Assessment of Pesticide Residues in Snake Melon
(*Cucumis melo* L.) in Wad Medani Market, Sudan

By

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<table>
<thead>
<tr>
<th>Name</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Prof. Ahmed Eltayeb Abdalla</td>
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<td></td>
</tr>
</tbody>
</table>

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DEDICATION

TO MY MOTHER

TO MY BROTHER ABDELRAHMAN

AND HIS WIFE MAHASIN

TO ALL MY BROTHERS

AND MY FRIENDS
Acknowledgement

Praise and thanks are due to Allah who gave me the power and health to complete this work. I would like to express my gratitude to my supervisor prof Ahmed Eltayeb Abdalla.

Deep Acknowledgment with appreciation are due to Dr Mohamed G. Elzorgani the co-supervisor of the study. Grateful thanks go to my dear Mawahib E. Mohammed a technician in central laboratory in Khartoum.
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Abstract

Pesticide residues is a major problem in Gezira area. Improper agricultural practices e.g. not observing the safety period of the pesticide could raise the risk of consuming pesticide residues in daily diets. The aim of this study was to detect the residues of two carbamate insecticides (carbaryl and carbosulfan) in cucumber produced in some farms around Wad-Medani city. Nine samples representing all the locations of cucumber production were collected (Hantob, Abu haraz, Fadacy, main market, central market, new market, public market, Atra, and Um sunut) The samples blended and pesticide residues were determined using high performance liquid chromatographs (HPLC). Four samples out of the nine samples were contaminated with residues of carbosulfan (main market, central market, Hantob and Abu haraz). Residues of carbaryl pesticide were not detected in all samples. The study recommended that vegetables should be periodically analyzed to make sure that they are free from pesticides.
ملخص البحث

تعتبر متبقيات المبيدات مشكلة رئيسية في منطقة الجزيرة. الممارسات الزراعية الخاطئة مثل عدم التقيد بفترة الأمان للمبيدات قد تزيد من خطر استهلاك متبقيات المبيدات خلال الطعام اليومي.

هدفت هذه الدراسة لتحديد متبقيات مبيدات الكاربامات الحشرية (الكارباريل، الكاربوسلفان) في نباتات العجور في بعض المزارع حول مدينة ود مدني. تم جمع تسعة عينات تمثل كل موقع إنتاج العجور (حنتوب و السوق الكبير والسوق الجديد والسوق المركزي والسوق الشعبي وعترة وأم سنط وفداسي وابو حراز). استخلصت العينات وتمت تنقيتها ثم تم الكشف عنها بواسطة جهاز الكروماتوغراف السائل ذو الأداء العالي. أربعة عينات من التسعة كانت ملوثة بمتبقيات مبيد الكاربوسلفان (السوق الكبير وحنتوب وابو حراز والسوق المركزي). بينما خلت التسعة عينات من متبقيات مبيد الكارباريل. أوصت الدراسة بضرورة تحليل الخضروات دوريًا للتأكد من خلوها من المبيدات.
Table of content

<table>
<thead>
<tr>
<th>Contents</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedication</td>
<td>i</td>
</tr>
<tr>
<td>Acknowledgment</td>
<td>ii</td>
</tr>
<tr>
<td>English Abstract</td>
<td>iii</td>
</tr>
<tr>
<td>Arabic Abstract</td>
<td>iv</td>
</tr>
<tr>
<td>List of contents</td>
<td>v</td>
</tr>
<tr>
<td>List of figures</td>
<td>viii</td>
</tr>
<tr>
<td>Chapter one: Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Chapter two: Literature Review</td>
<td>3</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>3</td>
</tr>
<tr>
<td>2.2 Pesticides application and their residues</td>
<td>4</td>
</tr>
<tr>
<td>2.2.1 Pesticide application methods</td>
<td>4</td>
</tr>
<tr>
<td>2.2.2 Pesticide side effects</td>
<td>5</td>
</tr>
<tr>
<td>2.2.3 Pesticide residues in food stuff</td>
<td>6</td>
</tr>
<tr>
<td>2.2.4 Safety period of pesticides</td>
<td>10</td>
</tr>
<tr>
<td>2.2.5 Carbamates insecticides</td>
<td>11</td>
</tr>
<tr>
<td>2.3 Presence of carbamate pesticide in the human Food supply</td>
<td>13</td>
</tr>
<tr>
<td>2.3.1 Carbamate Residues detective methodology</td>
<td>14</td>
</tr>
<tr>
<td>2.3.2 Carbaryl</td>
<td>14</td>
</tr>
<tr>
<td>2.3.3 Carbosulfan</td>
<td>16</td>
</tr>
<tr>
<td>2.4 Vegetables</td>
<td>17</td>
</tr>
<tr>
<td>2.4.1 Vegetable production in Sudan</td>
<td>18</td>
</tr>
<tr>
<td>2.4.2 Pests and diseases in Vegetables</td>
<td>19</td>
</tr>
<tr>
<td>2.4.3 Family <em>cucurbitaceae</em></td>
<td>20</td>
</tr>
</tbody>
</table>
2.4.4 *Cucumis melo*

2.4.5 Snake melon in Sudan

Chapter three: Material and methods

3.1 Sampling

3.2 Apparatus

3.3 Reagents

3.4 Sample preparation

3.5 Extraction

3.6 Clean-up

3.7 Thin layer chromatography method

3.8 HPLC analysis

Chapter four: Results and Discussions

4.1 Results

4.2 Discussions

Chapter five: Conclusions and Recommendations

5.1 Conclusions

5.2 Recommendations

References
## List of figures

<table>
<thead>
<tr>
<th>Fig. No.</th>
<th>Page No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>Carbaryl chemical structure</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>Carbosulfan chemical structure</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>Chromatogram showing peak of Carbaryl</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>Chromatogram showing peak of Carbosulfan</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>Chromatogram showing peak of Carbosulfan in sample from Main Market</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>Chromatogram showing peak of Carbosulfan in sample from Hantob</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>Chromatogram showing peak of Carbosulfan in sample From Abu –Haraz</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>Chromatogram showing peak of Carbosulfan in sample From Central Market</td>
</tr>
</tbody>
</table>
Chapter one

Introduction

Chemical contaminants are chemicals or compounds that can potentially harm the health of human, wildlife, and other organisms. The number of chemical contaminants are increasing and they enter into all life needs like food, water, air, and soil. Important source for chemical contamination is pesticides use especially for agricultural production such as vegetables. FAO has defined the term of pesticides as: Any substance or mixture of substances intended for preventing, destroying, or controlling any pest including vectors of human or animal disease, unwanted species of plant or animal causing harm during or otherwise interfering with production processing, storage or marketing of food and agricultural commodities. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.

