http://doi.org/10.4038/agrieast.v14i1.83

Pesticide residue analysis in Okra (*abelmoschus esculentus* L.) and brinjal (*solanum melongena* L.) in the selected areas in Batticaloa district, Sri Lanka

P. A. N. P. Pandipperuma[1], R. F. Niranjana[1*], D. Senevirathne[2], K. P. G. T. Guruge[2]

[1]*Department of Agricultural Biology, Faculty of Agriculture, Eastern University, Sri Lanka.*
[2]*Food Laboratory, Government Analyst's Department, Sri Lanka*

ABSTRACT

The reference studies showed that the farmers of Batticaloa district are using synthetic and cocktailed pesticides in high frequencies and high dosages. In order to evidence that the present study was undertaken in the Batticaloa district. Okra and brinjal are major crops cultivated in the Batticaloa district. A questionnaire survey was conducted by using 1200 farmers (2% of vegetable farmers from each DS Division) to aware of the ways adopted by farmers during the application of pesticides. Results of the questionnaire survey revealed that 90% of the farmers didn't follow the recommended practices while applying pesticides. Further, they did not get recommendations about pesticides from an appropriate source/center. In addition, Okra and brinjal pod samples were randomly collected from 40 farmer fields and 10 markets in the Batticaloa district to determine pesticide residues by using Gas Chromatograpgy/Mass Spectrometry/Mass Spectrometry (GC/MS/MS). The samples were extracted according to the AOAC official method 2007.01. The presence of pesticide was confirmed by using retention time and mass spectrum. Reported residue levels were compared with EU MRL. Analyzed reports revealed that there were no okra samples contaminated with pesticide residues, which were analyzed during the study period. However, one brinjal sample collected at the Kaluwanchikudy market and 4 brinjal samples of Kaluthavalai farmer fields were contaminated with Profenofos residues. The study recommended that analyze the residue of pesticides which frequently used by farmers including banded and restricted pesticides in Sri Lanka for major cultivating vegetables in the Batticaloa district and expand it into other districts in Sri Lanka.

Keywords: *Okra, Brinjal, Pesticide residue, Profenofos*

INTRODUCTION

The accretive agriculture sector in the world has led a worldwide concern on food safety and enforcement of strict pesticide regulations. Though the concept of food safety is in functional the insect pest management in vegetable cultivation in Sri Lanka is highly pesticide dependent (Smith, 2005; Nagenthirarajah & Thiruchelvam, 2008), which is not exceptional to the Batticaloa district. The Batticaloa district is located in the Eastern province of Sri Lanka, which falls under dry zone climatic conditions. ManmunaiSouth and Eruvilpattu is the highest vegetable growing divisional secretariat in the Batticaloa district where brinjal (*Solanummelongena* L.) and okra (*Abelmoschusesculentus* L.) are among the major crops cultivated and supplied throughout the Batticaloa district (High land crop statistics report, 2017). As it was reported that pesticides have extensively been used in agricultural production in Sri Lanka to control pests and diseases it is obvious that indiscriminate use of pesticides leads to the accumulation of pesticide residues in crop production, and causes numerous health and environmental impacts (Lakshani *et al*.,2017).

Corresponding author: niranjanaf@esn.ac.lk https://orcid.org/0000-0002-4272-4870
Received: 29.01.2020 Accepted: 06.05.2020

Although the provisions have been set out to declare safe limits for pesticide residues in local consumptions of fresh fruits and vegetables under the control of pesticides Act No. 33 of 1980 in Sri Lanka the use of pesticides in the agricultural sector is inevitable. The use of pesticides in a dissentient manner has created several health and environmental impacts and may disrupt the ecological balance of pest control by natural enemies and may lead to secondary pest upsurges.

In the Batticaloa district,t here is no documentary evidence to show the protective use of pesticides in vegetable cultivation. However, it was suspected that farmers are using cocktail mixtures of pesticides to vegetables without relying on the recommendation of the department of agriculture while using pesticides (Sutharsan *et al*., 2014). Thus, the present study was planned to analyze the selected pesticide residues in okra and brinjal, which are highly consumed by the people of Batticaloa district.

