/factsheets/imidagen.html#whatis[Retrieved on September 8, 2021]

Imran A, Hussain T, Nadeem A. Chromatographic determination of residual contents of pesticides in rice samples from different geographical regions of Punjab. *FUUAST Journal of Biology*. 2016;6(2):155–60.

Jabbar, Abdul, and Seeme Mallick. Pesticides and environment situation in Pakistan. Sustainable Development Policy Institute. 1994.

Lozowicka, B., Kaczynski, P., Paritova, A. E., Kuzembekova, G. B., Abzhalieva, A. B., Sarsembayeva, N. B., & Alihan , K. (2014). Pesticide residues in grain from Kazakhstan and potential health risks associated with exposure to detected pesticides. *Food and Chemical Toxicology*, *64*, 238-248.

Mahmood A, Malik RN, Li J, Zhang G. Human health risk assessment and dietary intake of organochlorine pesticides through air, soil and food crops (wheat and rice) along two tributaries of river Chenab, Pakistan. *FoodChemToxicol*[Internet].2014;71:1725.Availablefrom:http://dx.doi.org/10.1016/j.fct.2014.05.008

Mahmood, I., Imadi, S. R., Shazadi, K., Gul, A., & Hakeem, K. R. (2016). Effects of pesticides on the environment. In Plant, soil and microbes (pp. 253-269). *Springer, Cham*.

Munshi AB, Siddiqi HA, Ansari FA, Begum R, Usmani TH. Multiresidue analysis of pesticides by gas chromatography with electron-capture in rice samples from different geographical regions of Pakistan. Pakistan *Journal of Nutrition.* 2011;10(9):851-9.

Munshi AB, Siddiqi HA, Ansari FA, Begum R, Usmani TH. Multiresidue analysis of pesticides by gas chromatography with electron-capture in rice samples from different geographical regions of Pakistan. Pakistan *Journal of Nutrition*. 2011;10(9):851-9.

Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P, Hens L. Chemical pesticides and human health: The urgent need for a new concept in agriculture. *Front Public Health* [Internet].2016;4:148.Availablefrom:http://dx.doi.org/10.3389/fpubh.2016.00148 NSW EPA.*WhatArePesticidesandHowDoTheyWork?*(2013).http://www.epa.nsw.gov.au/pesticides/pestwhatrhow.htmPesticides,BYJU'Shttps://byjus.com/chemistry/pesticides/ [Retrieved on September 8. 2021]

Pingali PL, Roger PA. Impact of pesticides on farmer health and the rice environment. Tucson, AZ: Kluwer Academic; 1995.

Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GPS, Handa N, *et al*. Worldwide pesticide usage and its impacts on ecosystem. *SN Appl Sci* [Internet]. 2019;1(11). Available from: http://dx.doi.org/10.1007/s42452-019-1485-1

Sharma R, Peshin R, Shankar U, Kaul V, Sharma S. Impact evaluation indicators of an Integrated Pest Management program in vegetable crops in the subtropical region of Jammu and Kashmir, *India. Crop Prot* [Internet]. 2015; 67:191–9. Available from: http://dx.doi.org/10.1016/j.cropro.2014.10.014

Singh VK, Singh R, Lichtfouse E, editors. Sustainable Agriculture Reviews 50: Emerging Contaminants in Agriculture. Springer Nature; 2021 Mar 12.

Sreeramulu K. Evaluation of pesticide residue in rice, wheat and pulses of Bidar district Karnataka. India; 2015.

Tang H, Ma L, Huang J, Li Y, Liu Z, Meng D, *et al*.Residue behavior and dietary risk assessment of six pesticides in pak choi using QuEChERS method coupled with UPLC-MS/MS. Ecotoxicology and Environmental Safety, 213, 112022.

Teló GM, Marchesan E, Zanella R, Prestes OD. <b>Fungicide and insecticide residues in rice grains. *Acta Scientiarum Agronomy*. 2017;39(1):9–15.

Teng P, Heong K, Moody K. Rice pest science and management. Los Baños. 1994;256–7.

Tzanetou EN, Karasali H. A Comprehensive Review of Organochlorine Pesticide Monitoring in Agricultural Soils: The Silent Threat of a Conventional Agricultural Past. *Agriculture*. 2022 May;12(5):728.

Upadhayay J, Rana M, Juyal V, Bisht SS, Joshi R. Impact of pesticide exposure and associated health effects. Pesticides in crop production: physiological and biochemical action. 2020 Apr 6:69-88.

Zhang J-M, Chai W-G, Wu Y-L. Residues of chlorantraniliprole in rice field ecosystem. *Chemosphere* [Internet]. 2012;87(2):132–6. Availablefrom:http://dx.doi.org/10.1016/j.chemosphere.2011.11.076

Gupta PK. Pesticide exposure--Indian scene. *Toxicology*. 2004 May 20;198(1-3):83-90. Doi: 10.1016/j.tox.2004.01.021. PMID: 15138033.

Sharma R, Peshin R, Shankar U, Kaul V, Sharma S. Impact evaluation indicators of an Integrated Pest Management program in vegetable crops in the subtropical region of Jammu and Kashmir, *India*. *Crop Prot* [Internet]. 2015; 67:191–9. Available from:http://dx.doi.org/10.1016/j.cropro.2014.10.014

Imran A, Hussain T, Nadeem A. Chromatographic determination of residual contents of pesticides in rice samples from different geographical regions of Punjab. *FUUAST Journal of Biology*. 2016;6(2):155–60.

Tang H, Ma L, Huang J, Li Y, Liu Z, Meng D, *et al*.Residue behavior and dietary risk assessment of six pesticides in pak choi using QuEChERS method coupled with UPLC-MS/MS. Ecotoxicology and Environmental Safety, 213, 112022.