Chemical pesticides were first used in war (World War II) against lice but scientists saw their pesticidal effect on other insect, after that they were used to fight or kill the pest as insect in the farms. Due to this use rate of pesticides increased for long time (Hassal, 1982). At present, pesticides are indispensable for maintaining and increasing agricultural production particularly food, to meet the need of the growing world population. Pesticides also play important role in the protection of human health. Consequently, pesticides usage has greatly increased during the last few decades as marked by the annual consumption of pesticides in the developing countries which exceeded 14000 tons since the mid seventies (Ahmed, 2005).
The large-scale usage has become source of great concern because of their possible effect on human health and non-targeted organisms. This concern is heightened by non-specificity and high toxicity of many pesticides. Chemically, pesticides are classified to several groups such as organochlorines, organophosphates, carbamates and Pyrethroids. All these compounds affect on the nervous system. Pesticides in general has the problem of residues in edible parts of the crops and vegetables. Some pesticides were banned from use such as DDT even so some of them are illegally used by some farms (Lobe 2006). Pesticides usage can be of positive effect like with improvement of production and farmers income. They control weeds and pests on plant animal and human. The negative effects are killing of non-target organisms and contamination of water, soil, and many crops. The accumulation of these chemicals lead to cancer. This effect comes due to bad uses of pesticide or indirect procedure like residues on food or vegetable, residues are detected in human blood, soil water, and food.

**Objectives:**

1. determine the magnitude of pesticidal contamination of seasonal snake melon
2. Find out the most prevalent Carbamate insecticide (Carbaryl, Carbosulfan) in Snake melon and guidance to farmers and policy makers for future use.
Chapter two
Literature review

2.1 Introduction:
Pesticide were defined by the United States Federal Insecticide Fungicide Rodenticide Act (FIFRA) as “any substance or mixture of substances intended for preventing, destroying or repelling any pest,” where pests are defined as organisms that may be deleterious to human or the environment (Tadeo, 2008). It can be insects, mice and other animals unwanted plants (weeds) fungi, or microorganisms like bacteria and viruses. Pesticides can be classified into insecticides, herbicides, fungicides, and various other substances used to control pests.

Chemical substances were used by human to control pests from the beginning of agriculture. Initially, inorganic compounds such as sulfur, arsenic (Ar), mercury (Hg), and lead (Pb) were used (Tadeo, 2008). The discovery of the compound dichlorodiphenyltrichloroethane (DDT) as an insecticide by Paul Müller in 1939 caused a great impact on the control of pests and soon became widely used in the world. At that time, pesticides had a good reputation mainly due to the control of diseases such as malaria transmitted by mosquitoes and the bubonic plague transmitted by fleas, both killing millions of people over time. Nevertheless, this opinion changed after knowing the toxic effects of DDT on birds. Since 1983 DDT was banned in many countries, but it is still used in some developing nations to prevent malaria and other tropical diseases by killing disease-carrying insects (Lobe, 2006).

The most convenient and economical way to increase production and reduce cost for farmers is applying pesticides on crops. However, because of the
potential hazardous effects on public health, Monitoring programs for pesticide residues in fruits and vegetables was the routine work of food safety related authorities.

2.2. Pesticide application and their residues:

2.2.1 Pesticide application methods

Pesticides are used to control organisms that are considered to be harmful. For example, they are used in grocery stores and food storage facilities to manage rodents and insects that infest food such as grains. Many methods are used in application of pesticides, which means good methods by timing selection and the manner of appropriate usage of the pesticides. The application of pesticides may be before the planting or post emergence after crop appearance and that as agronomy or foliar application in which the pesticide is applied on the plant leaves. The main ways to apply the pesticides can be explained as follows:

1. Soil processing (application by soil) in which the pesticide is sprayed or sown or integrating the pesticide with the soil by whatever ways like plowing to mix the pesticide with the soil.

2. The application in fine lines or limited scope in which the pesticide is applied in splatters, stripes or batches on or along the ranks of the crop.

3. Full coverage where the pesticide is regularly distributed over all the targeted areas.

4. The direct spray in which the pesticide is directly applied on the plant and in the case of herbicides is known as the oriented spray where the paces between rows and lines are sprayed or by treating growing’s found in ridges between the rows.

5. Dipping method, its one of the best methods that enables being sure of the target total coverage or the full or partial immersion of the plant in the pesticide solution.

