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Detection of organochlorine pesticide residues in black pepper and clove using tolidine paper

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Abstract

A simple method was developed for the quick detection of organochlorine pesticide residues in black pepper and clove using tolidine paper, prepared by wetting filter paper strips in 2% orthotolidine in acetone. Colour development when exposed to sunlight, showed the presence of the pesticide residues. The lower limit to which it can be detected was also estimated using gas chromatography fitted with electron capture detector. The tolidine paper detection technique was quick, reproducible and easy to adopt for the detection of organochlorine pesticides like benzene hexachloride, aldrin, endosulfan and dichloro diphenyl trichlro ethane also in black pepper and clove.

Kew words : black pepper, clove, organochlorine pesticides, pesticide residue, tolidine paper.

Abbreviations

BHC : Benzene hexachloride
DDT : Dichloro diphenyl trichloroethane
OC : Organochlorine

OC pesticides are widely used in agricultural practices of spices and vegetables. BHC, though recommended only for seed and ground preparation prior to sowing, is also used for the control of insect pests in these crops. When used for spices, the pesticide residues are retained on the essential oil rich surfaces for a long time. The permitted level of BHC in selected agro products is fixed at 5 ppm and the lindane isomer at 10 ppm (Patty 1962). Kannan *et al.* (1992) reports that FAO/WHO has estimated that the intake of lindane isomer as 600 μg per person of 60 kg weight. Reports on analyses have shown that the spices sold in the market contain varying amounts of these pesticde residues (Kaphalia *et al.* 1990). In developed countries the upper limits of different OCs are steadily being reduced due to the new information generated

on the mutagenic side effects and extremely high toxicity of these compounds (Bushway & Fan 1995). Hence, sophisticated instruments and complicated procedures are adopted for the detection and estimation of OCs in agro products, at very low levels. The problem of detection and estimation of these toxic compounds is complicated in agro products like spices and oilseeds, in which a large amount of organic extractables are present. The literature on the estimation of pesticide residues in spices is also scanty. Hence a modified, simple method for speedy detection of OCs, focussing on BHC was standardised, in black pepper. This method of pesticde extraction and cleaning is essentially based on the technique developed by Veierov & Aharonson (1978) for high fat natural products like milk and butter. The ortho tolidine reagent is specific for OC compounds, and hence used for their detection. After standardisation, the procedure was also adopted for the detection of other OCs such as aldrin, endosulfan and DDT residues in black pepper and clove.

Black pepper berries and clove buds were procured from the local market. Black pepper oleoresin for standardisation of the procedure (using BHC) was prepared in the laboratory. Formulated samples of BHC (50%), endosulfan (35%) and aldrin (20%) were procured from the local market and purified by eluting through silica gel column with hexane. Analytical grade DDT was procured from Sigma Chemicals, USA. Standard stock solutions were prepared by dissolving a known weight of the above pesticides in hexane and diluting to the required level. The resulting standard solution of BHC had 0.01984 mg/ml, DDT 0.06924 mg/ml, aldrin 0.08 mg/ml and endosulfan 0.35 mg/ml. All these concentrations were arrived at by estimating active ingredients using GLC in presence of the internal reference standard, DDT. Tolidine was procured from Aldrich Chemical Company, USA. Chromatographic grade filter paper strips, 1 cm x 10 cm long were dipped in 2% tolidine in distilled acetone solution and used after air dryig within 30 min. Merck GR grade sulfuric acid (95-98%) was used.

For standardising the sulfuric acid treatment method, clean dry black pepper charged with a known amount of BHC (0.1984 mg/100 g) dissolved in hexane and air dried for 2 days, was used. This sample weighing 50 g was mixed thoroughly in 50 ml acetone - hexane (20:80 V/V) for 1 h in a separating funnel, shaken at intervals and extracted twice. The combined extract was concentrated at reduced pressure in a rota vac evaporator. The solvent-free residue was redissolved in 5 ml of double distilled hexane and transferred to a 10 ml stoppered test tube. The solution was slowly mixed with 1 ml conc. sulfuric acid (95-98% with a sp. gr. 1.8) shaken well and allowed to settle. The supernatant clear hexane layer was carefully decanted, concentrated and spotted using a capillary glass tube, on the tolidine paper. The spotted paper was exposed to mild sunlight or bright shade and observed for the development of bluish green colour within 30 sec. A blank using same volume of solvent system was also run.

