Pesticide Residues in Eggplant Fruit and Soil under Study

Yin Min[1], Ni Ni Sein[2],Aung Myint[3] and Kyaw Naing[4]

Abstract

Eggplant (*Solanummelogena Linn*) is a popular vegetable fruit in Myanmar. Persistence pattern of two non-systematic broad spectrum insecticides viz., malathion[O,O-dimethyl-S-1,2-dicarbethoxy ethyl dithiophosphate]O,O-dimethyl and chlorpyrifos [O,O-dimethyl O-(3,5,6-trichloro 2-pyridyl) phosphorothioate) were evaluated on eggplant fruit. Malathion and chlorpyrifos residues were determined by gas liquid chromatography equipped with the flame photometric detector. Initial deposit of malathion and chlorpyrifos on eggplant fruit was 0.4731ppm and 0.6213ppm, respectively. After 7 days and 9 days, initial deposit dissipated to 98.20% and 96.98% for malathion and chlorpyrifos, respectively. Physicochemical properties of eggplant and also in soil from experimental plots were firstly determined. The recovery rates (88.33±3.11 and 93.15±2.57) for fruits and soil, respectively, obtained for the extraction of pesticide revealed that the extraction procedure combined with GC analysis remained effective throughout the study period. The accuracy and precision of this method were good (1.72 and 3.52%, respectively). The preharvest interval (PHI) values of malathion and chlorpyrifos in eggplant fruits were found to be 1 day and 5 days, respectively. The reaction order of degradation, rate constant and half-life of each insecticide in fruit and soil were determined by graphical method. Metabolite of malathion was identified by gas chromatograph interfaced with mass spectrometer (GC-MS).Pesticide residues from retail markets from Yangon environs were determined by TLC.

Keywords: preharvest interval, malathion, chlorpyrifos, insecticides, half-lives, eggplant, recovery rate, TLC

INTRODUCTION

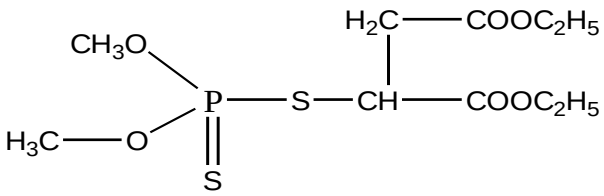
Pesticides are essential in agriculture with ever increasing for food, for demand, for consumption. Myanmar is predominantly an agricultural country. The Government emphasizes increasing production and productivity through the supply of agriculture inputs (Aung, 2003). Control on pesticide residues in crops are generally based on Maximum Residue Limits (MRLs) which are set using field trial data for a particular pesticide to arrive at the highest residue levels expected under use according to Good Agriculture Practice (GAP). Malathion and chlorpyrifos are two non-systemic broad spectra insecticides which are widely used in agriculture. They are used for controlling any insect pests such as aphids, thrips etc., (Hamilton, 1988) are listed in Tables (1). By pesticide law, out of 12 POPs, 9 pesticides have been managed to control in the country (Table 2.). Pesticide legislation has improved the safe and efficient use of pesticide in Myanmar. Most toxic products which are classified in class I of WHO toxicity class has been rejected for registration and less toxic compounds are allowed. Restricted and banned pesticides in Myanmar are often classified according to the organisms which they are used to control, e.g., insecticides (for insect control), herbicides (for weed control), rodenticides (for rodent control), fungicides (for control of plant disease fungi), molluscicides (for control of destructive effects of slugs and snails) and miticides (for mite control) as well as wood preservatives, disinfectants, products that control algae, etc

[1] Associate Professor, Dr, Department of Chemistry, Hinthada University
[2] Professor (Retired), Dr, Department of Chemistry, Yangon University
[3] Emeritus Associate Professor, Dr, Department of Chemistry, Yangon University
[4] Deputy Director General, Dr, Department of Higher Education, Yangon

Physical and chemical properties of malathion are described as follows.