6. Treatment of tunnels in the stems and branches of the trees and the cracks or other openings where pests can live.

7. Drenching where the soil, seeds, the plant
or parts of it are saturated with the pesticide. (8) Pouring where the pesticides are applied by pouring them in tunnels made in the stems of the tree. (Zometi, 1997)

2.2.2 Pesticides side effects:

The intensive use of pesticides leads to some negative impacts including (1) resistance property in some insect, some studies indicated that there are many agriculturally important insect species, which gained the pesticides resistance property (Baroni, 1991) and this property has developed for some organochlorine and the Carbamic compounds. (2) Human poisoning: when exposed to pesticides in several ways, during their transport, preparation for usage, during treatment or after treatment by eating contaminated food stuff. As a result, human problems with pesticides are liver diseases, effect on nervous system, causing of cancer tumors, fetus deformations and death. (3) Impact on honey bees, the bees are considered as the most useful insects for humans because they pollinate 80% of the flowering plants and vegetables. They lack some enzymes that work to destroy the pesticides and removing their poisonous effects. Therefore, they are highly sensitive to any pesticide concentration. (4) Impact on Wild Animals, a number of insecticides are highly toxic for vertebrates and invertebrates and their usage negatively affect the wild life. As these animals are directly exposed to pesticides or via contaminated food or plants and in both cases this leads to their death. (5) Impact on the Environment, when applying pesticides, some of their particles hang on air causing pollution and may fall on soil entering its pores causing intoxication of the living microorganisms. (6) Impact on plant, the impacts differ in accordance with the type of pesticides concentration, type of plant, cultivation time and methods of treatment. Primary studies depended on standard of plant morphological changes like
the relationship between pesticides treatment with the roots, stem height knots number, number of leaves and fruits. Scientists knew that the harmful impacts of pesticides are inevitably reflected on the rate of the dry matter formation in the treated plant and the product is affected. (Abdalhamed, 1995)

The physiological effects, e.g. the change of plant color to dark green after being treated with Carbamate compounds as a result of the increase of chlorophyll in plant tissues and also the decrease in the rate of exudation and photosynthesis (Abdalhamed, 1995). As pesticides have their impact on asThe plant essential elements: the treatment of plant with pesticides leads to incidence of reduction in the concentration of zinc (Zn), manganese (Mn) and copper (Cu) elements whereas activation in potassium (K) element occurs. The lack of the element will probably be in the plant seed. (Abdalhamed, 1995).

2.2.3 Pesticides residues in food stuff:

It was stated that 84.4% of pesticides residues in food during the period 1967-1969 were insecticides (Abdalhamed, 1995). Before the usage of most modern pesticides, the problem of residues in food stuff included the residues of arsenic (Ar) and mercury (Hg) and the first case of intoxication with arsenic was recorded in England. By the development and using chlorine pesticides in agriculture, the identification of their residues in food was assured. This led to the necessity of conducting the studies of residues and toxicity before permitting the registration of the new compound. There are two sources of food stuff contamination. The first resulted from direct usage of pesticides and depends on the nature of pesticides, vegetarian surface or the soil to which the pesticide is added. The second is by casual contamination of food with pesticides used for other purposes (Abdalhamed, 1995). In one of the studies conducted in the central laboratory
in Egypt, it was clear that about 81-82% of the amount of Novarcon pesticides sprayed on Jew's mallow plant penetrated into the leaf tissue one hour after spraying and cannot be removed by water washing, as it will change into the leaves to other more toxic products. The study targeted also the residues of some phosphoric pesticides in beans and okra and the extent of their durability and impact on public health. (Abdalhamed, 1995).

One hundred thirty two samples of fruits, vegetables, herbs and spices collected from Egyptian local markets were analyzed for pesticide residues. Contamination with pesticide residues reached 54.55% while samples free from contamination reached 45.45%. Only one sample from 132 analyzed samples violated the Maximum Residue Limits (MRLs) of the Codex Committee. From the 132 analyzed samples, 72 samples (54.55%) were contaminated, from which 43.18% contaminated with residues from one pesticide residue, 6.06% with 2 residues and 5.3% with more than 2 residues. In addition, two caraway and one fennel samples contained 4 pesticide residues, one sample of marjoram contained 5 pesticide residues and one mint sample contained 6 pesticide residues. Six of the pesticides detected as residues in the analyzed food items were considered to be carcinogens at different levels of assurance. (Farag, et al, 2011).

Pesticide residues and contaminants in selected leafy vegetables (lettuce garden rocket, and salad onion) were monitored at market level in Riyadh city in (Saudi Arabia) during the period June to July 2001. Fifteen samples of vegetables from the city vegetable market were collected and subjected to multi-pesticide residue detection and analysis by gas chromatography with mass spectrometer and electron capture detectors (GC/MS, ECD). Results of sample extracts analysis showed that the two vegetables of garden rocket and salad onion contained pesticide residues and contaminants which
has not Maximum Residue Limits (MRL) prescribed by Codex Alimentarius Commission (CAC) collaborate with World Health and Food and Agriculture Organizations (WHO/FAO). Whereas lettuce vegetable was found free of any identified pesticide residues or contaminants. Garden rocket was shown to contain Dibutylphthalate (0.04ppm) Stearylchloride (0.02ppm) and Tridecane (0.06ppm). The salad onion was found to contain octadecanamide (0.13ppm) Tridecane (0.15ppm) and Teradcan (0.16ppm). There are no established MRLs for these pesticides and contaminants detected in garden rocket and salad onion, although when impacts on human health were reviewed some of them were found probably hazardous. (Ibrahim and Ishag, 2005).

Monitoring of pesticide residues on cucumber, tomatoes and strawberries in Gaza Governorates: Three techniques of gas chromatography, either with flame photometric-detector (FPD), electron capture detector (ECD), or with mass-spectrometry (MS) were applied for identification and quantification of pesticide residues on 45 samples of cucumber, tomatoes and strawberries in fifteen locations in Gaza. GC-FPD analysis showed the presence of four different organo-phosphorus pesticide, their levels were very low and below maximum residue limits (MRLs) GC-ECD detected different pesticide at levels below the MRLs. Using the GC-MS technique, alpha and beta-endsulfan, chlorpyrifos, carbofuran, penconazole and iprodione were detected and confirmed on some samples of cucumber, tomatoes and strawberries. All GC-MS pesticide residues detected on tomato were below the MRLs except chlorfluazuron while on strawberry were below the MRLs except penconzole, chlorfluazuron, but on cucumber were slightly higher than the MRLs except alpha and beta endosulfan. Also statistical analysis of
pesticide residues in all samples showed that most of the detected residues mean were significantly lower than the MRLs (0.05). Generally tomatoes showed the least number and level of pesticide residues by all the GC-techniques. On the other hand strawberries showed greater number and levels of pesticide residues, particularly by the GC-MS technique. These results indicate that the protective period to elapse before harvesting should be increased especially on strawberry. The results also can help in risk assessment of consumers exposure to the expected pesticide residues. (Safi, et al, 2002).

