

GAS CHROMATOGRAPHY MONO SPECTROMETRY STUDY OF MALATHION RESIDUES IN *Centella asiatica*

A.M. Latifah, R. David Musa, P. A. Latiff

Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, Selangor, Malaysia

Received 21 April 2009; revised 20 July 2010; accepted 18 October 2010

ABSTRACT

Centella asiatica is a herbaceous plant and known as pegaga in Malaysia. It was commonly used as a healing agent and constituent to improve mental ability because contains polyphenols and triterpenes. Malathion is an insecticide that helped to increase value and yields of *Centella asiatica* by control the damage caused by crop pests. Study was done in MARDI Serdang to measure malathion residue in *Centella asiatica* with different treatment methods using GC-MS. Six plots were randomly selected and samples were taken a day before, a day, three days and five days after the application of malathion. The samples were divided into treated (soaked in tap water and salted water), and untreated groups. The sample was extracted through the liquid-liquid extraction and underwent a clean-up process by a silica gel. The residues were found in; three samples treated with salt water (A1= 19.78 µg/kg, C1 = 8.53 µg/kg and E1= 0.99 µg/kg), one sample treated with tap water (E1=0.44 µg/kg) and two unwashed samples (E1=0.0053 mg/kg and F1=0.0077 mg/kg). Therefore the safest way to consume is by soaked with tap water compared with soaked in salt water and unwashed. However the malathion residue found were below the Maximum Residue Limits set up by US EPA.

Key words: *Centella asiatica*; Pegaga; Malathion; Pesticide residue

INTRODUCTION

Pesticides are the chemicals or any agent to kill or control pests (U.S. Environmental Protection Agency, 2007) or undesired organisms like insects, weeds, rodents, fungi and bacteria. The usage of pesticides in agriculture sector worldwide can enhance greater productivity to fulfill the increase needs in foodstuff. However, the slow degradation rate of pesticides and with the influenced of improper usage by farmers can affect the environmental quality by contaminating soil, water, air, other non-target plants and possibly humans (Rissato *et al.*, 2007).

A lot of studies had been conducted to determine the pesticides residues in plants worldwide include honey (Rissato *et al.*, 2007), cabbage

(Zhang *et al.*, 2007), spring tomato (Gambacorta *et al.*, 2005), wine and fruit juices (Zambonin *et al.*, 2004), olive (Rastrelli *et al.*, 2002), and orange, white cabbage and wheat (Kocourek *et al.*, 1998). Environmental Protection Agency, EPA, (2007) also conducted various studies to determine the maximum levels of pesticides that may introduced into food when harvesting, processing and marketing, and during preparing to be served.

The values of pesticide residues are not similar in fruits and vegetables. This may be caused by the climatic condition and also the variation of the plants species (Tariq *et al.*, 2007). Norris (1969) indicates that the pesticide enters the plant when it makes a contact with the surface and compatibility with the cuticle and its behavior of pesticides on aerial portions of the plant, on the roots and

*Corresponding author: E-mail: latifah@env.upm.edu.my
Tel: + 603-8946 6747, Fax: + 603-8946 7463

inside the plant. Upon intercepted with the aerial portion of the plant, the pesticide might undergo adsorption, surface adsorption, volatilization, wash-off and degradation. It is almost the same to be happening on the roots. It only differs on the degree of the operation process and can depend on the pesticide solubility. Translocation, storage, metabolism and exudation are the processes that may happen inside the plant. The amount of pesticide absorbed into the plant will determine the residues.

Malathion

Malathion (C₁₀H₁₉O₆PS₂) is an insecticide and belongs to organophosphate classes (NPIC, 2001). The US EPA classified malathion as a Class III pesticide indicates the signal word of Caution of low toxicity (NPIC, 2001). The exposure to malathion can cause nausea, vomiting, stomach cramps, diarrhea, blurred vision, confusion, sweating, muscle twitching, irregular heartbeat and convulsions and fatality. Fig. 1 shows the structural formula malathion.

