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Detection and Identification of Spinetoram insecticide in Biological Material by Thin Layer Chromatography

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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 a thin layer chromatographic method is used for the detection of Spinetoram in biological material. A chromogenic reagent Potassium triodide has been used for the detection of Spinetoram.

Keywords: Forensic science, Spinetoram, Thin Layer Chromatography

Introduction:

Soyabean, Rice, Pulses and cotton are important crops cultivated in India. Due to the prevalence of a tropical climate across the country, these crops are attacked by numerous pests and pathogens that cause drastic yield losses annually. Pesticides play an important role in protecting the crops from these obnoxious pests and increase the yield. The use of new-generation insecticides in crops has been getting encouraged to tackle new challenges set by insect pests. The key advantage of these new chemical compounds lies in the differences in their unique mode of action that enables them to act strongly in the field when used rotationally. Spinetoram was recently registered in India by Dow Agro Science Ltd. for use in crops like cotton, soybean and chili [1]. Spinetoram is a mixture (approx. 3:1 ratio) of two macrocyclic compounds 3O-ethyl-5, 6-dihydro spinosyn J (spinosyn-J) and 3-O-ethyl spinosyn L (spinosyn-L) [2]. Spinetoram is a broad-spectrum insecticide effective against a wide range of pests, including thrips, leafminers, whiteflies, and lepidopteran insects. It's a semi-synthetic chemical

belonging to the spinosyn group and acts by disrupting neurotransmission in insects [3, 4]. Spinetoram is used in various crops including cotton, grains, vegetables and fruits.

Experimental:

Chemicals, reagents and solutions:

All reagents used were of analytical-reagent grade. Standard Spinetoram solution was preapared in Methanol. Potassium tri-iodide spraying reagent was prepared by dissolving 1.00 gram of Iodine (S.D.Fine-Chem Limited, Mumbai, India) and 2.00 gram of potassium iodide (Merck life science private limited, Mumbai, India) in a solvent mixture consisting of 25.0 ml of Ethanol, 25.0 ml of concentrated hydrochloric acid and 50.0 ml of distilled water. The resulting solution was mixed thoroughly until complete dissolution was achieved.

Extraction of Spinetoram from biological Materials:

A portion of about 100 gm of postmortem sample (pieces of stomach, intestine, liver, spleen, lungs and kidneys) containing Spinetoram was taken. Viscera were cut into fine pieces and minced carefully, 100 ml of Methanol 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 Methanol 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 Spinetoram. The extract of blank viscera and Spinetoram containing viscera were spotted on TLC plate along with the spot of Spinetoram standard with fine capillary tubes. The plate was dried and developed in a presaturated tank containing the Methanol (100%) as solvent system. After development the plate was removed from chamber, dried at room temperature and then sprayed with Potassium tri-iodide. After spraying the plate was kept in air. A Brown colour spot observed with yellow colour background at $R_F = 0.71$ (**Figure 1**)

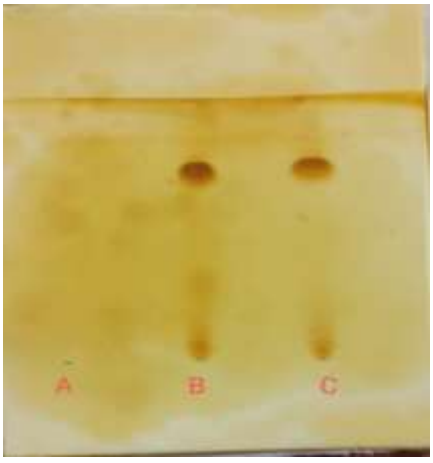

Figure 1. Showing spot of Spinetoram.
A) Blank Viscra Extract
B) Spinetoram poisoning Viscera Extract
C) Spinetoram Standard

Figure 1. Showing spot of Spinetoram.

Result and Discussion:

Spinetoram is a Spinosyn based insecticide particularly macrocyclic lactones having conjugated double bonds and an amine moiety. Spinetoram has no strongly chromogenic functional group like Nitro (-No_2) or phenol so it

requires oxidative derivatization for visualisation on TLC. Potassium tri-iodide is a strong oxidative agent, under acidic condition it can be oxidized primary, secondary alcohols and conjugated unsaturation and released I_2. This I_2 (elemental iodine) forms brown colored complexes with unsaturated and oxidized species as shown in **figure 2**.