Seasonal variation of pesticide residues in some salad vegetables in Khartoum State Seventy seven farmers were checked for the use of pesticides in salad vegetables such as tomatoes, cucumber, sweet pepper garden rocket, lettuce and snake melon. The survey covered summer autumn, and winter seasons with collection of samples of these salad vegetables for estimation of pesticides residue, as food contaminants in each. Almost one fourth of these farmers used the pesticide malathion with all salad vegetables tested. Pesticides detected in summer tomatoes included traces of ethorophos methyl parathion, and ethyl paraxon. Autumn tomatoes were also found to contain trace residues of chloryrifos, ethoprophos dimethoate, malathion, beside methyl parathion and omethoate. Only traces of chloryrifos were detected in winter tomatoes. The pesticides residues were detected in summer lettuce included ethoprophos, methyl parathion and ethylparaxon, whereas no pesticides residue were detected in other seasons of lettuce. Summer garden rocket was found to contain residues of ethoprophos (0.15ppm) and traces of methyl parathion, ethylparaxon, dimethoete and malathion. In Autumn garden rocket chloryrifos(0.46ppm) and traces of acephate residues were detected,
whereas the same salad vegetable was completely free from any pesticides in winter harvest. The summer harvest of sweet pepper was found to contain residue of ethoprophos (0.17ppm) and traces of methyl parathion, and quinalphos. In Autumn only traces of endosulfan and phosphamidon residues were detected in sweet pepper, while the same salad vegetables was completely free from any pesticides in its winter harvest. Residue of ethoprophos and traces of residues of malathion, methamidophos and methylparathion were detected in summer cucumber, whereas only phorate (0.3ppm) and traces of endosulfan were found in autumn harvest. Non of pesticides were detected in winter cucumber. The summer harvest of snake melon was found to contain residues of ethoprophos (0.36ppm) paraxon (0.12ppm) and traces of methyl parathion. In Autumn season traces of dichlorovos and phosphamidon residues were detected in snake melon whereas no pesticides were detected in the same salad vegetable during winter harvest. It was obvious that most salad vegetable farmers in Khartoum tend to use pesticides with summer and Autumn crops and most of these pesticides did not meet Codex specifications with respect to using pesticides in food plants. (Said and Ahmed, 2007).

2.2.4 Safety Period of pesticide Residues:

The residues are defined as the amount of pesticides left from the application process on the agricultural crops. They include the changed and unchanged pesticides particles. These pesticides residues continue to be stable for long periods depending on the nature of the pesticide, the treated surfaces and the weather condition. Thus, if these crops are provided for consumption, the residues will be directly transmitted with food to humans and animals. As their harmful effect on humans and environment cannot be eradicated, the
consumption of contaminated food and fodders should not be allowed directly after application. A chance should be given to growth increase and weather factors that act to eradicate them like rain and dew, in addition to the effects of destructive enzymes in plant tissues which act to reduce the level of residues to less harmful limits. Safety period is the period that should pass between the application and supplying the crop for marketing and consumption. This period depends on the type of the crop, pesticides formulation and the prevailing weather and environmental conditions. Therefore, it can differ from one place to another and from one crop to another. (Zumeti, 1997)

2.2.5 Carbamate Insecticides:

Investigations of chemicals that exert an anti-cholinesterase action on the nervous system similar to organophosphates led in the 1950s to the development of carbamate insecticides. Carbamate insecticides are derivatives of carbamic acid, $\text{HOCONH}_2$. They have the general formula shown below where $R^2$ is an alcohol, oxime or phenol and $R^3$ is hydrogen or methyl group.

$$\text{R}^2\text{O} \equiv \text{OC} - \text{NH} \equiv \text{R}^3$$

Carbamates vary in their spectrum of activity, mammalian toxicity and persistence. They are relatively unstable compounds that break down in the environment within weeks or months. Carbamates are commonly used as surface sprays or baits in the control of household pests. Carbaryl, the first successful carbamate, was introduced in 1956. Two distinct qualities have made it a widely used insecticide. First, it has very low mammalian, oral and
dermal toxicity. Secondly, it has a rather broad spectrum of insect control. This has led to its wide use as a lawn and garden insecticide. Propoxur is highly effective against cockroaches that have developed resistance to organophosphates. It is commonly used by pest control operators for the control of cockroaches and other household insects in restaurants, kitchens and homes. Bendiocarb has found its greatest use as household, and ornamental insecticide. Several Carbamates have systemic use in plants because they have a high water solubility which allows them to be taken up by the roots and into the leaves of plants (Diazinon,2009). Carbamates are used as either dusts or sprays. They may be absorbed through the skin as well as by ingestion and inhalation. The immediate toxic effect of Carbamates is very similar to that of organophosphates, but the recovery is comparatively rapid. Spontaneous recovery without medical treatment occurs generally within 4hours of an exposure which has produced symptoms and sings of headache, weakness, nausea, or vomiting, and other. More severe symptoms and signs generally prompt medical treatment. Like organophosphates there is no clear evidence that Carbamates have adverse health effects from long-term exposure at levels that do not affect acetylcholinesterase levels. Carbamates are not regarded as mutagenic carcinogenic or teratogenic substances. If Carbamate insecticide is ingested, vomiting should be induced. If absorbed through the skin contaminated clothing should be removed and the skin washed thoroughly with soap and water. In Carbamate poisoning, the use of atropine is indicated, but oximes are contra-indicated. The most important human exposure route is dermal. Those most likely to be affected are occupationally exposed workers, such as insecticide formulators and applicators, and farm workers (Diazinon,2009).
Low level exposure to residues in foods may occur wherever Carbamates are used on crops or food producing animals. These residues should be within the maximum residue limits prescribed by the food standards code. A low level exposure may result from use of Carbamates in control of household pests through the use of surface sprays or baits. Such low level exposures would not be expected to be of any clinical significance.

