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Risk Alleviation Of Pesticides In Agriculture: From History To Analytical Techniques.

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ABSTRACT

Population explosion leads to the exploitation of agricultural field to meet the supply of food products (mainly vegetables and fruits). Before first century onwards the usage of pesticide was started to promote the growth of food products and in the present, biotechnological strategies are adopted for pest control along with synthetic pesticides. The demand for maximization of productivity is achieved by the application of synthetic pesticides. Apart from its benefits, its misuse predominates and generates serious consequences. The categorization of pesticides can be done according to their source, mode of action, nature of pest and also on their chemical nature. Excessive and inappropriate usage of pesticides undoubtfully leads to poisoning and various instances of pesticide poisoning are being reported every year. These incidences of pesticide poisoning tragedy include Endosulphan poisoning, Tragedy of Tauccamarca, Seveso disaster etc. These pesticides create serious health hazards ranging from short term effects like vomiting to long term effects including cancer and neurological problems. Analytical laboratories contribute for reducing its intensity to humans and to environment through preliminary screening techniques and comprehensive screening procedures like HPLC, LCMS, GC and GCMS. These are efficient and powerful methods of pesticide determination in vegetables and fruits. The scenario of pesticide usage is complex and hence, care has to be taken by the government and public to lower its usage and to promote environmental stability and social health.

Keywords: Pesticides, Chromatography, vegetables, fruits, poisoning

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INTRODUCTION

The human population is growing continuously and it has been observed that there is a tremendous increase in population for the last 50 years. Increase in population demands more agricultural productivity [1] and for the quantum yield of crops, infestation of the pest on fruits and vegetables has been decreased with the help of pesticides. To limit the loss of crops produced due to the attack of pests there is a need to widen the plant protection umbrella and propagate the judicial use of crops. The judicious use of pesticides can protect the crops and increases their yielding capacity [2]. Pesticides are biocides which are used to kill pests. They may be of natural or artificial in origin. They can be insecticides, rodenticides, herbicides, fungicides etc [3]. Pesticides have innumerable benefits. These include crop protection, improvement of food quality and also the prevention of vector-borne diseases [4]**.** But these are misused nowadays largely. Misuse of pesticide includes the selling of banned pesticide, over usage of pesticides and also its improper usage without following the guidelines. Illiterate distribution patterns of pesticide in agricultural practices lead to its persistence in the agricultural fields leading to environmental pollution. These can also create serious consequences on food samples (vegetables and fruits) finally reaching man through food [5]. Due to its widespread usage in agriculture for more food products, it can be indirectly considered as "Poison in Plates". According to the data extracted in 2014, the statistics of pesticide sale reveal the use of 43.8% of fungicides and bactericides, 33.2% of herbicides and haulm destructors, 14.2% of other plant protection products, 5.2% of insecticides and ascaricides, 3.2% of plant growth regulators and 0.4% of molluscicides.

The origin of the use of pesticides and its advancement till the present scenario is given in table below [6]

Table.1. Origin of pesticides and its advancement

Time period	Pesticide used
Ancient times	Ashes, common salts, and bitters
1st century	Arsenic, suggestion of soda and olive oil for treatment of legumes (Pliny the Elder, a Roman naturalist-Historia naturalis)
16th century	Arsenicals and nicotine in the form of tobacco extracts (Chinese farmers)
1850	Pyrethrum, soap and a wash of tobacco, sulfur, and lime also used
1867	The pigment Paris green (impure form of copper arsenite), Paris green and kerosene oil emulsion
1896	Bordeaux mixture ($CuSO_4$ & $Ca(OH)_2$), and selective chemical herbicides
1900	Dilute Sulfuric acid, copper nitrates, potassium salts
1900 -1950	Sodium arsenite solutions become the standard herbicides and are used in large quantities
1913	Organomercury seed dressing
1913- 1939	Dithiocarbamates fungicides used in US
1939	Insecticidal potential of DDT discovered in Switzerland. Chlorinated hydrocarbons (DDT, BHC, dieldrin, Aldrin & chlordane)
1950s	Fungicides captan, glyodin and organophosphorous insecticide Malathion
1961	DDT registered for use on 34 different crops as pesticide usage dramatically increases
1962	Bio accumulation and long-term toxicity and pest resistance became evident. Stoppage of DDT usage and other chlorinated compounds by farmers. Favor of the use of Organophosphates and Carbamates
1972	Environmental Protection Agency revoked the use of DDT on all food sources in the United States. The World Health Organization, however, still reserves the right to use DDT on particularly virulent outbreaks of malaria