Molecular Formula - $C_{10}H_{19}O_6PS_2$

Molecular Structure -



Molecular Weight - 330.3 g/mol

Physical State - Clear amber liquid

Melting Point - 2.85°C

Boiling Point (0.7mmHg) - 156-157 °C

Specific Gravity (25 °C) - 1.23

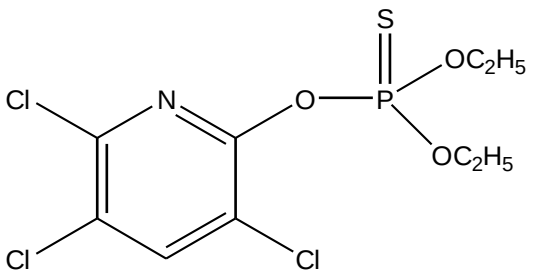
Vapour Pressure (30 °C) - 4×10^{-5} mmHg

Solubility (25 °C) - 145 mg/L water

Physical and chemical properties of chlopyrifos are described as follows.

Molecular Formula - $C_9H_{11}Cl_3NO_3PS$

Molecular Structure



Molecular Weight - 350.62 g/mol

Physical State - White, granular, crystalline solid

Melting Point - 42-43.5°C

Vapour Pressure (25°C) - 1.87×10^{-5} mmHg

Solubility (25°C) - 14 mg/L water readily soluble in acetone, benzene, chloroform, methanol and iso-octane

Table (1). List of restricted pesticides in Myanmar

No.	Pesticide	Remarks
1	Methyl bromide	Fumigant to be handled only by Certified Pesticide Applicator
2	Phosphine	Fumigant to be handled only by Certified Pesticide Applicator
3	Bromadiolone	Highly toxic, to be handled only by Certified Pesticide Applicator
4	Zinc Phosphide	Intensely poisonous to mammals, to be handled only by Certified Pesticide Applicator
5	Brodifacoum	Fumigant to be handled only by certified Pesticide Applicator
6	Fenthion	Restricted to vector control
7	DDT	Restricted to vector control of Malaria

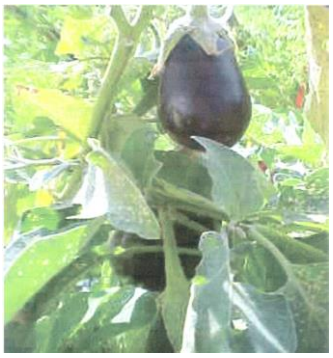
Table (2). List of pesticides which have been managed to control in Myanmar

No.	Pesticide	Remarks
1	Aldrin	Banned
2	Chlordane	not used
3	DDT	restricted to malaria control
4	Dieldrin	Banned
5	Endrin	Banned
6	HCB	not used, no registration
7	Heptachlor	not used, no registration
8	Mirex	not used, no registration
9	Tsoxaphene	Banned

EXPERIMENTAL

The chemicals used in this work were from "British Drug House Chemicals Ltd., Poole , England", "Kanto Chemical Co., Inc., Japan", "Hopkin and Williams Co., Ltd., England", "Merck, Darmstadt, Germany", and "Hi media Laboratories Pvt,Ltd., India", "Sigma-Aldrich Co., Germany" and "Riedel-deHanCo., Germany", which are simply abbreviated as BDH,Kanto, Hopkin and Williams, Merck, Hi Media, Sigma, and Riedel-deHan , respectively.

