ANALYSIS OF CYPERMETHRIN RESIDUES IN FRESH TOMATOES SOLD IN NAGONGERA FOOD MARKET IN NAGONGERA TOWN COUNCIL, TORORO DISTRICT IN EASTERN UGANDA

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DECLARATION

I, Mudume Isihaka, declare that this research project is my own original work otherwise cited, and where such has been the case reference has been stated and it has not been submitted for any award in any other university or other tertiary institute of higher education.

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This project report has been submitted for examination with the approval of the following supervisor:

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MR. MUSAGALA PETER

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ABBREVIATIONS

CPA: Cyclopropane carboxylic acid

CPB: cyano-3-phenoxy-benzyl ester

DDCC: 3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropanecarboxylic acid

DDE: Dichlorodiphenylethanes

GAP: Good Agricultural Practices

IPM: Integrated Pests Management

OCP: Organochlorine pesticides

PBA: 3-phenoxybenzoic acid

UV-Vis: Ultra violet visible spectrophotometer

ABSTRACT

Cypermethrin residues in fresh tomatoes were determined using uv-visible spectrophotometry. The method is based on hydrolysis of cypermethrin in alkaline solution to cyanid ion and reacts with iron (III) ions to form colored complex compounds. The results confirmed the residues were present in the tomatoes and the concentration was found to be 0.0096 mg per 250 g of tomato. Cypermethrin residues may lower the quality of tomatoes and pose health risks to humans. Therefore, regulation and monitoring pesticide residues in tomatoes produced should be done to avoid effects on human health.

CHARRPTER ONE

INTRODUCTION

1.1 Background to the Study

Pesticides refer to any toxic and poisonous substance or a mixture of substances, chemicals that is used to repel, control or destroy any pest such as rodents, insects, fungi and plant weeds that are harmful to health and environment. Pesticides have different physicochemical characteristics, structures, modes of action and use in agriculture. Depending on their molecular structures, pesticides are categorized into various types such as organochlorines, organophosphates, neonicotinoids, triazine, carbamates, urines, phenoxyacids, pyrethroids, and briazoles. The classifications of Pesticides depend on their use and application in agriculture for example are classified as insecticides, fungicides, nematicides and herbicides. Currently, pesticides are commonly used in agriculture to eliminate the effects of pests, weeds and diseases in tomatoes so as to increase on the yield and quality of tomatoes. However, the improper use of pesticides involves risks for human health, as pesticides remain in fresh vegetables, food, soil and water bodies after harvest (Essumang, Dodoo et al. 2008).

Most of the pesticides such as organochlorine compounds are non-degradable or slowly degraded thus their residues remain and accumulate in the food chain which are later transferred from one organism to another in the ecosystem leading to bioaccumulation in organisms. Therefore, the over use and misuse of pesticides can greatly affect the environment and leads to serious risk for human health since short or prolonged exposure to pesticide residues may cause acute or chronic toxicity. The effects of chronic exposure to pesticide residues may include metabolic disorders such as

genotoxicity, carcinogenesis, neurological disorders and endocrine disruption for instance chlorpyrifos (organophosphate) has been reported to cause neurological, deficit hyperactivity and fetus development disorders, carbofuran (carbamate) is linked to reproductive abnormalities, while carbaryl causes nausea, vomiting, blurred vision, coma and death (Elgueta, Valenzuela et al. 2021).

1.2 Statement of the problem

Tomatoes are consumed worldwide by almost every household but majority of the local consumers cannot determine the presence of residual pesticides in tomatoes. These pesticide residues may accumulate into human bodies which may later result into various human health problems such as breakdown of the central nervous system, respiratory system, eye and skin irritation, endocrine disruption, interference of fetus development in pregnant women and cancer related problems(Chowdhury, Banik et al. 2012). Thus the purpose of this study was to determine cypermethrin residues in tomatoes grown in Tororo.

1.3 Objectives of the Study

The overall objective was to determine the cypermethrin residues in tomatoes sold in Nagongera food market.

The overall objective was achieved by the following specific objectives

i) To determine the nature of cypermethrin and its residues in the environment.

ii) To assess the impacts of excess consumption of pesticide residues in human beings.

