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EXTRACTION / ISOLATION AND DETECTON OF CARBOFURAN FROM KOHLRABI (GATH GOBI)

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ABSTRACT

Pesticides are used to protect the fields, crops and grains from the pests, insects, rodents, fungus etc. Different kinds of pesticides have been used for crop protection for centuries. Pesticides protect the crops but have serious health effects on humans, animals and environment through the direct or indirect exposure. Excessive use of pesticides may lead to the destruction of biodiversity. Pesticides are a concern for sustainability of environment and global stability. Carbofuran is a carbamate pesticide which is used to control insects on a wide variety of agricultural crops. An attempt has been made to develop a new method for analysis of carbofuran in kohlrabi (gathgobhi) by using Thin Layer Chromatography (TLC). Developed TLC plates were viewed under UV light followed by spray of chromogenic reagents which successfully increased the sensitivity without dispensing with the simplicity of the method. The method developed is a simple, rapid, inexpensive, non-destructive and reproducible which can be performed easily in any laboratory.

Keywords: Drug, Pesticides, carbofuran, kohlrabi, extraction, TLC etc.

INTRODUCTION

A pesticide that are intentionally used into the environment in order to protect/avert, control/ kill/destroy populations of insects, weeds, rodents, fungi, herbs or other harmful pests. These are harmful/toxic chemical substances or a mixture of substances or biological agents example are organochlorines, carbamates, organophosphates. Pesticides are a broad class of bioactive compounds important for food and crop production and for human health (1,2). A "pest is any animal/insect or plant that could damage/ destroy the crops/fields or food, forestry". The use of pesticides has increased many folds over the past few decades. According to an estimate, about 5.6 billion pounds of pesticides are used worldwide every year (2-4). Pesticide use for pest mitigation has become a common practice all around the world. Their use is not only restricted to agricultural but they are also being used in the form of sprays, poisons and powders for controlling cockroaches, mosquitoes, rats, fleas, bees, ticks and other harmful bugs. Due to this reason, pesticides are frequently found in our food commodities/water bodies in addition to their presence in the air (5,6). Pesticides can be natural compounds or they can be synthetically produced. Pesticides major classes are organochlorines, carbamates, organophosphates, pyrethroids and neonicitinoids. Pesticides break down into substances known as metabolites

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when they are released in environment that are more toxic. Non-selective pesticides kill non-target plants and animals along with the targeted ones (11).

CLASSIFICATION OF PESTICIDES

Pesticides are classified on the basis of their action mechanism. Nerve targets of insects which are known for development of neuroactive insecticides include acetylcholinesterase for organophosphates and methylcarbamates, nicotinic acetylcholine receptors for neonicotinoids, gamma-aminobutyric acid receptor channel for polychlorocyclohexanes and fiproles and voltage-gated sodium channels for pyrethroids and dichlorodiphenyltrichloroethane (7-9). It is an observation that the use of neonicotinoid/ acetylcholinesterase pesticides is increasing. These pesticides are associated with different types of toxicities. Pesticides are considered to be more water soluble, heat stable and polar which makes it very difficult to reduce their lethal nature (12-14).

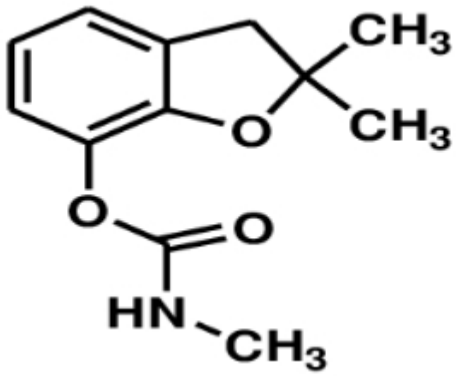


Fig.1: Chemical structure of carbofuran

Carbofuran, an anticholinesterase carbamate. Its two major metabolites (3-hydroxycarbofuran and 3-ketocarbofuran) on overall toxicity. Biochemical (cholinergic and noncholinergic), hematological, and immunological effects induced by carbofuran. To date, a combination of atropine and memantine remains the most effective antidotal treatment against acute carbofuran toxicity (11-16).

.Herein, an attempt has been made to develop a method for extraction and detection of carbofuran from gath gobhi using TLC.

EXPERIMENTAL

Material Required

Chemicals/Reagents: Acetone, Acetonitrile, n-hexane, Anhydrous sodium sulfite, Cyclohexane, Benzene, Silica gel, Medical charcoal, Sodium chloride, Dichlormethane (DCM), from Merck Life Sciences Pvt. Mumbai India were used.

Apparatus: Centrifuge (Remi centrifuge Mumbai, India), Vortex (York Sahibabad), Pipettes (Magnus Enterprise, India) were used.

TLC Plate: TLC plates of Pre-coating Silica gel 60 F254 Merck, Germany were used.