The samples of eggplant fruits (Fig.1) were grown in Myanma Agriculture Service Insein Township, Yangon. (Fig.2) Standard malathion and chlorpyrifos were obtained from Sigma Company Ltd. For degradation study, malathion and chlorpyrifos were purchased from Marlarmyaing pesticide selling shop. Moisture content and pH of fruit and soil were determined by the oven drying method and pH meter, respectively. Ash content of fruit was identified by the ashing method. The protein content of fruit and total nitrogen of soil were determined by Kjeldahl digestion method (Jones and Benton, 1991). The fat content of fruits was determined by Soxhlet extraction method. Organic carbon and humus of soil were identified by chromic acid wet-oxidation method (McLeod, 1973).Texture of soil from experimental plot were determined by Pipette method (Gee and Bouder, 1986).For degradation study, pesticide residues of fruit and soil were determined by GC-FPD. Moreover, metabolite of malathion can be detected by GC-MS and market samples of eggplant were determined by enzyme inhibition TLC method.



Myanmar Name - Kayan
English Name - Eggplant
Botanical Name - *Solanum melongena* Linn Family
Family - *Solanaceae*

Figure (1). Eggplant fruit (*Solanum melogena* Linn) sample.

Figure (2). Eggplant fruit (*Solanum melogena* Linn) sample.

Six test plots: one for control and five for treated plots, 3 ft apart were used for growing eggplant (Figure 2). A single application of malathion (57 EC) was performed when the eggplants had reached the picking stage. The application was conducted by a person with more than five years of experience in the field of pesticide application. The malathion pesticide was applied with the recommended dose (20 cm^3 of emulsifiable concentrate per gallon) by hand spray from a spray tank. Samples from non-sprayed control plot and five treated plots were collected at random at 0, 1, 3, 5, 7, 9 days after application of malathion. In order to avoid taking samples at the beginning or at the extreme end of plot, eggplants grown along the border were left from sampling area. Eggplant samples (1 kg) were chopped into small pieces and mixed thoroughly. The samples were stored in individual, labeled plastic sealed bags for freezer storage (-20^{o}C) until extraction.

The Chopped sample (50 g) was put into a 250 ml tall beaker. Anhydrous sodium sulphate (30 g) and 50 ml of ethyl acetate were put into the above beaker and it was homogenized for 3 minutes with an ultra-turrax homogenizer. It was shaken with the shaker for a few minutes. The pulp in the extract was allowed to settle. After filtration, the volume of the extract was measured and then transferred into a round-bottomed flask. Toluene 2 ml was added to the extract and then concentrated under the vacuum of about 1 ml by the rotary

evaporator. The concentrate was transferred into a 5 ml graduated test tube. The round-bottomed flask was rinsed with about 1 ml toluene and the rinsate was transferred into the above test tube. And then it was evaporated off just to the point of dryness with a slight nitrogen stream, after which the volume was made up to 2 ml with toluene.

Clean up 1- A chromatographic column was prepared with a mixture of activated charcoal and anhydrous sodium sulphate in weight ratio of 1:15. It was pre-washed with 20 ml dichloromethane. A 1 ml of aliquot from the extract was loaded onto the column followed by elution with 150 ml dichloromethane. The eluate was collected in a round-bottomed flask and the volume was reduced to 1 ml by rotary evaporator. It was transferred into the test tube and evaporated off using a nitrogen gas stream to less than 0.5 ml.

Clean up 2 - Deactivated silica gel (0.3 g) and a small piece of cotton wool pre-treated with n-hexane were placed into a short Pasteur pipette. The concentrate (0.5 ml) was transferred into the column and eluted with 5 ml of n - hexane: ethyl acetate (1 : 1) mixture and the eluate was discarded .After elution with 20 ml of ethyl acetate, the eluate was collected in a round-bottomed flask. It was then concentrated (less than 1 ml) in a rotary evaporator and the round-bottomed flask was rinsed with about 2 ml of ethyl acetate and the rinsate was transferred into the 5 ml graduated test tube. Finally it was evaporated off using a nitrogen gas stream to 0.5 ml and this solution (1 μl) was injected into the GC-FPD. For the spiked solution, 1 ml standard malathion (4.49 μg/ml) was added into the mixture of control sample, ethyl acetate and anhydrous sodium sulphate before homogenization. The remaining procedure was the same as that mentioned above.Perkin-Elmer Clarus 500 GC with flame photometric detector was used for the determination of malathion residue in eggplant. GC conditions were described as follows.