1.4 Justification of the study

Most farmers in Tororo have no alternative pest control mechanism, thus synthetic pesticides are commonly used to control pests and diseases. Failure to comply with good agricultural practices

put local consumers at a risk of consuming tomatoes with high levels of pesticide residues. Thus this project was aimed at developing an idea about the alternative ways of improving tomato productivity and ways of avoiding the spread of diseases caused by pests which are destroyed using pesticides.

1.5 Literature review

1.5.1 Pesticides

Pesticides refer to any toxic substance and poisonous substance or a mixture of substances, chemicals or biological in nature that is used to control or destroy any pest such as rodents, insects, fungi or plant weeds that affect health and environment. Pesticides are compounds consisting of one or more substances developed with an intention of eliminating pests, for example carriers of human, animal or plant diseases(Davies, Smith et al. 1978).

1.5.2 Pesticide residues

When pesticides are applied in the soil or on plants, they are gradually lost by several processes such as leaching and evaporation and what remains after application is known as pesticide residues (Gambacorta, Faccia et al. 2005) . Some pesticides do persist in the environment due to their longer period of residual activity while others don't persist in the environment due to their shorter residual activity. It is therefore possible to find pesticide residues in the environment and vegetables such as tomatoes which may get into food chain resulting into complications to lives of humans and animals.

1.5.3 Ways through which pesticide residues get into vegetables

The pesticide residues in the environment and vegetables occur as a result of application of insecticides to the vegetation with the aim of eliminating the insects that attack vegetables. Also

pesticides may get into vegetables through erosion of soils contaminated with high concentration of pesticide or through air during spraying of vegetables (Gambacorta, Faccia et al. 2005).

1.5.4 Ways through which pesticide residues get into human bodies

Pesticide residues may get into human body through inhaling polluted air or direct penetration by eating contaminated food or taking drinks contaminated with pesticide residues. They also enter human body via food chain from food sources of both plant and animal origin like fruits vegetables, fishes, cereals and meats (Rano and Singh 2021).

1.5.5 Organochlorine pesticides

1.5.5.1 Composition of Organochlorine Pesticides

The constituents of organochlorine pesticides include carbon, hydrogen and chlorine. The persistent type of organochlorine pesticides is found to possess one or more rings in their structure, the polychlorinated biphenyls and Dichlorodiphenylethanes(DDE) are made up of two rings (Doong, Peng et al. 2002).

1.5.6 Pyrethroids pesticides

Pyrethroids are a group of compounds naturally isolated from the flowers of the plant Tan acetum cineraria folium. Pyrethroids are pesticides commonly used in crops protection, in the forestry, wood and textile industries as well as in veterinary medicine to treat parasitic crustacean infestations. Pyrethroids are recommended for personal protection against malaria and virus Zika by World Health Organization (Hołyńska-Iwan and Szewczyk-Golec 2020). Examples of pyrethroids include; cypermethrin, deltamethrin, and permethrin.

1.5.6.1 Classification of Pyrethroids

Pyrethroids were divided into groups depending on type of intoxication symptoms that appeared in the vertebral organism after their administration i.e. type 1 pyrethroids, including permethrin cause the symptoms known as the tremor type syndrome (T), which is characterized by tremors throughout the body, hypersensitivity, aggressive behavior, and ataxia(Wright, Forshaw et al. 1988).

Type II pyrethroids represented by deltamethrin and cypermethrin, are associated with the choreoathetossis-salivation syndrome, in which salivation and muscle dysfunction occur.

1.5.6.2 Characteristics of pyrethroids

Pyrethroids have the following characteristics; Low in toxicity to mammals and birds, highly toxic to fish if applied directly to water, require very low doses to kill insects (high arthropod toxicity), have low solubility in water and also bind tightly to soil and organic matter thus are not effective in penetrating soil to kill underground pests.

1.5.6.3 Effects of Pyrethroids on Mammals

Pyrethroids are considered to be relatively non-toxic to humans in all stages of life. However, recent data shows that they are not completely harmless to human's health as they may enter the body through skin contact, by inhalation and food or water, and absorption level depending on the type of food. Permethrin has an adverse effect on fertility, immune system, cardiovascular and hepatic metabolism as well as enzymatic activity (Chrustek, Hołyńska-Iwan et al. 2018).

Deltamethrin induces inflammation, nephro and hepatotoxicity and influences the activity of antioxidant enzymes in tissues. Alpha-cypermethrin may impair immunity and act to increase glucose and lipid levels in blood (Pimpão, Zampronio et al. 2007).