2.3 Presence of Carbamate Pesticides in the Human Food Supply:
Pesticides are used widely in agriculture to control a variety of detrimental organisms such as microscopic fungi, weeds, insects, and rodents. Their application has preserved crop yields and led to increased consumption of fresh fruits and vegetables, thereby contributing to improvements in public health. Furthermore, it is widely acknowledged that the use of pesticides has permitted the control of pathogenic microorganisms, which produce highly toxic or carcinogenic metabolites and hence reduces the potential hazards of their presence in the food supply. However, because they are designed to kill living organisms, pesticides are potentially hazardous chemicals which can cause injury to humans if exposed to exceedingly high levels. Human dietary intake of pesticide residues comes from different sources such as: on-farm or postharvest use, or use on imported foods, and use of banned pesticides in the environment. Carbamates are used to control pests (insects, fungi, nematodes, grasses) on fruit, cereals, vegetables, ornamentals, and other crops. As consequence, the use of Carbamate pesticides and their presence in foods are subject to stringent regulations.

2.3.1 Carbamate Residues methodology:
N-methyl carbamates (NMCs) have been widely used for pest control due to their effectiveness and broad spectrum of biological activity. Because most NMCs are acutely toxic, their residues in foodstuffs have a potential hazard to the health of consumers. As a result, it is necessary to establish a simple sensitive method for the determination of NMCs residues in vegetables. Many analytical methods have been developed for the determination of NMCs and their metabolites in the food stuffs. NMCs pesticides are thermally labile and not amenable to gas chromatography. High-performance liquid chromatography (HPLC) is preferable and often used to monitor NMCs with various detectors. Currently, the hyphenated techniques such as HPLC–fluorescence and HPLC–mass spectrometry (MS) offer the prevailing schemes for the determination of residual pesticides, due to their specific identification, good selectivity, and sensitivity (Xucong, et al 2008)

2.3.2 Carbaryl:

Common name: carbaryl, trade name sevin, UPAC name: 1-naphthyl methylcarbamate. Mol.wt.201.2 Solubility in water 120mg/l (20°C).Readily soluble in polar organic solvents. In dimethylformamide, dimethyl sulfoxide 400-450, acetone 200-300, cyclohexanone200-250, isopropanol100 xylene100 (all in g/kg,25°C). Carbaryl, is weak cholinesterase inhibitor. Mode of action insecticide with contact and stomach action, and slight systemic properties.

![Fig(1) Carbaryl chemical structure](image-url)
uses in control of Lepidoptera, coleoptera, and other chewing and sucking insects, different crops including vegetables, tree fruit, mangoes, olive, okra, cucurbits, cotton, potatoes, ornamentals, forestry. Ecotoxicology toxicity to Carbaryl oral LD$_{50}$ 250mg/kg to 850mg/kg for rats, 100mg/kg to 650mg/kg for mice. Birds Acute oral LD$_{50}$ for young mallard ducks 2179mg/kg young pheasants 2000mg/kg. Fish LC$_{50}$(96h) for rainbow trout. Plants metabolites of carbaryl are 4-hydroxycarbaryl, and methylol-carbaryl. In Soil/Environment under aerobic conditions, carbaryl at 1ppm degraded with DT$_{50}$ 7-14 in a sandy loam and 14-28d in a clay loam. Carbaryl kills both targeted (malaria-carrying mosquitoes) and beneficial insect (honeybees) as well as crustaceans. Although approved for more than 100 crops in the Us Carbaryl is illegal in several countries, including UK Austria, and USA. Carbaryl is often produced by using methyl isocyante as intermediary. A leak of methyl isocyante used in the production of carbaryl caused the Bohpal disaster (1984). The largest industrial accident in history. This accident caused around 11,000 death and over 500,000 injuries. Carbaryl is cholinesterase inhibitor and is toxic to human. It is classified as likely human carcinogen (Tomlin, 2000)
2.3.3 Carbosulfan:

Common name: carbosulfan, Trade names Marshal, UPAC name: 2,3dihydro-2,2dimethylbenzofuran-7-yl(dibutylaminothio)methylcarbamate

Physical chemistry: Mol .wt 380.5. It is color From orange to brown clear, viscous liquid. Density1.056(20°C), Solubility in water 0.3ppm(25°C). Miscible with most organic solvent, e.g.methanol, ethanol, acetone, hexane.

Fig(2) Carbosulfan chemical structure

Carbosulfan biochemistry is cholinesterase inhibitor; it is activity is due to in vivo cleavage of the N-S bond, resulting in conversion to carbofuran.
Carbosulfan is a systemic insecticide with contact and stomach action.
Used in control of wide range of soil dwelling and foliar insect pests.
Examples of uses include control of millipedes, springtails, aphids, flea beetles, caterpillars. The product is used in a wide range of crops, e.g. cotton, sugar beet, top fruit, coffee and vegetables. It is compatible with liquid fertilizers. Mammalian toxicology of Carbosulfan are:(1)oral acute oral LD$_{50}$ for male rats 250mg/kg, female rats 185mg/kg. inhalation LC$_{50}$ for male rats 1.53, female rats 0.61mg/l air. and it Ecotoxicology for: birds acute oral LD$_{50}$ for mallard ducks 8.1mg/kg, quail 82mg/kg.
**Fish** LC$_{50}$ for bluegill sunfish 0.015, trout 0.045mg/l. **Bees** toxic to bees. Environmental fate of Carbosulfan in: **Rats**, following oral administration, rapidly metabolized by hydrolysis, oxidation and conjugation, forming Carbofuran methylol, Carbofuran phenol, and 3-hydroxy and 3-keto derivatives; the metabolites are rapidly excreted. **Plants** metabolites include Carbofuran and 3-hydroxycarbfuran. **Soil/Environment** in soil, rapidly degraded under both aerobic and anaerobic conditions. Under field conditions Carbosulfan and Carbofuran are unlikely to leach to groundwater (Tomlin, 2000)

### 2.4 Vegetables:

Vegetables are an essential component of the human diet. Mostly, vegetable crops are cultivated under high pressure for achieving higher production. This involves repeated application of pesticides for the control of various insect-pests. Chemical pesticides have been widely employed for effective controlling of pest complex of various vegetable crops, but their indiscriminate use may create health hazards due to toxic residues that may persist in amounts above allowed Maximum Residue Limits (MRL). The problem becomes more acute if the pesticides are applied close to harvest as well as during transport and in vegetable yards. Hence, it becomes imperative to regularly monitor the vegetables for pesticide residues.