1972-1980	Herbicidal sulfonylureas, neonicotinoids, glyphosate, synthetic fungicides such as metaxyl and triadimefron,and light-stable pyrethroid pesticides introduced
1990s	Integrated pest management, intensified research on biological pest control methods and other alternatives to pesticides.
1990-1995	Increased interest in Integrated Pest Management (IPM) programs
2000	Wide spread usage of IPM techniques organic farming excluding the usage of synthetic pesticides.
2010-15	Involvement of genetic engineering and biotechnological methods to control the usage of pesticides eg. baculoviruses

Integrated Pest Management (IPM)

IPM is an expansive approach urged mainly by ecologists and environmentalists for the pest control in an economical manner. The main objective of IPM is pest control giving consideration for the acquirable pest control techniques without causing any threat to the ecosystem and human beings. It is mainly employed in agricultural and horticultural practises with the main intention of controlling pests, incorporating organic farming and barring the synthetic pesticides [7]. IPM focuses on the long term pest control which involves the process of checking the admissible level of pests and then monitoring the economic injury level or the safety standards. If it is crossed, then mechanical and biological methods of pest control are initiated and then if the condition requires the use of synthetic pesticides, then synthetic ones harmless to the surroundings are employed. IPM techniques are advantageous since it is facile, cost saving and economically compactable.

Classification of Pesticides

Pesticides can be classified into different types depending upon the source from which they are derived, mode of action, nature of the pest, on the basis of its chemical nature, functional group etc. Mainly they are classified as fungicides, herbicides, insecticides, molluscicides, rodenticides and insect repellents. Herbicides are used for killing weeds in domestic as well in commercial agricultural and horticultural practices. Eg. Dequat, pentachlorophenol, paraquat. Insecticides are widely used in agriculture for killing of insects. Although some insecticides cause serious health hazards, these are beneficial in the treatment of ectoparasitic infections and scabies. They enter the human body through skin or through systemic absorption. Insecticides may further be classified as carbamates (eg.carbosulfan, bendiocarb), chlorinated (chlordane, dieldrin, endosulfan), organophosphorus (ethion, phosmet, malathion, temefos), pyrethroid (Eg. permethrin, cypermethrin, bifenthrin). Molluscicides are used to control snails, for eg. bulinus (a freshwater snail vector) is controlled by the applying of nicloamide. As the name suggests, rodenticides are used to kill rodents (rats and mice). Eg. aluminium phosphide, squill, norbormide, difenacoum. Certain rodenticides (such as warfarin) are used as an anticoagulant medication [8]. On chemical basis pesticides are classified into four classes as Organophosphorus, Organochlorine, Carbamates and Pyrethroid pesticides.

Present studies specify that Organophosphorus pesticides are more used when compared with organochlorine pesticides in vegetables for the control of pests. The widespread usage of pesticides in the agricultural field and also during the time of transportation and handling in the selling market can drastically create a boon to all living organisms including human beings. Studies reveal that the vegetables and fruits purchased from markets are intensively contaminated with traces of synthetic pesticides. In a decade, the vegetables purchased from the supermarkets are found to double its content of pesticide use. Although these amounts are below the internationally recognised safety levels, its bioaccumulation even at low levels proves harmful.

Adverse Effects of Pesticides

The impact of pesticides is not limited to human beings, but it also affects the environment, mainly the water (both surface and underground water), soil and soil microorganisms [9]. But when human beings are taken into consideration, the preliminary symptoms of the effects of pesticide exposure include short term effects like irritation to eyes and mucous membrane, nausea, diarrhoea, fatigue, sweating, vomiting and

giddiness. Certain pesticides such as dibutyl phthalate, diethyl toluamide may create hypersensitivity and convulsions. Long term exposure to various types of pesticide can create severe neurological problems (Parkinson's disease) including birth defects [10]. It has been reported to cause dermatitis, whole body tremors, aggressiveness, nervousness, reproductive effects including impaired fertility in males and females [11]. It may also lead to carcinogenic effects which develop several types of cancer like those of non-Hodgkin's lymphoma [12], skin, lungs, blood, ovaries, prostrate etc[13].Food and even may get contaminated with banned pesticides may lead to congenital paralysis,loss of response to stimuli,reduced motor skills and kidney damage.[14,15] Acute Poisoning is characterised by pinpoint pupils, uncontrollable muscle twitching, increased pulse, respiratory failure, coma and dizziness.