1.5.7 Cypermethrin

1.5.7.1 Cypermethrin as pesticide

Cypermethrin belongs to the class of pyrethroid II insecticides that are synthetic analogues of pythrethrins, the naturally occurring insecticidal compounds in the flowers of Chrysanthemum species because of its cyano group. It occurs in the form of a crystalline powder and as a dense yellow mass. It is used as an insecticide to control insect pests on crops and it can also be used as a veterinary drug to treat ectoparasites on livestock. Therefore, cypermethrin residues have the potential to occur in a wide range of foods (Ferre, Quero et al. 2018). Like any other pesticide residue, the risk of cypermethrin residues in food to consumers depends on residue exposure level, which is determined by the concentration of residues remaining in food after the food has been prepared and ready for consumption.

1.5.7.2 Structure of Cypermethrin

Chemically, cypermethrin is the alpha-cyano-3-phenoxy-benzyl (CPB) ester of the dichloro analogue of chrysanthemic acid, 3-(2, 2-dichrovinyl)-2, 2-dimethylcyclopropanecarboxylic acid and the alcoholic hydroxyl group of hydroxyl (3-phenoxyphenyl) acetonitrile. It is an organochlorine compound, nitrile, an aromatic ether and a cyclopropane carboxylate ester. It is functionally related to a 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid(Liu and Gan 2004).

The molecule embodies three chiral centers, two in the cyclopropane ring and one on the alpha cyano.

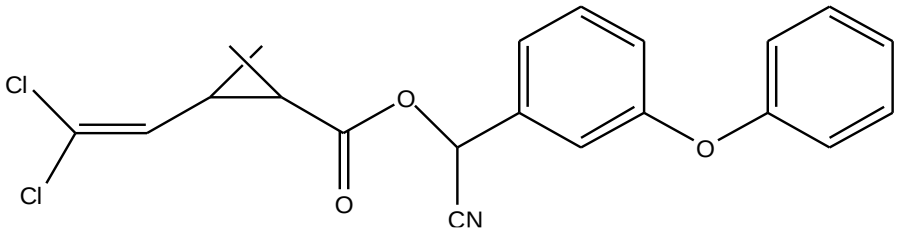

Figure 1 Structure of Cypermethrin

1.5.7.3 Stability of Cypermethrin

Unlike the natural pyrethrins, cypermethrin is relatively stable to sunlight though it is probable that photo degradation plays a significant role in degradation of the on leaf surfaces and in surface waters, its effects in soils are limited. The most important photo degradation products are, 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylic acid(CPA) and 3-phenoxybenzoic acid(PBA)(Yong-fei, Ming-ming et al. 2010).

Degradation in the soil occurs primarily through cleavage of the ester linkage to give CPA, PBA and carbon dioxide gas. Some of the carbon dioxide is formed through cleavage of both the cyclopropyl and phenyl rings under oxidative conditions. The half-life of cypermethrin in a typical fertile soil is between 2 to 4 weeks.

1.5.7.4 Mode of action of cypermethrin

Cypermethrin is a pyrethroids so its mode of action is by contact or ingestion. This ingredient interferes with the central nervous system, through disruption of the sodium ion transport through the cell membrane thus causing muscle spasms and inflicting paralysis, creating inability to eat or function which eventually leads to pests' death(Smith and Soderlund 1998). Depending on the formulation, cypermethrin may leave a long-lasting residual that will continue to control pests for up to 90 days.

1.5.7.5 Effects of excess consumption of cypermethrin residues

Cypermethrin is used to protect crops against insects, fungi and other pests. However, cypermethrin is potentially toxic to humans as it may induce adverse health effects including cancer, effects on reproduction, immune or nervous systems. Before it can be authorized for use, it should be tested for all possible health effects and the results should be analyzed by experts to assess any risks to humans(Hotchkiss 1992).

CHARPTER TWO

EXPERIMENTAL

2.1 Apparatus

Weighing of the masses of crystalline salts such as potassium chloride, sodium hydroxide was done using a beam balance. Equipped 1cm path length quartz cells were used. Absorption was recorded using ultra violet visible (UV-Vis) spectrophotometer. $100cm^3$ measuring cylinders were used to prepare 96% ethanol and 10% sodium hydroxide. A tomato paste was obtained using a motor and a pestle. Filtering of the sample solution was done using a funnel and a filter paper.