Vegetables are plants that differ in their kinds, types, and benefits. It could be said that vegetables are basic economic and food source in the world, and their environmental need differ from kind to another. They also differ in serving methods and crop caring from fertilization, soil plowing and spraying of pesticides to get rid of pest. Vegetables are herbal plants most of them are annuals or biennials and few of them are long-lived. Vegetables
differ among each other by the part used for food. They are divided into three divisions including:

1) Botanical division according to the monocotyledonae like onion
   (*Allium Cepa*) or dicotyledonae like broad bean (*Pisum Sativum*) Jews mallow (*Corchorus Olitorius*)

2) According to the edible part like root plant carrot (*Daucus Carota*) or leaves plant lettuce (*Lactuca Sativa*).

3) According to temperature needs i.e. summer and winter, the winter vegetables are solid like broad bean, garlic, or onion. The summer vegetables are tender like cucumber, snake melon, and others.

**2.4.1 Vegetables production in Sudan:**

The agricultural sector is considered the backbone of the Sudan economy. It contributes with nearly 45% of the Gross Domestic product and employs about 65% of the working force. This sector plays the primary role in food production like cereal crops, vegetables, fruits, and legume crops (Seid Ahmed, *et al* 2003). Vegetables production is gaining an increasing importance in the Sudan. Vegetables area have greatly expanded due to the increased demand, influenced by urbanization and arising awareness of vegetables nutritional status and potential for export to the neighboring countries. In Sudan, there are many big aspects for the expansion in vegetable production as natural resources like fertile lands, water, or various climates. All these allow the opportunity to produce numerous types of vegetables, and there are huge capabilities to increase the productivity and production by horizontal and vertical expansion. Sudan is growing vegetable especially in the irrigated areas and high-rain regions since long time ago. Vegetables growing are concentrated in the irrigated sector and it occupies the most fertile lands and most of the output comes from privately owned
lands, spreading along the rivers banks and valleys and are artificially irrigated. Sudan has become self-satisfied of vegetables. Sudan started to export some of the types to markets in Europe and Gulf Countries since the beginning of the 1975. They export green pepper, beans and others. In the last years the quantity exported increased especially for muskmelon, hot pepper (*Capsicum frutescens*) okra, and cucumber (*Cucumis sativus*). Vegetable crops contain important and essential nutritional ingredients for the human life and health like salts, minerals, or others and therefore they are essential elements of balanced food that leads to healing the individual and increasing productivity and in turn increasing the national product. Vegetables play an important role in food security and solving the problem of nutrition. The increase in vegetables production also leads to supplying raw materials for food industries. The importance of vegetables come from the fact that they: (1) contains various nutritional substances like starches, fats, proteins. (2) The existence of the fibers in vegetables activates the stomach and facilitates the digestion process. (3) They contain salts, minerals, and vitamins. (Said Ahmed *et al*, 2003)

### 2.4.2 pests and diseases of vegetables:

The combat processes against agricultural pests should be carried out either by protective procedures to protect the plant and the production or by curative procedures. Plant diseases are either viral, bacterial, or fungal all these are cured by the resistant varieties, and/or chemical uses such as fungicides, bactericides, Insecticides and solar sterilization. Insects that hurt plants include Aphids and Thrips which are absorbing insects and worms which are leaf feeders. Different methods of insecticides were applied against insects. The sucking insects are sprayed with systemic insecticide while chewing insects are sprayed with contact insecticides (Said Ahmed *et al*, 2003).
Pesticides use on vegetables differ according to the pest types that affect the product, they could be insecticides, fungicides, or herbicides. Most of these pesticides are highly toxic and their effect on the plant and soil lasts for a long time which is known as pesticides residues that cause a lot of harms for human, water, and environmental constituents. The most important pesticides used on vegetables are groups of organophosphorous(malathion) Carbamates and pyrithroids. Carbamates are more used and they are less toxic than the organophosphorous group, they include carbaryl, methiomylin. These pesticides are frequently used in combating the pests of pumpkins and the insects of Aphids and Thrips. (Said Ahmed et al 2003)

2.4.3 Family Cucurbitaceae:

The Cucurbitaceae is a large family found mainly in the warmer parts of all continents. It consists of about 119 genera, with around 825 species. Many of which are eaten in one form or another. Cucurbits are among the most economically important vegetables grown throughout the world, they rank next to the solanaceous group in terms of popularity and share in the international vegetable export business.

2.4.4 Cucumis melo: Snake melon

Is one of the important species of the genus cucumis. Many authors showed that cultivated species of cucumis are indigenous to the tropics and subtropics of Africa and India. Melon, like most cucurbits are epigeally germinated, and have trailing stems, hirsute, scabrous and usually angled with primary branch, the main axis sympodium, leaves are simple alternate tendrils are borne on axils of the leaves. Flowers are monoecious, the pistillate flowers occur singly at nodes of secondary branch, seed are whitish, 5-15mm long. The uses of vine crop in family cucurbitaceae range
from fresh dessert and salad (cucumber, melon,...) to baked vegetables utilizing immature fruit (summer squash) or hardened mature fruit (winter cucumber). Snake melon like other cucurbit plants, provide significant amount of vitamins and minerals (vitamins A and C).