MATERIALS AND METHODS

Questionnaire Survey

A questionnaire survey was conducted in the Batticaloa district from July – August 2018 (*Yala* 2018) by using 1200 farmers (2% of farmers in each DS Division of the Batticaloa district). This questionnaire survey was carried out by personal interviews with farmers in their own cultivation field. The questionnaire was arranged to collect relevant information *viz*., socio-economic background, safety measures while applying pesticides and the types of frequently used pesticides, farmers' common practices during the application of pesticides, the common media used by farmers to get information about pesticides and their knowledge regarding pesticide usage. The data obtained from the questionnaire survey were analyzed by using *SPSS* 19.0 statistical software.

Sample Collection

Okra and brinjal pods were collected from 40 selected farmers who are supplying a considerable quantity of vegetables to markets in the DS Divisions of Manmunai South, EruvilPattu and PoratheevuPattu in Batticaloa district.

Table 1: Extent and production of okra and brinjal in selected DS divisions of Batticaloa district

DS division	Okra		Brinjal	
	Extent (Ha)	Production (MT)	Extent (Ha)	Production (MT)
Manmunai south and Eruvilpattu	52.8	739.2	53.5	856
Porathivupattu	14.5	203	15	240

(Source: High land crop statistics report, 2018)

And 10 markets were selected where consumers are highly aggregated in the Batticaloa district. The selected market places were Batticaloa (Manmunai North DS division), Vellavely (Poratheevu Pattu DS Division), Arayampathy (Manmunai Pattu DS Division), Kaluwanchikudy and Kaluthavalai (Manmunai south and Eruvilpattu DS Division), Eravur (Eravur Town DS Division), Chenkalady (EravurPttuDS Division), Oddamavadi (Koralai pattu west DS Division),Valaichchenai (Koralai Pattu DS Division) and Kattankudy

(Kattankudy DS Division). One kilogram of each vegetable sample from each selected farmer field was collected at their farm gate. Similarly, 1kg of each vegetable was collected from each selected market in the Batticaloa district. The collected samples were separately packed in cleaned polyethene bags with proper labels and within 24 hours the samples were taken to the food laboratory of the Government Analyst's Department of Sri Lanka for pesticide residue analysis.

Pesticide Residue Analysis in Vegetables

Bifenthrin, Carbofuran, Chlorpyrifos, Chlorothalonil, Diazinon, Difenoconazole I, Difenoconazole II, Dimethoate, Dimethomorph 1, Dimethomorph 2, Fenobucarb, Fenthion, Flutolanil, Isoprothiolane, Malathion, Metalaxyl, Oxadixyl, Phosalone, Profenofos, Pyraclostrobin, and Thiamethoxamwere selected for the study due to their extensive usage, toxicological effects and available analytical facilities. Among the selected pesticides Dimethoate and Fenthion, are banned in Sri Lanka whereas the usage of Carbofuran, Chlorpyrifos, and Diazinon are restricted in Sri Lanka.

The samples were extracted according to the AOAC official method 2007.01 (AOACofficial method 2007.01, 2010). 1kg of samples was homogenized by blending and 15g of it was transferred into a 50 ml centrifuge tube. Fifteen ml acetic acid in acetonitrile was added into it for the sample extraction. Then by adding a QuEChERS AOAC salt packet containing 6g of anhydrous magnesium sulfate ($MgSO_4$) and 1.5g of sodium acetate (NaOAc) sample drying and buffering was done. Then for the phase separation, it was centrifuged at 4000 rpm for 5 minutes. Dispersive-Solid-Phase Extraction (SPE) cleanup was done by transferring the 1ml of supernatant into a Dispersive SPE 2ml tube, which contained 150mg of $MgSO_4$, 50 primary secondary amines (PSA) and 50mg of $C_{18.}$ And it was centrifuged at 13000 rpm for 2 minutes, then immediately 250µl was transferred into 1ml glass auto-sampling vials. Then 25µl of 500ng/ml Tri-phenyl Phosphate (TPP) internal standard and 15 µl D-sorbitol analyte protectant was added. Thereafter the vial was capped and transferred for GC/MS/MS analysis.