Spinetoram is used in crops like cotton, soybean and chili as insecticide which reacts with Potassium tri-iodide gives brown colour spot (**Figure 1**). This spray reagent is stable, easily available and useful for the detection of Spinetoram from biological material. This spray method is economic, single step spray, reproducible and does not involve in any critical reaction condition. This reagent can also be used for the quantitative estimation of Spinetoram in biological samples. Hence, this reagent can be used routinely for detection of Spinetoram in biological samples. limit of detection of this insecticide is up to 10 µg.

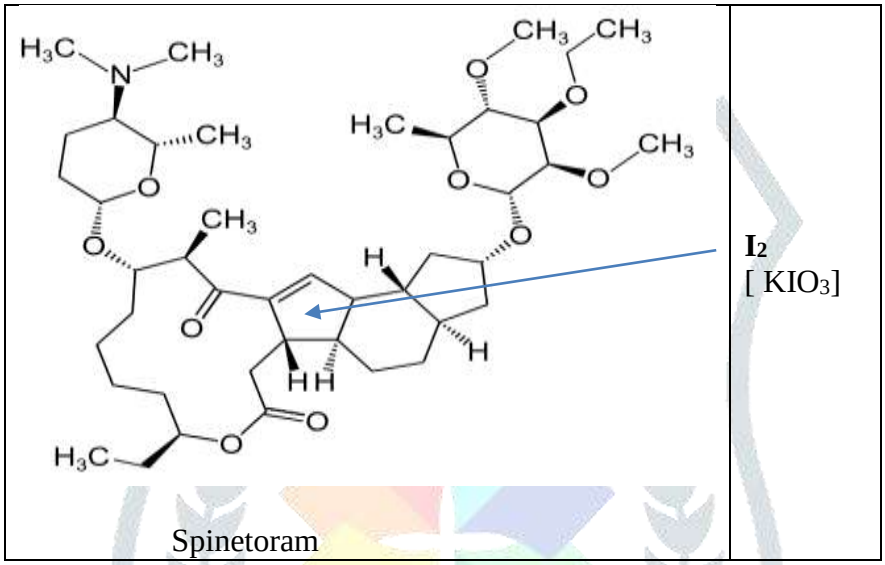

Figure 2: Possible Reaction Mechanism

Conclusion

A TLC method employing potassium iodate as a chromogenic spray reagent was successfully developed for the detection of Spinetoram in biological matrices. The method provides a visible brown spot corresponding to Spinetoram under oxidative conditions. It can serve as a cost-effective screening tool for Spinetoram based poisoning cases in forensic toxicology.

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Abbreviations

TLC Thin layer chromatography

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Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

References:

1. Central Insecticide Boards and Registration Committee. Major Uses of Pesticides; Directorate of Plant Protection, Quarantine and Storage, Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, Government of India: Faridabad, India, 2019. Available online: http://ppqs.gov.in/divisions/cib-rc/major-uses-of-pesticides (accessed on 12 October 2022).
2. Yee, W.L. Spinosad versus spinetoram effects on kill and oviposition of Rhagoletis indifferens (Diptera: Tephritidae) at differing fly ages and temperatures. J. Insect Sci. 2018, 18, 15.
3. Sparks, T.C.; Crouse, G.D.; Benko, Z.; Demeter, D.; Giampietro, N.C.; Lambert, W.; Brown, A.V. The Spinosyns, Spinosad, Spinetoram, and Synthetic Spinosyn Mimics—Discovery, Exploration, and Evolution of a Natural Product Chemistry and the Impact of Computational Tools. *Pest Manag. Sci.* **2021**, *77*, 3637–3649.
4. Campos, M.R.; Silva, T.B.M.; Silva, W.M.; Silva, J.E.; Siqueira, H.A.A. Spinosyn Resistance in the Tomato Borer *Tuta Absoluta* (*Meyrick*) (*Lepidoptera*: *Gelechiidae*). *J. Pest Sci.* **2015**, *88*, 405–412.