Detector	-	Flame photometric detector
Attenuation	-	6
Column	-	PE-1
Temperature	-	Column 200-250^{o}C,Injector 250^{o}C, Detector250^{o}C
Carrier gas	-	Nitrogen (10mL/min)
Coolant gas	-	Air (90mL/min)
Inject volume	-	1μL

Malathion standard was injected first, followed by control sample, and spiked sample. Malathion pesticides were qualitatively identified according to the retention time of standard malathion. For quantitative determination, the malathion contents were determined from peak areas of standard and sample by using the standard comparison method.

Malathion and Chlorpyrifos pesticide residues in soil under pesticide-treated eggplant fruit were determined by the Soxhlet extraction method. Soil sample under pesticide- treated plants were collected according to the sampling schedule of fruits. The soil samples were collected by using 6.3 cm internal diameter, stainless steel auger approximately 10 cm into the soil. The soil portions were cut off using clean steel scissors and placed into a polyethylene plastic sealed bags for freezer storage (-20^{o} C) until extraction. A 100 g of each soil sample were obtained by using coning and quartering method.

RESULTS AND DISCUSSION

The physicochemical analysis of eggplant fruits was shown in Table (3). The moisture content of eggplant fruits was found to be about 93.85±0.40%. The reported moisture content was found to be 93% for eggplant. The pH values of eggplant were in the range of 4.6 to 5.00. The ash of a foodstuff, the inorganic residue remaining after the organic matter has been burnt away. The ash obtained is not necessarily of exactly the same composition as the mineral matter present in the original food as there may be losses due to volatilization or some interaction between constituents. Too high a temperature may cause the volatilization of certain elements,such as particularly potassium, sodium, sulphur, chlorine and phosphorus and may also cause the mineral matter to melt and fuse. Table (3) described ash content in eggplant and sweet pepper fruit. The ash content in eggplant fruit was 0.23±003. The classical Soxhlet method with or without acid hydrolysis is an important routine-procedure. The Soxhlet extraction is a method to extract a soluble fraction from a solid medium. In this study, the fat contents in eggplant fruits were 0.38±0.07%. The protein content of eggplant was found to be 1.08±0.03% as shown in Table (3). These are in agreement with the reported nutritional values of 1% for eggplant. Recovery rates obtained for the analytes ranged between 81.93±3.81% to 88.33±3.11% and 88.73±3.11% to 93.15±2.57%, for fruits and soil, respectively (Fig.3). These data reveal that the pesticide extraction procedure remained effective throughout the study period.

Table (3). Physicochemical analysis of eggplant fruit

No.	Parameters	Eggplant
1	Moisture (%)	93.85±0.40
2	pH	4.85±0.17
3	Ash (%)	0.23±0.03
4	Fat (%)	0.38±0.07
5	Protein (%)	1.08±0.03

Diminution of malathion and chlorpyrifos in eggplant as a function of time are shown in Table (4). MRL value of malathion in both fruits is 0.5 ppm. Malathion residues in eggplant fruit reached below the MRL value of 0.5 ppm after one day application. Thus, preharvest interval (PHI) for malathion in eggplant fruits was one day. MRL value of chlorpyrifos in eggplant was 0.2 ppm .Chlorpyrifos residue level on eggplant reached below the MRL of 0.2 ppm after five days. Thus, PHI of chlorpyrifos in eggplant was five days.