The presence of pesticide was confirmed with Retention Time (RT) and Mass Spectrum. Recovery studies were performed at range from 0.01mg/kg to 0.1mg/kg and recoveries were obtained within the range of 70- 120%, with an associated repeatability $RSD_r \leq 20\%$ (Relative Standard Deviation RSD) for all analytes. The recoveries were obtained with a linearity criterion of $R^2 \geq 0.98$-0.99 and the 0.01 mg/kg was used as the Limit of Quantification (LOQ). Reported residue levels were compared with the EU MRL values. The data from sample analysis were statistically analyzed by using SPSS 19.0 statistical software.

GC/MS/MS instrument configuration

For analysis, an Agilent 7890A GC gas chromatography system equipped with Agilent 7000 C triple quadrupole GC/MS and Agilent 7693A automatic liquid sampler was used. Residues were separated through Agilent J&W HP-5MS 5% phenyl Methyl Silox; 30m× 250µm, 0.25µm N_2 collision gas flow rate: 1.5 ml/min. the carrier gas used was He and He quenches gas flow rate: 2.25 ml/min.

Temperature program: Initial temperature 70 ^{0}C for 0.06 min followed by 600 ^{0}C/min to 310 ^{0}C (10 min hold) and then 20 ^{0}C/min to 150 ^{0}C (until the end of the analysis). Oven temperature program: 60 ^{0}C for 1.0 min; then 40 ^{0}C/min to 120 ^{0}C;

then 5 ^{0}C/min to 300 ^{0}C: then 50 ^{0}C/min to 280 ^{0}C (1.5 min hold).

RESULTS AND DISCUSSIONS

Questionnaire survey

According to the data observed in the present study, most of the farmers did not have formal education. Only 24.6% studied up to G. C. E. Ordinary Level and 19% of farmers studied up to G. C. E. Advanced Level. Among the interviewed farmers, the majority of them (55%) represented the age group between 31 to 50 and 16% were above 50 and it represented the minority of them and others were below 31. Most of the farmers engaged in farming as their main occupation. Annual income varied from Rs. 80,000 to Rs.400,000 and many families (65%) were in the income level between Rs.100,000 to Rs.200,000. The data related to experience in farming showed that 47.5% of farmers had below 10 years of experience, 32.5% of them had 10 to 20 years of experience and remaining with more than 20 years of experience.

The survey revealed that during cultivation 100% of the fields had pest attacks in their fields and all the farmers relied on chemical control measures. The majority of them (90%) used chemical pesticides as soon as they observed pests in the field. The study of Nagenthirarajah and Thiruchelvam (2008) also reported that almost all the farmers in Sri Lanka depended on chemical pesticides to control pests in their cultivation.

Further, the survey stated that the majority of the farmers (90%) obtained recommendations from sellers rather than getting through proper channels. The findings of the present study par with findings of Kumarapeli (2006) and suggested that farmers ignore technical recommendations and apply pesticides by their own experience.

The survey also pointed out that cocktailed pesticides were used by 75% of farmers in the study area. It is obvious that the indiscriminate application of pesticides and the use of incorrect pesticide mixtures may cause many problems to human health (Kumarapeli, 2006; Lakshani *et al*,2017).

Although the majority (70%) of the farmers well aware of safety measures while applying insecticides *viz*., wind direction and safety clothes, 30% of them are ignorant of the safety measures and imprecisely handling the toxic insecticides.

According to observed data, it was noted that the application doses were very much higher than the recommendations. Seventy percent (70%) of brinjal farmers used Acetamiprid at the dosage of 16- 30 ml per 16 L of water where the recommendation is10 ml per 10 L of water. Further 70% of brinjal farmers applied Chlorantraniliplore with a dosage of 1-15 ml per 16 L of water while the recommendation is 1.9 ml per 10 L of water (Pest management recommendations, 2015).

Farmers in the research area applied certain pesticides more frequently based on pest infestations on vegetable crops. The study stated that brinjal was the highly pesticide sprayed crop than the okra. The frequently used pesticides were Acetamiprid (75%), Diazinon (15%), Abamectin (10%), Profenofos (10%), Etofenprox (20%) and Chlorantraniliprole (50%). Though Diazinon is a restricted pesticide in Sri Lanka (Department of Agriculture, 2016) using it in cultivation is totally unethical. Moreover, it was observed that most of the pesticides used by the farmers are not recommended for okra and brinjal, which clearly showed

that the farmers had a lack of knowledge about the relevant pest and on which the pesticides have been recommended. Khan *et al* (2006) discovered that 90% of vegetable growers were found with lack of knowledge regarding the recommended doses, pre-harvest intervals and the harmful effects of these chemicals on human health.