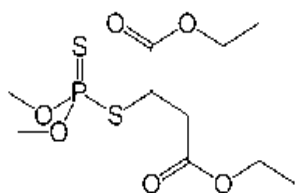


Fig. 1: Structural formula of malathion (Wikipedia, 2007)

Malathion may enter the body through inhalation, contact with skin and ingestion with the major route through skin and eyes. The toxic effects can occur after the inhalation of malathion sprays or dusts at threshold of 13.5 mg/m³. Therefore in a plantation area, EPA requires at least 12 hours after the application before an entry into the area to avoid exposures to the worker. In 1967 – 1968, 35 cases of malathion poisoning reported in Indore where 5 were dead due to myocardium damages (Gupta, 2004).

The variation of the LD50 value (LD50 2100 mg/kg) for malathion is dependant to its impurities where isomalathion is the major toxic component. Malathion does not degrade readily by sunlight. It breakdown rapidly in soil but does not pose a threat to groundwater. Under certain conditions, it may breakdown in water. On plant surfaces, its half-life ranges from less than one day to about a week.

Centella asiatica classification and functions

Centella asiatica (also known as gotu kola, Indian Pennywort, Mandookaparni or pegaga in Malaysia) has been used for centuries as a medicinal herb and was referred to in the French pharmacopoeia in 1884, as well as the ancient traditional Chinese Shennong Herbal some 2,000 years ago, as well in Indian Ayurvedic medicine some 3,000 years ago (Sallamander Concepts (Pty) Ltd 2010). These species can be found almost all over the world. Table 1 describes the scientific classification of *Centella asiatica*.

Table 1: Scientific classification of *Centella asiatica* (Wikipedia, 2007)

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Apiales
Family	Apiaceae
Genus	Centella
Species	<i>Centella asiatica</i>
Binomial name	<i>Centella asiatica</i>

It has been used for: wound healing, better circulation, memory enhancement, cancer, vitality, general tonic, respiratory ailments, detoxifying the body, treatment of skin disorders (such as psoriasis and eczema), revitalizing connective tissue, burn and scar treatment, clearing up skin infections, slimming and edema, arthritis, rheumatism, treatment of liver and kidneys, periodontal disease, strengthening of veins (varicose veins), blood purifier, high blood pressure, sedative, anti-stress, anti-anxiety,

an aphrodisiac, immune booster, anabolic and adaptogen (Sallamander Concepts (Pty) Ltd., 2010).

There are many different uses for the plant especially in Ayurveda and traditional Chinese medicine (Wikipedia, 2007) because the whole plant contains polyphenols and triterpenes (Subathra *et al.*, 2005) especially asiatica acid and madecassic acid as active components (Inamdar *et al.*, 1996). Therefore, *Centella asiatica* have been used as healing agent and a constituent to improve mental ability of mentally retarded children.

In pharmacological studies, the plant extract has shown to have central nervous depressant activity. It enhances learning and memory in children and adults (Rao *et al.*, 2005). *Centella asiatica* is also able to lower blood pressure, purify blood, cure indigestion and nervousness, treat skin disorders, as a diuretic, anti-hypertensive agent, remedy for asthma, leprosy, anemia and inflammations (Hussin *et al.*, 2007).

In Malaysia, *Centella asiatica* is a well-known multipurpose herb especially among the Malays, Chinese and Indians. To treat high-blood pressure, the roots and the stems of *Centella. asitica* are grounded and squeezed into a glass of water. An amount of salt is added to be drink every morning. The mixture also can be used to get rid of nausea. The Indians in Selangor drink it with a glass of milk after two hours to enhance memory for 40 days. The Chinese drink *Centella asiatica* soup to treat stomachache by boiling the whole plants with ginger.

The objective of this research was to measure malathion residue in *Centella asiatica* using Gas Chromatography Mass Spectrometry (GC-MS). Besides that, the comparison of malathion residue in *Centella asiatica* with different treatment methods will also determined.