The minimum level to which the tolidine paper responded for OC pesticide residue detection was estimated as follows. A batch of black pepper oleoresin (0.2-0.3g) containing BHC ranging between 0.01984-1.984 mg/100 g was dissolved in 10 ml hexane and treated with 3 ml

conc. sulfuric acid. The supernatant hexane layer was separated after 3 h, mixed with known amount of internal standard, DDT solution in hexane and analysed by GC fitted with ECD. The minimum dilution of BHC at which the colour development takes place in the tolidine paper was compared with the GC data (after giving due correction for the response factors). The modified method optimised for the estimation of BHC residue in black pepper has been reported in our earlier publication (Gopalakrishnan & Narayanan 1997). The method in a nut shell is extracting a known weight of black pepper containing the pesticide residue using the acetone - hexane solvent system, removing the solvent at low pressure, redissolving the residue in hexane after mixing with known quantity of internal standard DDT, and analysing by GC fitted with ECD as explained above.

The procedure was adopted for black pepper treated with endosulfan, aldrin and DDT equivalent to 0.35-3.50 mg, 0.8-8.0 mg and 0.600-6.924 mg per kg, respectively, for their residue detection. Black pepper and clove samples procured from the market were also subjected to the examination for the presence of OCs. In all the above trials, pesticide residues present only on the surface of spices were examined and hence they were extracted without grinding.

The extracts obtained from black pepper and clove were greenish yellow in the dilute form and became dark viscous, when concentrated. Hence the conventional method of cleaning the pesticide residues by adsorbing on florisil, alumina, etc., was not very effective. Presence of even traces of organic components interfere the detection and estimation of the pesticide residues (Stimac 1979). Treatment of the spice extract containing the pesticide residue, with conc. sulfuric acid, decomposed the organic spice extractives and they settled with the acid at the bottom. This principle is adopted for the estimation of pesticide residues in fat materials like milk, butter, etc. (Veierov & Aharonson 1978). The conventional procedure has a number of unit operations to be performed repeatedly for which the time ranges between 16-24 h. But the advantage of the procedure is that BHC and other OCs are not affected by the sulfuric acid treatment. In the modified method too when the extracts were examined after sulfuric acid treatment, with TLC, it was found that the hexane layer containing the pesticide residue was free from organic impurities. The BHC peaks in the GC chromatogram were also well marked without the interference of any co-eluting substances.

Hence it could be concluded that the simplification adopted in our procedure did not affect the detection and estimation of BHC and other OCs. In our earlier paper we could prove that 95.0-97.5% of the pesticide residues could be extracted and analysed by the modified sulfuric acid treatment method using GC fitted with ECD. This observation is very well in agreement with the reported data and support adopting the technique for the detection of OCs in black pepper and clove.

Tolidine paper gave intense bluish green colour spots for BHC. Aldrin gave more prominent greenish blue spots. The colour development for endosulfan was similar to BHC. Even at a concentration of 0.01 ppm BHC gave the characterised colour. The quantitative data was also

examined with GC for BHC. The minimum level of detection was systematically carried out by GC, only for BHC. For other pesticides, standard solutions were prepared using the purified technical grade sample, diluted to different concentrations as given above and used for examining the level to which it can be detected. By this method it was found that endosulfan, aldrin and DDT could be detected even at 1 ppm level using the tolidine paper. In all the above observations, colour development in the tolidine paper, by the residues of different OCs obtained from black pepper and clove and that for their standard references was similar. This also confirms the absence of interfering compounds.

The response of the pesticide residues on colour development in the tolidine paper was optimum when tolidine was used at 2% concentration. It is important to note that tolidine paper gave good results when used within 30 min or preparation. Among the four black pepper samples analysed using tolidine paper, two responded for the OCs. However the clove samples were free from OCs. This observation was also well in agreement with that of GC data of the samples. Since the detection test is extremely sensitive to the chlorinated compounds, all the glasswares, solvents, distilled water, etc. should be absolutely free from chlorinated compounds.

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