Due to the hardly surveillance, it also leads to kidney dysfunction and enzyme inhibition. It can also pave way to attention deficient hyperactivity disorder (esp. in children), autism, respiratory problems, depression, memory disorders and several skin disorders. Long term exposure to pesticides like clofenotane (a Chlorinated Insecticide) may lead to neuropsychological and psychiatric symptoms.

Recently published data (April 2016) from US. Department of FDA reveals that strawberries rank first for containing the largest quantity of pesticides among the fruits and vegetables. Findings reveal that the dirtiest sample of strawberries had 17 different kind of pesticides and about 98% of the pesticide sample contained detectable amount of at least one pesticide. The most detected pesticides in strawberries include carbendazim, (a hormone-disrupting fungicide), bifenthrin and malathion. Other items in their decreasing order of the amount of pesticides are apple, nectarines, celery, grapes, cherries, spinach, tomatoes, sweet bell pepper etc. The detected pesticides in these vegetables and fruits are known to cause cancer and reproductive damage.[16]

Another shocking case of pesticide poisoning includes the death of 26 black bucks in gummadan village in Mahbubnagar district of Telangana, India on 1st week of august, 2016.The dead bodies of black bucks were found in fields located on backwaters of Srisailam reservoir in River Krishna. Post-mortem reports reveals that the death was caused due to the toxic level of local pesticide granules called gulikalu in the maize crop field. Gulikalu was used for warding off rodents, black bulbs, insects that would attack the maize crop. The amount of pesticide found in the crop sample was analysed and it was found that if consumed, would be fatal to humans [17]]

Another recently reported case of pesticidal tragedy happened on April 17, 2016 in Layyah, Punjab Province of Pakistan A resident of the village purchased sweets from local sweetshop for the birthday celebration of his grandson which caused the death of about 30 people. The incident happened because of the pesticide shop nearby the sweet shop. The shop was on renovation and the pesticide products were kept in the bakery. When the owner of the bakery left the shop, an employee at the bakery inadvertently mixed the pesticide (chlorfenapyr) to the sweet mix which turned to a great tragedy.[18]

A reported case of pesticide poisoning was in Preah Vihear, Cambodia in January 2015.It happened in such a manner that the pesticide used for hunting caused poisoning effects on human beings as well as animals, including endangered species.Carbofuran was the pesticide responsible for the poisoning. It caused health issues like abdominal cramps, dizziness and neurologic problems finally leading to death [19]

One of the drastic cases of pesticide usage reported in Kerala, India, is Endosulfan poisoning in Cashew plantations (owned by State Plantation Corporation of Kerala) in Kasargod district .This caused highly responsive change to the environment. It also caused the extinction of a large number of wildlife species, reproductive abnormalities and malconformations among human beings especially in children. The poisoning case was reported in February 28, 2001 and the Kerala government banned Endosulfan usage among plantations in the state in August 25, 2001.[20]

Another tragedy of accidental pesticide poisoning was reported on October 22, 1999 in an Peruvian village named Tauccamarca.This was caused due to the illiteracy of a village woman who mixed a milk powder bag with Methyl Parathion(a white coloured powder pesticide)to kill a dog that attacked her chickens. A child accidently took it to school, thinking the packet to be milked powder. It was served along with the school food

which in turn caused the death of 48 children. This incident marked as a cause for the ban of Methyl Parathion in Peru on October, 2000[21].

Other reported case of pesticide tragedy was Seveso disaster which happened on July 10, 1976 in Seveso, Italy [22] it occurred due to a small pesticidal factory which caused the exposure of 2,3,7,8 Tetrachlorodibenzodioxin (TCCD) to the residents of the area. The instant effects included skin inflammation and lesions whereas the long term effects included chloracne, liver enzyme inhibition, respiratory and cardiovascular diseases.

Analytical laboratories

Although these pesticides are used as an effectual methodology for pest control, these may have deadly impacts to humans and to environment. Testing of pesticides after its application to a field is pointless, since its effects are long-drawn and are carried to future generations. Hence proper analytical techniques are required to ensure that these pesticides introduced into the market fulfil the quality and work offensively.

Moreover due to its adverse effects, care has to be taken in order to control its effect to the living organisms. Hence, the need of residual analysis of vegetables and fruits are important due to its severe health hazards. The world of pesticide residue detection is complex and hence advanced analytical instruments are needed for the detection of even the traces of pesticide residue throughout the testing process. The analytical laboratories use high quality analytical methods. These Scientifically developed analytical ways pave way for its identification in-vitro and in-vivo.