MATERIALS AND METHODS

Sample collection

This study was conducted on 7th (Day 0), 9th (Day 1), 11th (Day 3) and 13th (Day 5) January at *Centella asiatica* plantation site in MARDI Serdang. The plants were grown in open field 6th month prior to sampling in rows. For this study purpose, the site was divided into six plots as in Fig. 3. The

application of malathion was done on 7th January by a worker using a backpack-spraying pump at 0730 – 0800 am in a bright, sunny and windless day with the temperature of 28.5 °C.

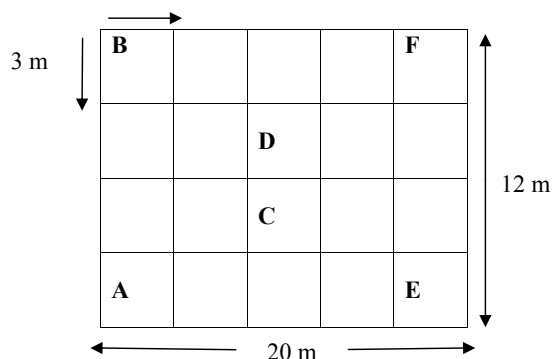


Fig. 3: The plots of sample collection in study area

The sample was taken on day one, day three and day five after malathion was sprayed to the plant. Summary of time and temperature during the sampling are presented in Table 2. The samples were collected randomly from six plots (plot A, B, C, D, E and F) for day one sample collection. However for day three and day five, only three plots (plot A, D and F) were selected randomly for samples collection. The weight of each sample was 20g. As for control of the experiment; one sample was collected from plot A, a day before malathion spray to the plant. After collection, samples were wrapped with aluminium foil to avoid loss of malathion to environment.

Table 2: Time and temperature recorded during the sampling

Day	Time		Temperature (°C)	
	Start	Finish	Start	Finish
0	09:30 am	10:20 am	30.0	31.0
1	09:40 am	13:00 pm	30.0	35.0
3	09:40 am	12:30 pm	30.0	31.5
5	08:45 am	11:30 am	30.0	33.0

Sample treatment

There were three treatment methods used in sample preparation:

- I. *SW method* - sample been soaked in 200 mL of 0.01 g/mL salt water one hour before extraction
- II. *TW method* – sample been soaked in tap water for one hour before extraction.
- III. *UN method* - sample left unwashed

Samples with the weight of 5 g were ground with mortar.

Table 3 summarize the treatment methods for samples.

Table 3: Summary of sample treatment

Date	Sample plots	Sample preparation
Day 0	A	SW, TW, UN
Day 1	A1	SW, TW, UN
	B1	SW, TW, UN
	C1	SW, TW, UN
	D1	SW, TW, UN
	E1	SW, TW, UN
	F1	SW, TW, UN
Day 3	A3	SW, TW, UN
	D3	SW, TW, UN
	F3	SW, TW, UN
Day 5	A5	SW, TW, UN
	D5	SW, TW, UN
	F5	SW, TW, UN

SW – Salt water; TW – Tap water; UN – Unwashed

1, 3 and 5 – indicate number of day after spraying of malathion

Sample Extraction

The grounded sample was mixed with 30 mL of acetonitrile in a 125 mL conical flask and was shook for a while. It was then sonicated for 15 minutes at room temperature. The liquid extract was then centrifuged at 2000 rpm for 10 minutes and transferred into another conical flask after passing through a filter funnel contained glass wool and anhydrous sodium sulfate.

The leftover sample extract was added again with 30 mL of acetonitrile, sonicated for 15 minutes, centrifuged and the liquid extract was collected in the same flask with the first extracted liquid. The process was repeated for another one more time. The collected liquid was concentrated for about 3 mL with rotary vacuum evaporator and ransferred into a 10 mL glass test tube using a

Pasteur pipette. The flask was rinsed with 2 mL n-hexane. The concentrated extract was then blow down to 1 mL using nitrogen gas. Fig. 4 shows sample of *Centella asiatica* before the extraction.