Furthermore, the study specified that all brinjal farmers applied pesticides more than 16 times during a cultivation season. Heavy usage of pesticides may result in infrequent contact with pesticides, which may ultimately result in acute or chronic health problems. Excessive application of pesticides may lead to concentrate in the plant and it may result in residues remaining in products of the plant. This practice may put both farmers himself and consumer into danger.Ingestion of such highly contaminated vegetables either in raw or processed form may result in some serious health hazards to the consumer (Kumari *et al*, 2003). Regarding human health such a large quantity of sprays per crop indicates a seriouscondition (Tariq *et al*, 2007).

Pesticide residues in okra samples

The GC/MS/MS analysis (Table 2) showed that there were negligible amounts or no residues of tested pesticides in okra samples collected at farmer fields at Manmunai South and Eruvilpattu D.S. Divisionand Porathivupattu D.S Division and the markets at various DsS Divisions in Batticaloa district.

Table 2: Pesticides residues in brinjal and okra samples collected in the selected D.S Divisions in the Batticaloa district

Name of the pesticide	Brinjal farmer field samples(mg/kg)	Okra farmer field samples (mg/kg)	Brinjal market samples(mg/kg)	Okra market sample(mg/kg)
Dimethoate	0.000	0.000	0.000	0.000
Diazinon	0.000	0.000	0.000	0.000
Metalaxyl	0.000	0.000	0.000	0.000
Malathion	0.000	0.000	0.000	0.000
Fenthion	0.000	0.000	0.000	0.000
Chlorpyrifos	0.000	0.000	0.000	0.000
Isoprothiolane	0.000	0.000	0.000	0.000
Flutonil	0.000	0.000	0.000	0.000
Profenofos	0.043	0.000	0.0012	0.000
Oxadixyl	0.000	0.000	0.000	0.000
Bifenthrin	0.000	0.000	0.000	0.000
Difenoconazole I	0.000	0.000	0.000	0.000
Difenoconazole II	0.000	0.000	0.000	0.000
Dimethomorph 1	0.000	0.000	0.000	0.000
Dimethomorph 2	0.000	0.000	0.000	0.000

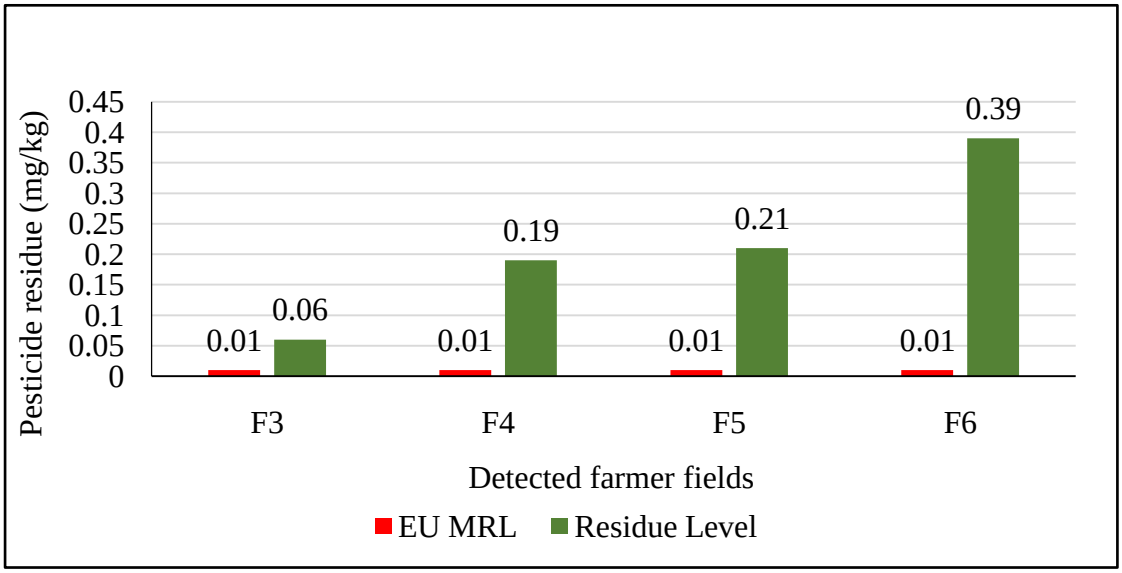

Figure 1: Profenofos residue in brinjal samples collected at the farmer fields in the selected D.S Divisions in the Batticaloa district