2.2 Materials

All chemicals used for analytical grade. They included ethanol, Standard solutions of cypermethrin, Sodium hydroxide solution, iron (III) chloride, potassium chloride, phosphate buffer, distilled water and tomato paste from fresh tomatoes

2.3 Sample preparation

Tomato paste (250 g) and 100 mL of ethanol (96%) were mixed to homogeneous slurry form. The waste was filtered and rinsed with ethanol. The filtrate was collected and evaporated for 20 minutes in order to concentrate the sample solution and then put in a 50 mL flask followed by adding small volume of ethanol.

2.4 Preparation of Phosphate buffer solution at pH 7

50.0 ml of 0.2 M potassium dihydrogen phosphate solution was pipetted into a 200.0 ml volumetric flask, followed by 29.1 ml of 0.2 M NaOH solution and then water was added to the mark.

2.5 Preparation of cypermethrin standard solution.

Cypermethrin standard solutions of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/L were prepared using 5% cypermethrin solution as follow;

1.0 ml of 5% cypermethrin was added to a 500.0 ml volumetric flask and distilled water was topped up to the mark to prepare a 0.1 mg/L cypermethrin solution.

2.0 ml of 5% cypermethrin was added to a 500.0 ml volumetric flask and distilled water was topped up to the mark to prepare 0.2 mg/L cypermethrin solution.

3.0ml of 5%cypermethrin solution was added to a 500.0 ml volumetric flask and distilled water was topped up to the mark to prepare 0.3 mg/L cypermethrin solution.

4.0 ml of 5%cypemethrin was added to a 500.0 ml volumetric flask and distilled was topped up to the mark to prepare 0.4 mg/L cypermethrin solution.

5.0 ml of 5% cypermethrin solution was added to a 500.0 ml volumetric flask and distilled was topped up to a mark to prepare 0.5 mg/L cypermethrin solution.

2.6 Preparation of 10% sodium hydroxide solution

Sodium hydroxide (10 g) was weighed and transferred into a 100 mL volumetric flask. Distilled water was added to dissolve. The resultant solution was made to mark with of more distilled water.

2.7 Preparation of 0.1 M potassium chloride solution

Potassium chloride (5.45 g) was weighed and transferred into a 1000 mL volumetric flask. Distilled water was added to dissolve. The resultant solution was made to mark with more distilled of water.

2.8 Procedure

5.0 mL of sample solution was pipetted into 6 different 50 mL conical flasks. 2 mL of 0.1 mg/L cypermethrin solution was added to flask 1, 2 mL of 0.2 mg/L of cypermethrin to flask 2, 2 mL of 0.3 mg/L cypermethrin solution to flask 3, 2 mL of 0.4 mg/L cypermethrin to flask 4, 2 mL of 0.5 mg/L cypermethrin was added to flask 5 and without addition in flask 6.

1 mL of NaOH solution (10%) was added to each flask, followed by adding 2 mL of phosphate buffer solution at PH 7, 1 mL of $FeCl_3$ solution (0.1M), 2 mL of KCl solution and 4 mL of distilled water.

5.0 mL of each of the solutions in the 6 different flasks was transferred into 6 different cuvette cells, which were taken to the uv-visible spectrophotometer for analysis. The absorbance corresponding to the respective concentrations were recorded.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Table of results

Concentration of Cypermethrin (mg/L)	Absorbance
0.1	0.05
0.2	0.10
0.3	0.14
0.4	0.22
0.5	0.25
Unknown	0.001