Glassware: Chromatographic chamber, test tube, beakers, glass funnel, bowls from Borosil India was used.

Miscellaneous: Blotting paper, Porous glass filter paper, Ultrapure water from Rions India Biotech Solution, New Delhi, India were used.

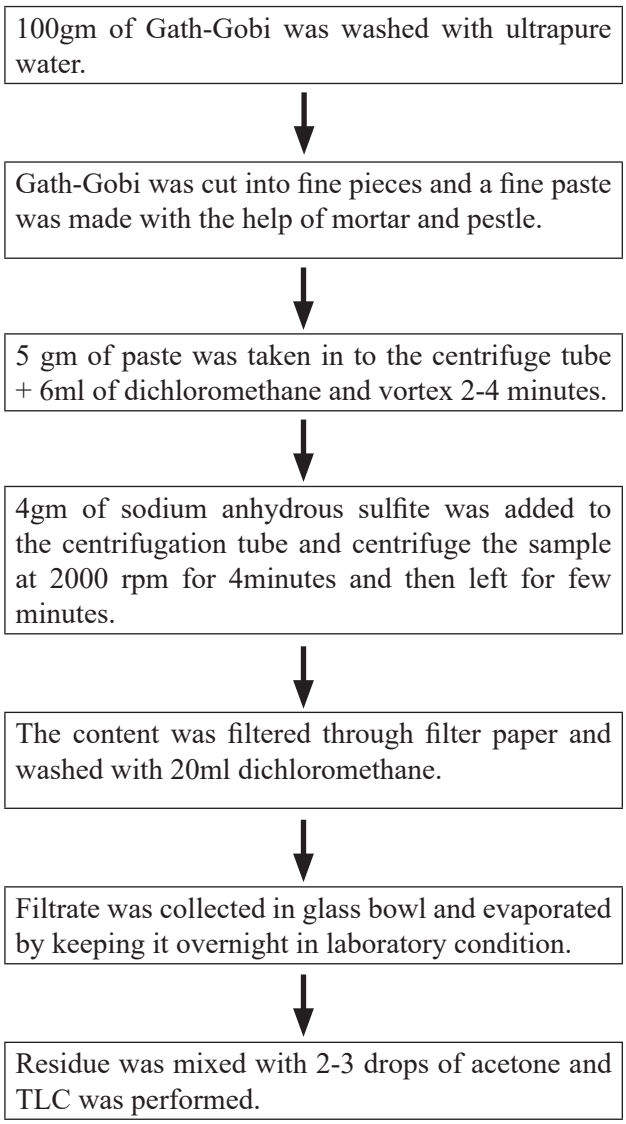
Preparation of Standard: Carbofuran standard were prepared by dissolving 100 mg of the standard in 100 ml Acetone or Benzene, and stored in dark containers.

Sample preparation

Pesticide was extracted from gath gobhi using liquid-liquid extraction. The steps are as follows:

1. 100 gram of Gath-Gobi was washed with water followed by ultrapure water and then cut into fine pieces and taken in mortar and pestle. A fine paste was made with the help of mortar and pestle.
2. 5 gram of paste was taken in centrifuge tube.
3. 6ml dichloromethane was added to the paste in centrifuge tube and vortex for 2-4 minutes.
4. 4gm sodium anhydrous sulfite was added to the centrifuge tube, and centrifuged at 2000 rpm for 4 minutes, and then left for few minutes.
5. The content was filtered through filter paper and washed with 20ml dichloromethane.
6. Filtrate was collected and evaporated by keeping it overnight in laboratory condition.
7. Residue was mixed with 2-3 drops of acetone and TLC was performed.

Same process has been represented in flow chart manner:



Spotting of samples and standard on HPTLC plates

Carbofuran extracted from gath gobhi was loaded on TLC plates along with the standard.

Development of HPTLC plates

The plates were kept in different solvent system which were taken in different ratios. After developing on TLC plates, the plates were taken out of the chamber and dried in air then the plates were subjected to UV light at 254nm.

Visualization of HPTLC plates

Dried HPTLC plates were sprayed with bromophenol blue reagent. Blue colored spots were observed.

RESULTS AND DISCUSSION

Dried TLC plates were sprayed with bromophenol blue reagent and blue spots were observed. The mobile phase (Benzene) used gives a compact spot. Pesticides are widely used in agriculture and can thus accumulate in crops and vegetables. High level of pesticides in food can lead to the kidney and lung ailments. Thin layer chromatography (TLC) proved to be an excellent choice because it is a simple equipment, less time consuming, low cost of TLC plate and can be easily performed in every laboratory.

CONCLUSION

The method developed for analysis of carbofuran in Kohlrabi (Gath Gobi) using liquid-liquid extraction followed by thin layer chromatography (TLC) is a rapid, simple and economical technique and can be performed easily in very less time in comparison to other sophisticated instruments.

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