Table (4). Diminution of malathion and chlorpyrifos in eggplant fruit as function of time

Packing interval (days)	Malathion Residue(ppm)	Chlorpyrifos residue(ppm)
0	0.4731±0.0997	0.6213 ±0.0515
1	0.2741 ±0.0667	0.4032 ±0.0437
3	0.1000±0.0080	0.2537 ±0.0219
5	0.0355±0.0097	0.1520 ±0.0307
7	0.0085 ±0.0006	0.0606 ±0.0130
9	ND	0.0189 ±0.0063
	MRL 0.5 ppm	**MRL 0.2 ppm**

In this study, single application of malathion (EC) and chlorpyrifos pesticides, formulated as 57% and 50% emulsifiable concentrate, respectively, were applied to the eggplant. The soil under malathion-treated eggplant were 3.65±0.15% (Table 5). In the case of soil under chlorpyrifos–treated eggplant were 0.92±0.18%. It was found that the soil under chlorpyrifos–treated eggplant field was the lowest moisture content among them (Table 5).

Table (5). Physical and chemical properties of soil under pesticide-treated eggplant fruit

No.	Parameters	Malathion	Chlorpyrifos
1	Moisture (%)	3.65±0.15	0.92±0.18
2	pH	6.75±0.08	5.00±0.04
3	Total N_2(mg/g)	0.30±0.06	0.18±0.03
4	Organic carbon (%)	4.17±0.22	0.10±0.20
5	Humus (%)	8.34±0.15	2.20±0.18

Texture refers to the relative proportions of particles of various sizes such as sand, silt and clay in the soil. The percentages of sand, silt and clay in a soil could be determined by the modified pipette method. Texture is an important characteristic of soil and affects water holding capacity, drainage properties, root development and more. Because texture has a great effect on water movement through soil, it can also affect the rate at which pesticides and nutrients move through the soil. Soil particles such as clay, can also bind up certain particles causing differences in pesticide activity on soil with different soil textures.

The proportions of the separates in classes commonly used in describing soils are given in the textural triangle shown in figure (4). In using the diagram the points corresponding to the percentages silt and clay present in the soil under consideration are located on the silt and clay lines respectively. The name of the compartment in which the two lines intersect is the class name of the soil. In this study, soil under malathion and chlorpyfifos-treated eggplant plot contained 67.36±7.34% and 67.36±6.84% of sand, 15.92±5.23% and 12.96±4.65% silt and 14.25±3.18% and 17.37±5.13% of clay, respectively (Table 6). Therefore, both eggplant and sweet pepper plots, types of soil were found to be sandy loam according to USDA texture triangle. Furthermore, it was observed that soil under chlorpyrifos-treated plots had slightly higher percentages of clay than those of malathion-treated plots.

Table (6). Texture of soil under pesticide-treated experimental plot.

No.	Treated pesticide	Sand (%)	Silt(%)	Clay (%)	Soil Type
1	Malathion	67.36±7.34	15.92±5.23	14.25±3.18	Sandy Loam
2	Chlorpyrifos	67.36±6.84	12.96±4.65	17.37±5.13	Sandy Loam

To address the extraction efficiency and analytical quality control, recovery tests were performed for each insecticide, at different sampling intervals. The known amount of standard pesticide was injected into the control sample before the extraction. The recovery test was conducted for the two insecticides during the degradation study. This experiment was necessary to establish if the insecticide residue extraction procedure remained effective throughout the study period.

The recovery rates obtained for the analytes ranged between 81.93±3.81%. The results of recovery tests were found to be good within the accepted range of 60-120%. In the present work, the precision was found to be 3.52% and the accuracy (the relative error)

percentage is 1.72 % Thus, the extraction procedure and the method of determination of the insecticide were found to be good.