The famous analytical laboratories in the world providing pesticide residue analysis include Anacon environmental consultancy (Central India), Euro fins (Global network of pesticide testing), SGS global testing Services, OMIC USA Inc. analytical laboratory etc. The various analytical techniques to determine the amount of pesticides are:

Preliminary screening tests

The preliminary screening tests are simple, mainly the colour tests used for the quantification of compounds present in a specific group. These mainly include ammonium molybdate test, Furfuraldehyde test, Phosphorus test and Sodium dithionate test. Ammonium molybdate test is used for the determination of phosphorus. Carbamates can be confirmed by Furfuraldehyde test and Organo phosphorus compounds by Phosphorus test. Sodium Dithionate test is used for the preliminary screening of diquat and paraquat.

Comprehensive screening procedures

Liquid Chromatography Mass Spectrometric analysis (LC-MS)

This method is an expeditious, powerful, efficient and standard highly sensitive method used for the identification and assay of different classes of pesticides. It is mainly used for the determination of Organophosphorus pesticides such as acephate, oxydementon methyl, monocrotophos, vamidothion etc. which are less sensitive to gas chromatography. [23]**.I**t can also be used for carbamate testing as well as for thermo labile and volatile substances [24]. The solvents use for LCMS must be highly pure and stable. LCMS grade water and solvents like acetonitrile and ion pairing agents are the commonly used solvents for this process. In the general method of analysis of pesticides in vegetables using LCMS, the sample is finely homogenised and extraction is carried out with a suitable solvent. When ethyl acetate is used for the extraction process, then sodium sufate must be added to remove microscopic amounts of water from the sample. Extraction is followed by centrifugation if an unclear liquid is obtained. The clear supernatant was injected into the instrument.

In a method of determination of less GC amenable Organophosphorus Pesticide, the following steps are carried out

1. Sample was finely homogenised and 25g was taken

2. Extraction of the sample with 50 ml ethyl acetate followed by the addition of sodium sulfate
3. Perform Centrifugation when unclear liquid phase is obtained. Transfer of supernant to the vial and evaporation at 35°C
4. Redissolution of the residue with 0.1% acetic acid in water
5. Filtration and analysis with LC-MS.[25]

In another method of determination of organophosphorus pesticide, Carbamates, neonicotinoids, sulfarylureas etc. the following method is followed

1. Small piece of the sample is taken without preaching and was homogenised.
2. To 20g of the sample 60ml methanol: water(80:20,v/v) 0.1% acetic acid was added and extracted
3. It was filtered using a filter paper and made upto final volume (100 ml)
4. LC-Grade water is added till the extract was diluted 8 times
5. Sample was passed through SFE and was evaporated to 0.5ml at 40°C using nitrogen as a carrier gas.
6. 10:90,v/v of methanol: water is added for reconstituting the sample and was analysed using LC-MS[26]

High Performance Liquid Chromatography (HPLC)

HPLC is an applaudable method for the determination of agricultural pesticides especially organophosphorus and Carbamates pesticides. In HPLC method of pesticide determination, first the sample is weighed and then it is extracted with a suitable solvent and filtered. The filtrate is diluted with the mobile phase and mixed well and the clear supernatant is injected to get the chromatogram.

When biological fluids such as blood or urine are analysed for the detection of pesticide, it must be mixed with at least two volumes of acetonitrile or methanol and is then centrifuged for the removal of the precipitated protein. Then the organic supernatant is evaporated and the required volume of the mobile phase was added for the sample reconstitution. Care must be taken when biological samples are injected as it may get trapped within the column and affect its performance. Then the sample was injected into the instrument and the analysis was carried out using HPLC with a suitable detector. [27]

In a method of determination of pesticides in fruits and vegetables extraction is done with an organic solvent of Supercritical fluid Extraction in modern laboratories. It was eluted with acetonitrile and the eluate was cleaned with the use of Sep-Pak ® Florisil and Bond Elut ® PSA cartridges. Elutation of the pesticide was performed from the cartridges with 15 % ether/n-hexane, Acetone /n-hexane (15 and 50% respectively). Elutation was done after washing with n-hexane. The samples prepared were analysed with HPLC with UV Detector. [28]

Gas Chromatography

Gas chromatography is more useful, simple and valuable analytical technique of determination of pesticides than HPLC because of its high sensitivity and the reproducible retention times. It is a sensitive and economical methology for the subjective and quantitative resolution of molecular species specific pesticidal compounds present in trace amounts. In this method, the pesticide sample is introduced into the carrier. The carrier is a stream of inert gas such as helium or argon and it is then passed through the column. The components are eluted from the column depending upon their relation with stationery phase and the additional analysis is meted out with a suitable detector. The various detector used for pesticide analysis are halogen specific detector(XSD), flame photo metric detector(FPD)[29],mass spectrometer(MS) and Inductively coupled plasma mass spectrometer(ICP-MS).