Fig. 4: *Centella asiatica* in aluminium foil before extraction

Clean-up process

The extract was subjected to clean-up process using a column containing 5% activated silica gel and anhydrous sodium sulfate where the column was wetted using 10 mL of n-hexane. The concentrated 1 mL extract then was pipette into the column and eluted with 10 mL ethyl acetate: hexane (1: 1). The collected eluents was concentrated by nitrogen to 1 mL before injected into GC-MS for analysis.

Preparation of standard solution for malathion

Standard solutions of malathion were prepared by dissolving the compound in hexane: acetone (1:1) (1 mg/mL). Three concentrations (1 ppm, 0.3 ppm and 0.1 ppm) of malathion were prepared as calibration standard solution from reference malathion standard using this formula below:

$$M_1V_1 = M_2V_2 \tag{1}$$

Where:

M_1 = initial concentration (ppm)

M_2 = final concentration (ppm)

V_1 = volume to be added (mL)

V_2 = volume of final solvent (mL)

The analysis of malathion residues in *Centella asiatica* was carried out by Gas Chromatography

Mass Spectrometer Shimadzu QP 5050A. The fused separation was performed using the operating conditions as mentioned in Table 4. The operations of GC-MS QP5050A (Shimadzu) for the detection of malathion residue in the leave extracts are given in the Table 4 below. The column used for this analysis is fired silica capillary column (30 m X 2.5 mm, 0.25 µm film thickness).

Table 4: The retention time and area of the analyzed samples

Sample	Retention time	Area	Concentration
A1 SW	22.132	74234	19.78 µg/kg
C1 SW	22.131	32043	8.53 µg/kg
E1 UN	22.132	1971	0.53 µg/kg
E1 TW	22.125	1642	0.44 µg/kg
E1 SW	22.092	3454	0.99 µg/kg
F1 UN	22.136	2886	0.77 µg/kg

SW – Salt water; TW – Tap water; UN – Unwashed

Quantitative and qualitative analysis

Full scan mode was used for qualitative analysis, that is for positive identification of target analyte which is based on the mass spectra and retention time of the malathion. Once the fragmentation ions of malathion are identified, 3 quantification ions were selected for SIM mode analysis. They were *m/z*: 125, 127 and 173 (with *m/z*: 127 being based peak ion). The selection of these three quantification ions was followed the level that suggested by Rissato *et al.*, (2007). The concentration of malathion residue was determine using external calibration and the formula below:

$$\text{Concentration in } \mu\text{g/L} = \frac{ABV_t}{V_i W_s} \quad (2)$$

Where:

A= Response factor (1/slopeslope from calibration curve)

B = Peak area

V_t = Extract volume in µL

V_i = Volume injected in µL

W_s = Weight of vegetable sample (kg)

