Quantitative Analysis of Pesticide Residues in Vegetables

U.S.Pujeri#,A.S.Pujar,K.G.Pujari,M.I.Kumbar and M.S.Yadawe
S.B.Arts and K.C.P.Science College Vijayapur
ulavayya452@gmail.com
mallikarjunyadawe82@gmail.com

Abstract: Brinjal (Solanum melongena L.) and tomato are cultivated all over India, North-east India is also famous for brinjal cultivation. The Bijapur district of Karnataka is specially known for an indigenous variety of brinjal, Longai Brinjal, local and hybrid variety of Tomato's. The farmers who cultivate brinjal and tomato in this place use a large number of pesticides, herbicides and fungicides for protection of the crop and better yield. In the process they are exposed to a large number of health hazards directly and indirectly. This work aims to study the pesticide residues in brinjal and tomato and to provide appropriate suggestions so that minimum harm is caused to the farmers and maximum yield is obtained.

Key words: pesticides, herbicides and fungicides, health hazards, brinjal, tomato etc.

INTRODUCTION

Pesticides are used worldwide to protect crops before and after harvest in agriculture and gardening. Variety of pesticides are used in current agricultural practice to manage pests and infections that spoil crops [1]. A wide range of pesticides (13–14%) are used for the production of fruits and vegetables in India due to heavy pest infestation throughout the cropping season of horticultural crops whereas cropped area is only 3% [2]. Pesticides have potentially adverse effects on vegetables, fruits, animal resources and human health [3]. Pesticide production and use in the country shows a different pattern from global trends insecticide use is around 75% in the country, compared to 32% in the world. Herbicide use is only 12% in the country while worldwide, consumption is 47%. Important to note is the fact that weeding is a critical agricultural operation that provides employment to millions of poor agricultural labour, especially women, in the country. Similarly, while carbamate and synthetic pyrethroid compounds are used the most globally [45% together], in India, organophosphates constitute 50% of the consumption. Similarly, bio-pesticides are used only up to 1% amongst all pesticides in India, while worldwide, it is 12%.Plant protection products (more commonly known as pesticides) are widely used in agriculture to increase the yield, improve the quality, and extend the storage life of food crops (Fern ndez-Alba and Garc a-Reyes 2008). Pesticide residues are the deposits of pesticide active ingredient, its metabolites or breakdown products present in some component of the environment after its application, spillage or dumping.

Residue analysis provides a measure of the nature and level of any chemical contamination within the environment and of its persistence. The pesticides must undergo extensive efficacy, environmental, and toxicological testing to be registered by governments for legal use in specified applications. The applied chemicals or their degradation products may remain as residues in the agricultural products, which becomes a concern for human exposure. Selected sampling programmes can be used to investigate residual levels of pesticide in the environment, their movement and their relative rates of degradation. The maximum residue levels (MRLs) or tolerances in the United States) limit the types and amounts of residues that can be legally present on foods are set by regulatory bodies worldwide. Pesticide residue analysis is tremendously an important process in determining the safety of using certain pesticides. Pesticides polluting the earth and causing problems in human beings and wildlife, the quantity of pesticide being consumed becomes a necessary knowledge. Analytical quality requirements like trueness, precision, sensitivity and selectivity have been met to suit the need for any particular analysis.

According to the latest government report - Monitoring of Pesticide Residues at National Level for the year 2014-15 revealed that out of the 20,618 samples analysed, residues of non-approved pesticides were detected in 12.5 % of the samples. While pesticides residues were detected in 3,857 (18.7%), and 543 samples (2.6%), pesticides residues above MRL (maximum residue limit) as prescribed by FSSAI were detected. No residues were detected in 16,761 samples (81.3%).The samples collected during 2014-15 have been analysed by 25 labs. In lab findings, non-approved pesticides like bifenthrin, acetamiprid, triazofos, metalaxyl, malathion, acetamiprid, carbosulfan, profenofos and hexaconazole, among others, have been detected. Residues of non-approved pesticides were detected in 1,180 vegetable samples collected from both retail and farm gate markets, 225 fruit samples, 732 spice samples, 30 rice samples and 43 pulses samples, it added.In vegetables, the tests detected the residues of non- approved pesticides such as acephate, bifenthrin, triazofos, acetamiprid, metalaxyl and malathion. In Karnataka, Bangalore centre collected 796 samples; 25.9 % of the samples analyzed contained measurable residues. 2.8 % of all the samples exceeded the MRL. The commonly detected pesticide residues which were found above MRL were chlorpyrifos and ethion. The main pests that attack plants are Brinjal Fruit Borer, Stem borer, Spider Mite, Aphid, Jassid, Whitefly and Roots-cutworm. For the control of these insect pests, the farmers are used different types of insecticides. The farmer community uses the major insecticides such as Acephate,Chlorpyriphos, Dichlorovos, Carbofuran and Imidachloprid. Residues of DDT, DDE, chlorinated hydrocarbons and other pesticides have been found indifferent vegetables included brinjal [4-7].