Table 1 of results

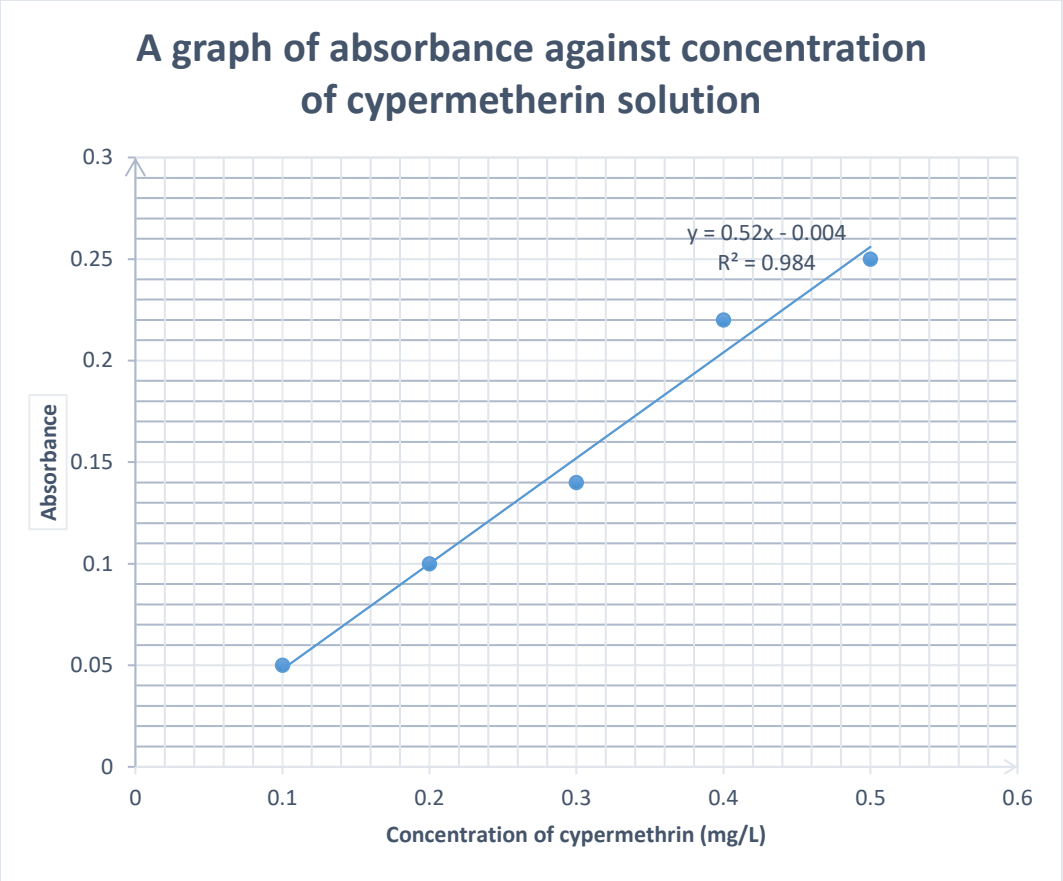

Figure 2 a graph of absorbance against concentration of cypermethrin

The standard curve was found to show a linear increase in absorbance with increase in concentration of cypermethrin solution. The equation for absorbance and concentration was found to be $y = 0.52x - 0.004$ where y is the absorbance of the unknown and x is the unknown concentration which was found to be 0.0096 mg/L.

The aim of this research was to analyze pesticide residues in tomatoes. To do this, it was necessary to sample tomato fruits that were readily available to the consumers and then analyze them to determine whether there were residues present. The results confirmed that pesticide residues were present in the tomato fruits.

There are three possible reasons for high levels of pesticides. First, applying pesticides at higher rates than specified by the manufacturers and harvesting tomatoes sprayed with this pesticide before the pre-harvest interval.

Secondly the pesticides are used to kill both insects and nematodes, to ensure the proper growth of tomatoes. Lastly, most of the farmers in the study area may not have enough knowledge about the chemical nature of pesticides that have been used or the effects of pesticides on the environment and the exposure effects of pesticides on public health, when using them indiscriminately. Therefore, it is advised that people living in this area do not consume tomato fruits that have just been harvested from tomato fields because they may be contaminated by these pesticides.

CHAPTER FOUR

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

Results indicated that consumers of tomato fruits produced in Nagongera as well as Tororo district are exposed to pesticide residues. Although these residues were detected in small amounts does not mean their presence in the fruits should just be ignored. The pesticide residues have the potential to affect human health and therefore we should be concerned and address the problem appropriately.

5.2 Recommendation

Farmers need to ensure that right pesticide quantities are applied on tomatoes as this will ensure that the pesticide residue levels in the tomatoes are within the recommend maximum residue limits at that time of harvest and consumption. Misuse and over-reliance on synthetic pesticide use to control tomato pests and diseases can be controlled by embracing Good Agricultural Practices (GAP) and Integrated Pests Management (IPM) together with periodic surveillance of pesticide use and their levels in the environment and produce.

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