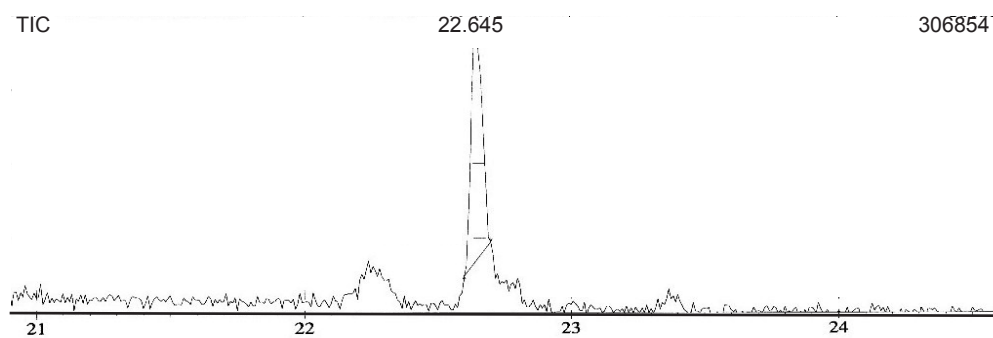


Fig. 5: Chromatograms of 3 mg/L standard and its retention time

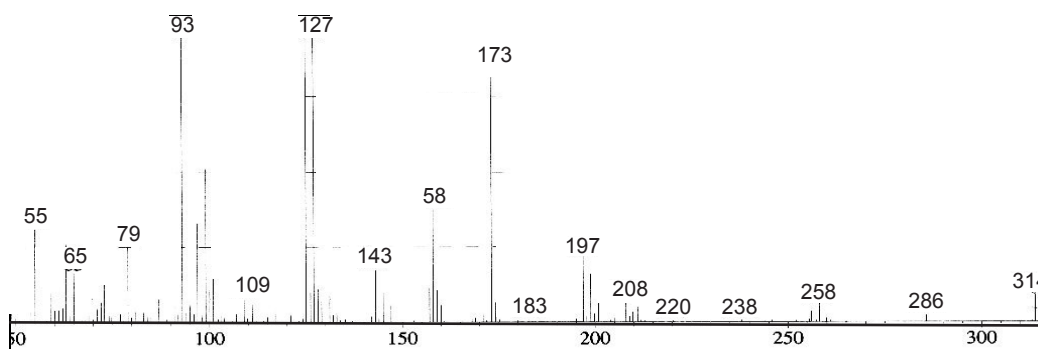


Fig. 6: Fragment ions of malathion

RESULTS

Below are results of GC-MS study on malathion residue in *Centella asiatica*. Fig. 5 shows chromatograms of 3 ppm standard and its retention time while Fig. 6 shows fragment ions of malathion. The response factor (rf) was generated by the slope of the calibration curve with the resolution of GC-MS is ± 1000 .

The control sample taken a day before the application of malathion do not has a residue left. This means that there was no residue from the previous spraying of malathion accumulate in leaves and affecting the study. However malathion residues were detected in 6 samples with different handling methods where in 3 samples treated with salt water (A1, C1 and E1), in 2 unwashed samples (E1 and F1) and in 1 sample treated with tap water (E1) (Table 5).

Table 5: The retention time and area of the analyzed samples

Sample	Retention time	Area	Concentration
A1 SW	22.132	74234	19.78 $\mu\text{g}/\text{kg}$
C1 SW	22.131	32043	8.53 $\mu\text{g}/\text{kg}$
E1 UN	22.132	1971	0.53 $\mu\text{g}/\text{kg}$
E1 TW	22.125	1642	0.44 $\mu\text{g}/\text{kg}$
E1 SW	22.092	3454	0.99 $\mu\text{g}/\text{kg}$
F1 UN	22.136	2886	0.77 $\mu\text{g}/\text{kg}$

SW – Salt water; TW – Tap water; UN – Unwashed

The malathion residue found in A1, C1 and E1 that had been treated with salt water contains highest concentration of malathion residue compared to other handling methods. Sample A1 contain the highest concentration of malathion residue (19.78 $\mu\text{g}/\text{kg}$) while C1 contain almost half of the residue in A1 (8.53 $\mu\text{g}/\text{kg}$). Sample E1 contains 0.99 $\mu\text{g}/\text{kg}$ of malathion residue. The high concentration of malathion residue found in sample A1 and C1 was due to time of extraction. Both of the samples were extracted within a week after the sample time while the other samples were extracted later than that. The time of storage would influence the amount of malathion residue because of its high volatilization rate. The malathion may be degraded to certain level influenced by the temperature of storage.

Only 1 sample treated with tap water has a malathion residue (E1) with concentration of 0.44 $\mu\text{g}/\text{kg}$. This concentration in E1 is the lowest malathion value detected in all samples. Therefore treatment with tap water is the most suitable handling method to clean *Centella asiatica* before consumption.

The unwashed samples from plots E1 and F1 also showed the existence of small concentration of malathion residue which are 0.53 $\mu\text{g}/\text{kg}$ and 0.77 $\mu\text{g}/\text{kg}$ respectively. The availability of malathion residue in the unwashed *Centella asiatica* was expected. Any unwashed foods or beverages have the better chances to be contaminated especially that were freshly taken from garden or farm. However, the concentrations are well below the Maximum Residue Limits (MRL).