The present study describe method of extraction, cleanup and determination of a pesticides by using gas chromatography(GC) equipped with mass detector (MS) for the separation, identification and quantification of chlorpyriphos, cypermethrin and monocrotophos on brinjal, tomato, were developed and validated. Finally, the method was applied for the determination of chlorpyriphos, cypermethrin and monocrotophos in the vegetable samples collected from the local market, Bijapur. Therefore, the purpose of this study was to develop an improved analytical method for the determination of the pesticide residues in brinjal and tomato by GC-MS.

MATERIAL AND METHODS

Assortment of Samples: The samples were collected from randomly chosen brinjal and tomato plants. After collection of samples, about 1kg sample was separated, sealed in polythene bags and stored at -4^{o}C for residues analysis of carbofuran, acephate, chlorpyriphos, dichlorovos, and imidachloprid. The extraction, clean-up and analytical work was followed by Asi. *et.al[8]*.

HPTLC Residual Analysis method: Brinjal and Tomato samples were extracted by ethyl acetate and analyzed by HPTLC with enzyme inhibition horse blood serum method(acetylcholine esterase enzyme) which was very sensitive for the detection of insecticide residues. The extract was spotted on silica gel plate, which was developed in mobile phase (ethyl acetate) and spot visibility was determined after spraying with acetylcholine esterase enzyme and tris-buffer solution. Rf value and average spot diameter was measured and imidachloprid, carbofuran, acephate, dichlorovos and chlorpyriphos residue concentration in ppm was calculated comparing the standard reagent spot diameter Asi. *et.al*. [8].

Fresh samples of Brinjal and Tomato were collected from various fields in local area of Bijapur. Samples were kept and wrapped in clean paper bag. Small sachet of silica gel helps to keep away it from moisture. A total of three different samples of each vegetable were collected for the analysis.Their detection and quantification by different analytical techniques are the major steps involved in pesticide residue analysis**.**

Reagents and Materials. Water and Acetonitrile were HPLC grade; Analytical grade Dimethyl formamide; anhydrous sodium sulphate were obtained from Fischer-Scientific. Acetic acid and sodium acetate from Merck were used for sample preparation. Analytical grade pesticide standards were obtained from Sigma-Aldrich. Crystalline standards were dissolved in acetonitrile for preparation of stock solution of standards. A standard mix solution was prepared from the individual stock solution to yield 10 mg/ml.

Sample Preparation. The acetate buffered sample preparation method for pesticide was applied to all samples. 50g samples were chopped and crushed in household grinder equipped with stainless steel blades and homogenize with 100 ml acetonitrile. Then 10 g of sodium chloride is added to it. Then, 6 g Na_2SO_4 were added to absorb moisture and shaken well. The extract was centrifuged at 5000 rpm for six minutes. Then ~15ml of the supernatant were filtered through a 0.45 mm PTFE filter. Pesticide was eluted with 20 ml acetonitrile. Sample was concentrated using a rotary evaporator. HPLC Condition Analytical Technologies 3000 series HPLC having UV/visible detector was used for identification and quantification of pesticides. Separation was performed on C18 (4.6ID x 250mm) column. Samples were injected manually through Rhyodyne injector. Detector was connected to the computer for data processing. The working condition of HPLC was binary gradient, mobile phase was acetonitrile: water (70:30), flow rate was 0.8ml/min, injection volume 20 µl, pressure 6-7 MPa and the wavelength of the detector was fixed at 254 nm for the residual analysis of three pesticides endosulfan, Carbendazim &Chloropyrifos. GC Condition Analytical Technologies GC 2012 model having FID detector was used for identification and quantification of pesticides. Separation was performed on Carbowax Packed column (SS 2meters). Samples were injected manually through Rhyodyne injector. Detector was connected to the computer for data processing. Following temperature conditions were maintained: Constant flow rate 1.0 ml/min, injection volume 1.0 µl, injector temperature 100^{o}C, Oven temperature110^{o}C, Detector temperature110^{o}C. Total MS running time 20 min. The individual constituents showed by GC were identified by comparing their MS with standard compounds of NIST library.

