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New chromogenic Spray for Detection and Identification of Carbendazim from Biological Material

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Abstract:

Thin layer chromatography(TLC) is a simple, rapid and reliable technique usually used in forensic science laboratory for detection of poison in biological material. It can separate many complex mixtures in a short period of time. In this study an effort has been taken to determine Carbendazim by using Thin Layer chromatography. A chromogenic reagent 1 % of sodium hydroxide followed by 0.2% of Bromophenol Blue in acetone has been used for the detection of Carbendazim with Chloroform:Acetone (7:3) as a solvent system for the separation.

Keywords: Forensic science, Carbendazim, Bromophenol Blue, Thin Layer Chromatography

Introduction:

Carbendazim (methyl 1H-benzimidazol-2-ylcarbamate) is a systemic broad-spectrum fungicide having chemical formula C9H9N3O2 and mol. wt. 191.19. It is also obtained as degradation products of thiophanate-methyl and benomyl fungicides, a benzimidazole fungicide, is generally used in treatment and control of fungal diseases in vegetables, flowers, fruits and several other plants such as banana, mango, strawberries, oranges, pineapples, pomes, cereals, sugar beet, fodder beet, rape seed, ornamental plants [1]. Further, carbendazim in combination with mancozeb exhibited an effective control of sunflower leaf blight, chilli rots and mango anthracnose, In addition, carbendazim is also used in paint, textile, paper and leather industries [2]. It is among the top five pesticides used in India with annual consumption of 1992 metric tonnes. It stands second after mancozeb in terms of most consumed carbamates in India. It is registered for 18 crops by The Central Insecticides Board and Registration Committee (CIBRC) in India. These crops are paddy, wheat, barley, tapioca, cotton, jute, groundnut, sugarbeet, peas cluster, beans, cucurbits, brinjal, apples, grapes, walnut, rose and mango [3]. Due to its severe toxicity and persistent nature, Carbendazim has been banned in Australia, most of European Union and USA. But developing countries such as China, Brazil and India are still permitting the production and use of carbendazim in various formulations [4].

Experimental:

Chemicals, reagents and solutions:

All reagents used were of analytical-reagent grade. Standard Carbendazim (S.D.Fine-Chem Limited, Mumbai, India) solution was preapared in N,N Dimethyl Formamide. Sodium hydroxide (S.D.Fine-Chem Limited, Mumbai, India) solution (1% w/v) was prepared by dissolving 1 g of sodium hydroxide in 100 ml distilled water. Bromophenol Blue (S.D.Fine-Chem Limited, Mumbai, India) solution (0.2% w/v) was prepared by dissolving 0.2g of Bromophenol Blue in 100ml Acetone.

Extraction of Carbendazim from biological Materials:

A portion of about 100 g each of different types of biological tissues (pieces of stomach, intestine, liver, spleen, lungs and kidneys) containing Carbendazim was taken. Viscera were cut into fine pieces and minced carefully, 100 ml of N,N Dimethyl formamide was added to homogenized visceral sample. The solvent was vigoursly mixed with viscera and left for about 1 hour, and then the liquid was filtered out using whatman filter paper. The extract was transferred to an evaporating dish and the liquid portion was evaporated. The residue was dissolved in 1 ml DMF and the solution was used for spotting.

Thin Layer Chromatography:

Chromatography was performed on pre-coated Aluminium TLC plate (silica gel 60 $F_{254,}$ Merck Ltd. Darmstadt. Germany)for detection of Carbendazim. The extract of blank viscera and Carbendazim containing viscera were spotted on TLC plate along with the spot of Carbendazim standard with fine capillary tubes. The plate was dried and developed in a presaturated tank containing the Chloroform: Acetone (7:3) as solvent system. After development the plate was removed from chamber, dried at room temperature and then sprayed with 1 % of sodium hydroxide followed by 0.2% of Bromophenol Blue in Acetone. After spraying the plate was kept in air. A Blue colour spot observed with purple colour background at $R_F = 0.8$ (Figure 1)

Result and Discussion:

Carbendazim is Systematic fungicide which reacts with 1 % of sodium hydroxide followed by 0.2% of Bromophenol Blue in Acetone gives Blue colour (Figure 1). The colour of spot remains stable. This spray reagent is highly sensitive, stable, easily available and specific for the detection of Carbendazim from biological material. Spots were not observed for Endosulfan (Organochloro insecticide), Monocrotophos, Chlorpyriphos, Triazophos, Quinolphos (Organophosphorus insectcide), Cypermethrin, Deltamethrin (pyrethroid). This spray method is economic, two step spray, reproducible and does not involve in any critical reaction condition. This reagent can also be used for the quantitative estimation of Carbendazim in biological samples. Hence, this reagent can be used routinely for detection of Carbendazim in biological samples.

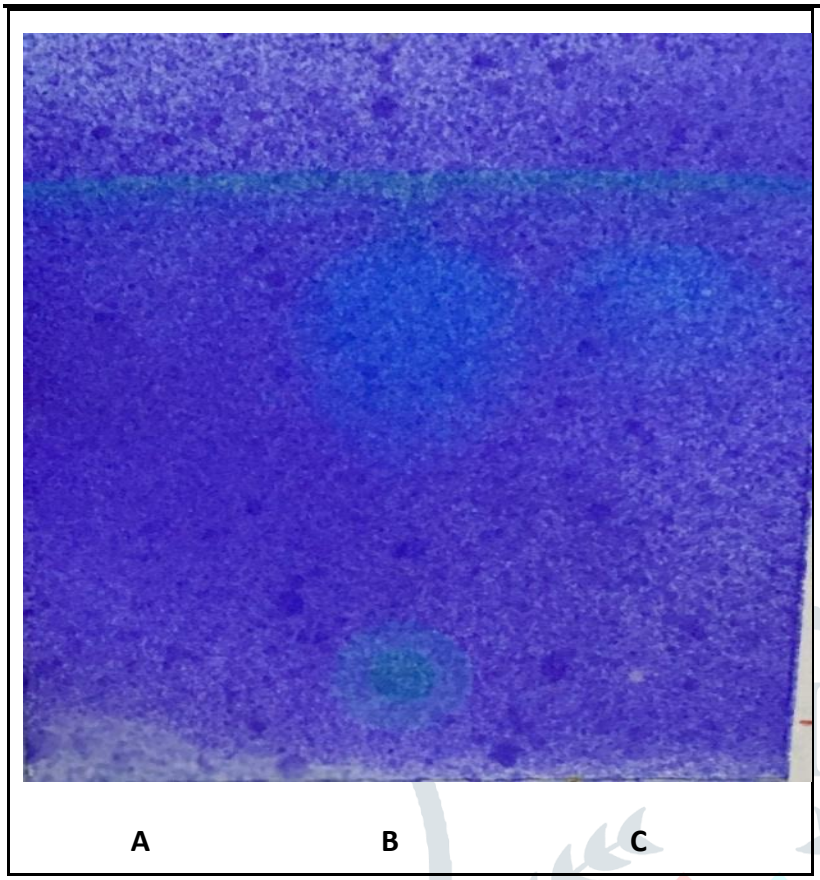

Figure 1. TLC showing spot of Carbendazim.

A) Blank Viscera Extract

B) Carbendazim poisoning Viscera Extract

C) Carbendazim standard

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