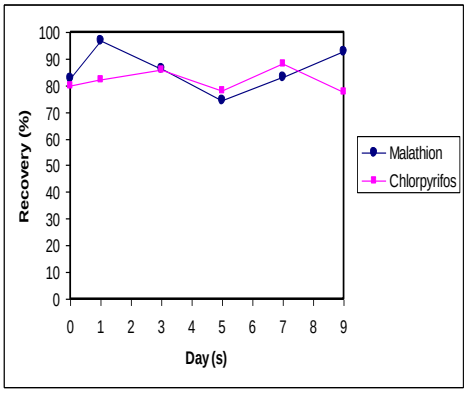


Figure (3). Recovery % of malathion and chlorpyrifos extraction

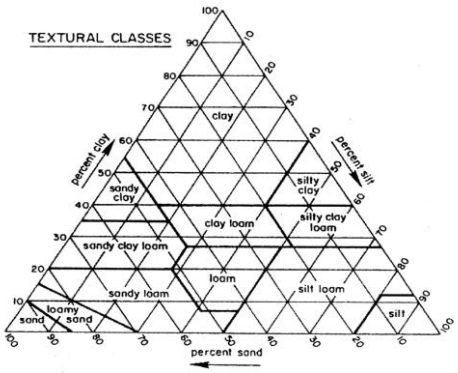


Figure (4). Textural classes according to USDA (1951) and adopted by FAO

CONCLUSION

This study concerns the determination of the concentration of pesticide residues namely, malathion, 57 % EC and chlorpyrifos, 50 %EC in eggplant fruit and soil under study. From the soil analysis, it was found that the soil under chlorpyrifos – treated eggplant field had the lowest moisture content among them. Thus, the soil under chlorpyrifos – treated plants was found to be slightly acid. From the texture diagram, the soil type of both plots was found to be sandy loam. Degradation of the pesticides in fruit and soil were monitored by gas chromatography. The recovery tests were conducted for the two insecticides during the degradation study. The recovery rates were (88.33±3.11 and 93.15±2.57) for fruits and soil, respectively, and it was found to be good. Malathion residues were below 0.5 ppm (maximum residue limit) in eggplant after one day and the preharvest interval (PHI) values for malathionin eggplant were found to be one day. Both soil under malathion treated eggplant fruit reached below the MRL value of 0.05ppm after three days treatment. However, soil samples from chlorpyrifos – treated eggplant reached below the MRL value of 0.03 ppm after five days. In this study, out of these two pesticides, chlorpyrifos was more persistent in soil ($t_{1/2}$< 2 days) than of malathion ($t_{1/2}$<1 days). From GC-MS spectrum of malathion, its metabolites were identified as malaoxon, malathiondicarboxylic acid and malathion mono

carboxylic acid. Pesticide residues in eggplant samples from Yangon environs were not detected in this investigation. Thus, the study on the pesticide residues from market samples revealed that consumers could safely consume eggplant fruits from markets. The findings of this work call for an awareness of the potential hazards in the vegetables that we consume daily.

Acknowledgements

We would like to express our deep gratitude to the Department of Higher Education, Ministry of Education, Yangon, for the Ph.D research grant. We are also indebted to Dr Tin Htwe (Rector) and Dr Theingi Shwe (Pro-Rector), Hinthada University. Our warmest thanks are also extended to Dr Theingi Nyo (Professor and Head), and Dr Cho Cho Than (Professor), Department of Chemistry, Hinthada University for their effective suggestions, comments, encouragement and allowing for submission of this paper.

References

Aye Aye Aung, 2003, Paper Presented to Workshop on monitoring of Pesticide Pollutants, Suwon, Republic of Korea.

Gee, G.W.and Bouder, J.W. 1986, Particle Size Analysis, 383-411. In A. L. Page (Ed). Methods of Soil Analysis. Part 1, Physical and Mineralogical Methods, 2nd Edn., Agronomy Monograph 9, American Society of Agronomy, Madison, W.I.

Hamilton, D.1988, Setting MRL Values for Pesticides in Food,. Queensland Agricultural Journal. 224 Jones.

J. Benton. 1991, Kjeldahl Method for Nitrogen Determination. Athens, GA: Micro-Macro Publishing.

Mcleod, S. 1973, Studies on Wet Oxidation Procedures for the Determination of Organic Carbon in Soil CSIRO Division of Soils, Notes on Soil Techniques,73.