The undetected of malathion residue in samples from plot B1 and D1 can be explained by the *Centella asiatica* growth coverage on that plots. The growth coverage was lesser in B1 and D1 compared to other plots which made the spray of malathion went directly onto the ground and less were remain on the leaves surface. The other plots had a crowded growth of *Centella asiatica* on them and given the chances of the malathion to be remained on the leaves surface.

The concentration of malathion residue in the samples treated with the 200 mL of 0.01 g/mL salt water were greater compared to the residue in unwashed samples and the samples treated with the tap water. It is noted that most Malaysian treated or mixed the *Centella asiatica* with salt water prior to consume it. However, the result of this study shows that salt water is not suitable to treat *Centella asiatica* as soaking because it not diluted the malathion.

There were no residue of malathion found in day three and day five samples. It can be explained by Newhart (2006) that the half-life of malathion on the plant surfaces ranges from 0.3 – 8.7 days and the volatilization of malathion that occurs 1.3 mg/m^2 and 0.9 mg/m^2 during the day and night respectively. Furthermore, the heavy rain on day two and three could be the cause malathion wash-off from the leaves.

DISCUSSION

The Food and Drug Administration (FDA) and the EPA allow a maximum amount of 8 ppm of malathion to be present as a residue on specific crops used as foods. From the result, the highest

residue found was 19.78 µg/kg and is far below the MRL. Although the residue is lower than the MRL, regular consumption may pose a threat to health. As most Malaysians consumed fresh *Centella asiatica*, rinse the plants thoroughly with water to enhance precaution and reduce exposure. Taking this safety steps will remove most of the existing surface residues, along with any remaining dirt. However, the surface cleaning like rinsing and scrubbing will not remove pesticide residues that are absorbed into the growing fruit or vegetable before harvest (EPA, 1995).

There is no detection of malathion found in the sample taken on day three and day five after the application of malathion. So, it is safe to harvest *Centella asiatica* at three days onwards after the application of malathion. As malathion can be dangerous to humans, the EPA requires that a certain amount of time must pass between the time of application of the insecticide and entry by a worker into a field where it has been applied. Usually, at least 12 hours must pass between application and entry, but in some cases, such as when workers are entering a field to hand harvest or hand prune the crops, time periods as long as 6 days must pass between application and entry into the field. California EPA restricted 12–24 hours before the workers or harvesting could be done after malathion been applied. The maximum pre-harvest is between 0–7 days.

Sample A1 that had been soaked in salt water for one hour had the highest residue that is 19.78 µg/kg. Only one sample soaked in tap water had a residue detected. However, the concentration is small and the *Centella asiatica* can be assumed to be safe to consume. Unwashed *Centella asiatica* is extremely not safe to consume. The result found the residue were small, however there may be dirt and other contaminants attach to the surface of *Centella asiatica* leaves. Salt water is not the best medium to wash or to be eaten with *Centella asiatica* as three of the samples were detected to have malathion residue even the concentration is small. Washing the *Centella asiatica* with tap water would be the safest way before consumed it especially fresh *Centella asiatica*. The wash-off can reduce the possibility of poisoning by removal of the malathion residue that may remain on the leaves surfaces.

Sanders (1993), in his study found that malathion concentration decline more rapidly on lettuce leaves compared to tomato fruit. The internal concentration only 13% after 12 hours after application and continue to degrade to 2% after two days of application. *Centella asiatica* is also a leafy vegetable like lettuce. It can be assumed that the degradation rate of malathion in *Centella asiatica* is the same with the lettuce which can explained with the absence of malathion residue in the *Centella asiatica* samples taken three days and five days after the day of application.

The decreasing rate of malathion concentration over time from a single spray application is affected by the type of material. Concentrations declined more slowly in soil than in sand, possibly because soil contains more organic matter which has been shown to slow pesticide degradation rates (Sanders, 1993). Newhart (2006) had stated that from 100% application, 8% of malathion unaccounted for either degraded before hitting the ground, volatilized before sample collection, or drifted outside of the target area.