Results and Discussion

Pesticide residues in food pose a significant health effect on human and animals. To provide adequate food for growing population, the usage of pesticide is necessary but dissemination of information regarding food safety, pesticide handling and good agricultural practices (GAP) among farmers is also a dire need. Moreover, good agricultural practices and is the important and effective tools in minimizing pesticide residues in food commodities. Therefore this study will provide adequate information to the farmers for safe harvesting period of the vegetables growing under agroclimatic conditions. On analyzing the data collected from the farmers we know that majority of the farmers are illiterate and a small group are educated/ literate. It has also been found that 99% of the farmers are not at all trained and do not know the proper use of agro-chemicals, they simply use these by learning from their elders, which may not always be correct. Most of the farmers mix two or more pesticides and spray them in the field. This is a very harmful practice both for the farmer and the environment. Generally the farmers do not enter a field for at least three days after spraying insecticides, but during peak harvest period, they harvest brinjals and tomato immediately after spraying (i.e. the next day). It has also been noticed that most of the family members of the farmers suffer from general ill health and chronic diseases. These can be due to the side effects caused by the handling of these harmful chemicals. As majority of the farmers are illiterate and unknowingly use their house for storing the pesticides. Moreover they hardly follow any precautions before and after spraying the chemicals in the field. Pesticides are dangerous for human health if it exceed from the calculated maximum residue limit [9]. Due to spray of pesticides on vegetables, it leaves their residues [10]. The result of the pesticide residue analysis of brinjal samples is represented in table 2. Bai et al [11] determined the concentrations of eight organophosphorous pesticides in 18 of 200 samples. Out Of 12 samples Table. No 1& 2 we have found Carbendazim 0.012, 0.015mg/kg in sample No.5 and sample No11, and Imidacloprid 0.077 and 0.053 mg/kg in the same Brinjal samples. Out 15 tomato samples we found Carbendazim 0.014mg/kg and 0.017mg/kg in sample No.2 and sample No.8 and Indoxacarb 0.015 in smple No.2 and Profenofos 0.022mg/kg and 0.172mg/kg in samples No.2&8 above MRL value. Brazil is still engaged, in some regions, with the problem of non-permitted compound use in tomatoes and the pesticide presence above the MRL[12]. Tomato and cucumber vegetable crops were sprayed at fruit stage with dimethoate or profenofos using a recommended field rate to study the persistence of such insecticide residues at different intervals. Residues on vegetable crops have been subjected to major concern. Various regulatory bodies to ensure safe use of pesticides and to protect consumers from adverse side effects have imposed numerous restrictions. The present work was designed to study the persistence of

Carbendazim and profenofos in tomato and brinjal fruits. Emphasis on the safety periods for these insecticides in the tested vegetables was considered.

Conclusion

It is concluded from this research work that pesticides can be adsorb in pulpy portion of vegetables. But the rate of adsorption is different in different vegetables. Pesticides also leech in vegetables with different rate. If vegetables used after peeling the pesticide residues become in less concentration. Checking for pesticides residues in different vegetable samples should be done after regular intervals. Vegetables like brinjal and tomato are infested with a number of insect pests and if left uncontrolled may cause heavy damage. The study has revealed that chemical control is the principal pest control method followed by the farmers in the study area. Biopesticides and botanical pesticides are applied by a limited number of growers, while application of weedicide has been observed absent. On an average, brinjal crops are each given 10-15 sprayings, and tomato is given 12 applications of pesticides.

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