2.4.5 Snake melon in Sudan:

All cucurbits are native to warm, humid, or sometimes arid regions indifferent parts of the world. In Sudan, snake melon is cultivated all over the country whenever irrigation water or sufficient rains are available to sustain its production. It is grown in small plots specially in western and central Sudan. In the Gezira, the area under cultivation is fluctuating from one season to another. Generally cucumber is a summer crop, and the plant thrives best at relatively high temperatures, but because it is a short maturing crop the snake melon could be grown during the slightly hot winter days when it becomes very rewarding and highly profitable (Ahmed 2005). It can be grown during the whole year, its production decreases in extreme coldness and extreme heat when the crop is exposed to hard weather factors and incidence of diseases and insects which reduce its productivity. Its cultivation works in light clay lands with good drainage like the lands of cliffs and islands. Varieties are local, like Gamoeya, Gadanbalya, Karary or Silka. Cucumber in northern and central regions is cultivated in all year.

1.5-2kg of seeds for one Fadden and in accordance with the method of cultivation, variety, and season.

Pests like Pumpkin fruits fly, lays its eggs in groups inside the fruits where they hatch and the larvae come out and start feeding on the contents of the fruits thus, elimination and exposing them to infections with fungi and bacteria causing rotting. Removal of all infected fruits and burn them take off all the withered plants and the collection of full insects and exterminate.
them. Powdery mildew and it is treated by spraying of sulfur compounds and intracowa pesticide. Snake melon harvested after 30-45 days from cultivation and in accordance with the variety and the season.
The Fadden produces from 10 to 20 tons according to the agricultural loop climate, and agronomy.
Chapter Three
Materials and methods

This study was conducted as a pilot project for residue analysis on vegetables ready for consumption. Sample collection was done in wad Medani market areas. Extract and standard material were prepared by the pesticide laboratory of the Agricultural Research Corporation (ARC) Wad Medani. Extract cleanup and analysis were done by High Performance Liquid Chromatograph (HPLC) in Central laboratory in Khartoum. The Study was conducted between July to November 2012.

3.1 Sampling:
A total of 9 sample of different type of cucumber were collected from baskets, ready for sale, of different random production area from various market places in wad-Medani town (Fadacy, Main market, Hantob, Public market, Abu haraz, Central market, Atra, Um sount, New market)

3.2 Apparatus:
1-waring blender
2-rotary vacuum evaporator
3-chromatographic columns- with Teflon stopcocks 10 mm id×50 cm long
4-laboratory shaker
5-readymade thin layer chromatography plats, silica gel F$_{254}$, 20×20 cm
6-developing tank

3.3 Reagents:
1-Solvents : (n-hexane, ethyl acetate, benzene, methylene chloride, acetone acetonitrile)
2-Chemicals: (sodium sulphate anhydrous LOBA CHEMIE , sodium bicarbonate Analar reagent (BDH Chemicals, Dorset, UK)
3-Silica gel: 5ml of water was added to 95g silica gel activity, and mixed thoroughly then shaked to get powdery material and kept tightly closed for 24hr before use.

4- Carbosulfan Pesticide analytical standards obtained from the formulation laboratory in ARC with quality certificate. Carbaryl standard were prepared from sevin by using methanol solvent and recrystallization (purity assay is 98%)

3.4 Sample preparation:
The samples were cut with kitchen knife. resulting materials were homogenized carefully and aliquots were taken for analysis.

3.5 Extraction:
Following Ambrus et al (2005) method with slight modification. A 60g portion of homogenized laboratory sample was extracted with 120mL ethylacetate, in presence of 15g NaHCO₃ and 60g anhydrous Na₂SO₄ using waring blender. Na₂SO₄ was added to remove the remaining water. The sample solvent mixture was kept for 15 minute to let the solvent separate from solid material. The separated solvent was filtrated through a small cotton wool plug (60mL filtrate). The filtrate was evaporated in a rotary evaporator to nearly dryness. The residue was dissolved in benzene (5mL).

3.6 Clean up:
5g of deactivated absorbent were placed in 10mm id column with gentle vibration. Pre-wet adsorbent with 15mL n-hexane. Pipet extract (equivalent to 10g sample) onto top of adsorbent. Pesticides were eluated by using the following solvent 16mL benzene, 20mL benzene-ethylacetate(1+1), and 50mL ethylacetate. The fraction was combined and evaporated to dryness, the residues were dissolved in 5mL acetone (Ambrus et al. 1981).
3.7 Thin layer chromatography method:

Spot 20µL from standard solution and extract on chromatographic plate 2cm from bottom. Place entire plate in 1cm eluting solvent (ethylacetate) in saturated developing tank. The plate was eluated until solvent front move 15cm from start. Detection of Carbaryl and Carbosulfan done under UV lamp, Carbaryl and Carbosulfan Rf 0.56, 0.66 respectively. (Ambrus et al. 1981, 2005)

3.8 HPLC Analysis:

Following the sample clean-up, aliquots of the final volume were quantified by using HPLC, equipped with waters 2487 variable wavelength UV-Vis. The analytical column was a C-18 reverse Phase Varian (150 × 4.6 mm, 5 µm) that was maintained at 30 °C in a column oven. The mobile phase, a combination of 50% acetonitrile and 50% water with pH 4, was filtered by using a cellulose filter of 0.45 µm before each use. The flow rate was 0.8 mL/min, and all solvents used were of HPLC grade. Prior to HPLC analysis, the samples were passed through 0.45 µm of nylon (Alltech Associates, IL, USA) syringe filters. The 20 µL samples were manually injected each time. The identification of the suspected pesticide was performed, relative to the retention time of the pure analytical standard. Quantification was performed based on the concentration of the two insecticide were calculated by the following equation

\[
\mu g/g = \frac{\text{peak height of the sample}}{\text{peak height with the standard}} \times \frac{\mu l \text{ of the standard}}{\mu l \text{ of the sample extract injection}} \times \frac{\text{final volume of sample extract (ml)}}{mass \ in \ g \ of \ sample} \times \text{Conc. in ppm of reference solution}
\]
Chapter Four
Results and Discussion

4.1 Results:

Residues of two insecticides namely Carbaryl and Carbosulfan were analyzed in samples of cucumber grown in production areas known around Wad Medni brought for sale in the local markets.