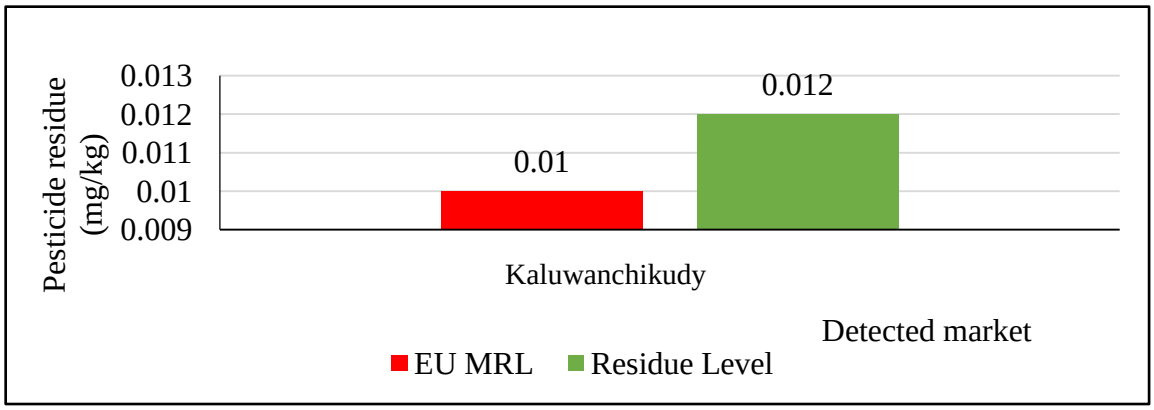

Figure 2: Profenofos residue in brinjal samples collected at the market in the Batticaloa district

In figure 1, F3, F4, F5 and F6 represent the farmer field samples collected from Thettathivu South, Kaluthavalai 1, Kaluthavalai 2, and Kaluthavalai 3 G. N. Divisions respectively. Among them, the samples collected from the farmer fields in Kaluthavalai 3 were contaminated with the highest residue level of 0.39 mg/kg whereas the brinjal samples collected from Kaluwanchikudy market contaminated with 0.012 0.39 mg/kg of Profenofos. This was due to the high frequency of insecticide applications.

CONCLUSION

Questionnaire survey revealed that 90% of the farmers in the Batticaloa district didn't rely on the recommendations given by the Department of Agriculture, Sri Lanka and the label of respective pesticides while applying them to the cultivation. *GC/MS/MS* analysis stated that none of the okra samples was contaminated with the pesticide residues. However some Brinjal samples were heavily contaminated with Profenofos residues.

Recommendations

As the study only restricted for certain pesticides viz., Bifenthrin, Carbofuran, Chlorpyrifos, Chlorothalonil, Diazinon, Difenoconazole I, Difenoconazole II, Dimethoate, Dimethomorph 1, Dimethomorph 2, Fenobucarb, Fenthion, Flutolanil, Isoprothiolane, Malathion, Metalaxyl, Oxadixyl, Phosalone, Profenofos, Pyraclostrobin, Thiamethoxamit was recommended to go for further analysis for some other pesticides namely, Acetamprid, Diazinon, Abamectin, Etofenoprox and Chlorantraniliprole which are highly used by the farmers in the Batticaloa district.

It cannot be stated that the okra is not 100% free from pesticide residues, as some of the other pesticides are needed to be tested.

Acknowledgement

Authors are grateful to the Academic and non-academic staff in the Department of Agricultural Biology, Faculty of Agriculture Eastern University Sri Lanka and all the staff in the food laboratory of Government Analyst's Department of Sri Lanka. Also we want to pay our special gratitude for the staff of the Department of Agriculture Extension, Batticaloa and for the Agriculture Instructors in Batticaloa District for their tremendous support given during the questionnaire survey and sample collection.

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