Gas chromatography can be used for the determination of Profenophos, Chlorpyrifos, Dimethoate, Malathion, Phosalone, Quinalphos, Lamda cyhalothrin in vegetables especially in tomatoes. Detectors used are Electron Capture Detector and Flame Photometric Detector. The injection temperature is 260°C and nitrogen is used as the carrier gas. The carrier gas flow is 1.0ml/min. Electron capture detector temperature is 300°C and the ECD make up is 25 ml/min.By using these techniques the above compounds were identified. [30]

Gas Chromatography-Mass Spectrometry

GC-MS is a recognized and a standard confirmatory method for the pesticide analysis. It is a powerful technique in which the characteristic of GC is coupled with MS (detector). It helps in the detection of pesticidal compositions with their determination limit down to ultra trace levels and also helps in unknown identification. In case when solids are used for analysis in GC-MS, extraction process is performed. But liquids and gases are directly injected into the stream of carrier gas in GC equipment and the analysis was carried out using MS as the detector.

It is a multiresidue method used for the detection of a wide range of pesticides including Organo phosphorus, pyrethroids, triazines, triazoles, chloroacetanilides and Organo chlorine pesticides in vegetables. The method involves extraction with acetonitrile, dichloromethane or with ethyl acetate. The injector temperature is 275°C .Helium is used as the carrier gas and the column carrier gas flow is 1 ml/min. After the extraction process, magnesium sulphate and sodium chloride must be added to the extract to separate the aqueous and organic phases. Solid phase Extraction (SFE) must be necessarily performed to remove the remaining water content. Following this process, an amine absorbent is added and then the sample is enriched. Then the analysis is performed with GC-MS [31].Individually, the pesticides are determined based on the retention time.

In another way of quantification and monitoring of pesticides such as OOPs, OCPs, carbamate and pyrethroids in vegetables, the extraction mixture contain toluene, hexane and ethyl acetate. The sample was extracted with the above extraction mixture taken in the ratio 3:1:1 and then the analysis was performed with GC-MS. [25]

Other Analytical methods:-

Apart from the above methods, various other methods are available for the pesticide determination. These methods include Liquid chromatography, Chemiluminescence method ,Supercritical Fluid Chromatography, Immunoassay ,Polygraphic Methods [32] ,Atomic absorption Spectrometric Detection ,Colorimetric Detection etc.

PLU code (Price look-up codes):-

These are labels or stickers attached to fruit which helps to determine how fruits are produced. It helps to determine whether the fruits are produced by genetic modification, organic farming or by cultivation with pesticides and harmful chemicals. They help to determine the food grade.
Four digit PLU code indicate traditionally grown pesticides by using pesticides.
Five digit PLU code starting with 8 denotes genetically modified fruits and vegetables.
Five digit PLU code starting with 9 denotes organically grown but not genetically modified fruits and vegetables. [33]

Although analytical techniques provide a way for the detection of the amount of pesticides, these pesticides cannot be simply controlled in agricultural practices. The harmful effects caused by these pesticides are of a mere knowledge to common farmer who has the only intention of increasing the yield of crops. So it is the duty of the agricultural department of every government to take necessary actions against its unlimited usage. Government should take necessary steps to promote the farmers in organic farming by providing grands and license.They should also be made aware of the harmful and adverse effects caused by pesticides to human and also to environmental balance. Pesticide control centres and analytical laboratories should be set up at low cost. Education campaigns regarding organic farming and controlled pesticide usage should be conducted at school and college level to create an awareness among students. Promotion of the cultivation of fruits and vegetables in the citizen's courtyard should be promoted by the government and for this purpose schemes should be initiated by the government for the supply of seeds seasonally. Financial aid should be provided for rain gathering techiques to water the plants.Thus, it is the need of the hour to have a very systematic agricultural system which gives equal stress to all scenarios including, community health, environmental balance and economical stability of the country.

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