The extraction was performed by ethyl acetate for both insecticides then the extract was subjected to column chromatography clean-up. Qualitative and quantitative analysis were performed by reverse phase high performance liquid chromatography with UV-detector at fixed wavelength has been used to detect Carbaryl and Carbosulfan on cucumber separately. The analytical parameters were chosen by using standard solution. The chromatographs of Carbaryl and Carbosulfan standard prepared in the laboratory are shown in Fig(4.1,4.2). The amount of Carbaryl and Carbosulfan in the samples was calculated according to the equation based on the ratio of the peak areas of the external standard and the sample. The isocratic conditions selected for the analysis result in a retention time of about 3.95 minute for Carbaryl and 3.63 minute for Carbosulfan. Recoveries of the analytical procedure are above %85 for both insecticides.
Figure(4.1) chromatogram showing peak of standard carbaryl(50ppm)
Figure (4.2) chromatogram showing peak of standard carbosulfan (500ppm).
Residues of Carbaryl and Carbosulfan detected in the selected samples are listed in table (1). Carbaryl residues were not detected in all samples, but Carbosulfan residues appeared in four samples with concentration between 0.069- 0.014 µg/g figure (4.3,4.6). Carbosulfan is well known in Sudan as trade name marshal is used as seed dresser in controlling cotton pests since season 90/91(Kennan and Omer, 1992), marshal was banned by the European Union in 2007.
Sarbani et al (2002) suggested that Carbosulfan is a potent genotoxic agent and may be regarded as a potential germ cell mutagen also. Carbosulfan has very low maximum residue limits for use in the EU and UK examples of this can be seen in apples and oranges, where it is 0.05 mg/kg.
Table (1): Concentration of Carbaryl and Carbosulfan in Cucumber Samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Carbaryl conc. (µg/g)</th>
<th>Carbosulfan conc. (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fadacy village</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Main market</td>
<td>ND</td>
<td>0.027</td>
</tr>
<tr>
<td>Hantob</td>
<td>ND</td>
<td>0.042</td>
</tr>
<tr>
<td>Public market</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Abu-haraz</td>
<td>ND</td>
<td>0.069</td>
</tr>
<tr>
<td>Central market</td>
<td>ND</td>
<td>0.014</td>
</tr>
<tr>
<td>Atra</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Um sount</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>New market</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

“ND” not detected
Figure (4.3): Chromatogram Showing Peak Of Carbosulfan In Sample From Main Market
Figure (4.4) Chromatogram Showing Peak Of Carbosulfan In Sample From Hantob
Figure (4.5) Chromatogram Showing Peak Of Carbosulfan In Sample From Abu-Haraz
Figure (4.6) Chromatogram Showing Peak Of Carbosulfan In Sample From Central Market
From figures there are co-peaks appear with the selected insecticide these peaks could be metabolites as the method used in this study could cover wide related compounds. There is a very sharp peak at RT of 2.38 minute representing a very polar compound (unknown) with high concentration appeared in chromatograms of all samples. Thin layer chromatography method was used as confirmation test. The maximum detected quantity (MDQ) are 1000ng under UV-lamp and 50-100ng with o-TKI for carbaryl, and carbosulfan 500ng under UV-lamp and 100ng with o-TKI. The ultraviolet (UV) detector has so far been the most frequently used for the LC determination of carbamate pesticides. However, confirmation of pesticides of the same class is made difficult by the high degree of similarity of their UV spectra. As a result, LC–UV based methods are most effective for fast screening of samples, but usually require additional confirmatory analysis in the case of positive samples (Hogendoorn and Zoonen, 2000).

4.2 Discussion:
In terms of pesticide residues some samples contained more than one residue. The reason for that might be that cucumber cultivated under field conditions are highly sensitive to pests and need for successive applications of pesticide treatments, leaving in consequence higher level of residues that tolerated. In the present study, the most irregularities were due to a poor compliance of the pre-harvest interval, especially after repeated treatments with some active ingredients. The Maximum Residue Limits (MRLs) as food standards differ widely for the same pesticide on the same commodity between countries as well as with the international Codex Committee standards (Codex, 2010). However, scientists cannot say for sure that there is ever a “safe” level of pesticide residues in food because many of the chemical messengers in
human bodies function at precisely minute quantities of ppm or even ppb (Boobis, *et al.*, 2008).
CHAPTER FIVE

Conclusions and Recommendations

5.1 Conclusions
In this study it was proved that some pesticides of the carbamates leave detectable residues in or on vegetables. Farmers do not stick to good agricultural practices. It is advisable to train them on the pesticides harmful effects and to monitor the application. Many of the pesticides which are used on vegetables are not prescribed for them and can cause residual effects.

5.2 Recommendations:

1- Farmers should be restrict to the good Agricultural practice or guidelines.

2-Vegetable and Fruit, should be rationally monitored for pesticide residue.

3-Farmer must be taken into account the safety period, during the time after pesticides spray and before harvest and consumption of vegetables especially these which are eaten fresh or cooked.

4-Continue in the markets for agricultural products and assessment of pesticide residues which traces amount, use high-technical device through laboratory analysis.
5-Wash vegetables under running water well for long time to get rid of the pesticide residues conjoined.

6-Do not buy vegetables that smell traces of pesticides.
References


Kannan, H O, and Omer, E E(1992) Marshal as Seed Dresser on Cotton. 58th Meeting of the National Pest and Diseases Committee. ARC (Sudan)


Tadeo, J T (2008) Analysis of pesticides in food and environmental samples, New York, CRC Press LLC,