As a conclusion, six from twenty five samples of *Centella asiatica* were detected to have a malathion residue with the ranges from 0.44 – 19.78 µg/kg by using GC-MS. The highest concentration of malathion found in sample A1 (19.78 µg/kg) that had been soaked in salt water for one hour prior to extraction. The availability of malathion residue in the unwashed *C. asiatica* was expected. Any unwashed foods or beverages have the better chances to be contaminated especially that were freshly taken from garden or farm. Unwashed *Centella asiatica* is extremely not safe to consume. Salt water is not the best medium to wash or to be eaten with *Centella asiatica* as three of the samples were detected to have malathion residue even the concentration is small. Washing the *Centella asiatica* with tap water would be the safest way before consumed it especially fresh *Centella asiatica*. The wash-off can reduce the possibility of poisoning by removal of the malathion residue that may remain on the leaves surfaces. Therefore, the safest way to consume *Centella asiatica* is to wash with tap water compared with the unwashed and upon soaked in salt water. However all the amount of malathion residue found on the samples were below the MRL set by the US EPA.

ACKNOWLEDGEMENTS

The authors thank the MARDI Serdang for allowing sampling of *Centella asiatica*.

REFERENCES

- Environmental Protection Agency (EPA). (2007). Protecting the public from pesticide residues in food. <http://www.epa.gov/pesticides/public/residues/food.htm>. Accessed on 11th December 2007.
- Environmental Protection Agency (EPA). (1995). *Citizen's Guide to Pest Control and Pesticide Safety*. Prevention, Pesticides, and Toxic Substances. United States.
- Gambacorta, G., Faccia, M., Lamacchia, C., Di Luccia, A. and La Notte, E., (2005). Pesticide residues in tomato grown in open field. *Food Control*, **16**: 629 – 632.
- Gupta, P.K., (2004). Pesticide exposure—Indian scene. *Toxicology*, **198**: 83–90.
- Hussin, M., Azizah Abdul-Hamid, A., Mohamad, S., Saari, N., Ismail, M., and Hair Bejo, M.H., (2007). Protective effect of *Centella asiatica* extract and powder on oxidative stress in rats. *Food Chemistry*, **100**: 535–541.
- Inamdar, P.K., Yeole, R.D., Ghogare, A.B. and de Souza, N.J. (1996). Determination of biologically active constituents in *Centella asiatica*. *Journal of Chromatography*, **742**: 127-130.
- Kocourek, V., Hajslova, J., Holadova, K. and Poustka, J. (1998). Stability of pesticides in plant extracts used as calibrants in the gas chromatographic analysis of residues. *J Chromatograph.*, **800**: 297–304.
- Newhart, K., (2006). Environmental Fate of Malathion. Department of Pesticide Regulation. California Environmental Protection Agency. California.
- Norris, L.A., (1969). *Behavior of pesticides in plants*. Symposium of Environmental and Physiologic Chemodynamics, Environmental Health Sciences Center at Oregon State University, Corvallis.
- NPIC, (2001). Malathion (Technical Fact Sheet). National Pesticide Information Center. Oregon State University, Corvallis, OR.
- Rao, S.B., Chetana, M., and Devi, P.U., (2005). *Centella asiatica* treatment during postnatal period enhances learning and memory in mice. *Physiology & Behavior*, **86**: 449 – 457.
- Rastrelli, L., Totaro, K., and De Simone, F., (2002). Determination of organophosphorus pesticide residues in Cilento (Campania, Italy) virgin olive oil by capillary gas chromatography. *Food Chemistry*, **79**:303-305.
- Rissato, S.R., Galhiane, M.S., Almeida, M.V., Gerenutti, M. and Apon, B.M., (2007). Multiresidue determination of pesticides in honey samples by gas chromatography–mass spectrometry and application in environmental contamination. *Food Chemistry*, **101**: 1719 – 1726.
- Sallamander Concepts (Pty) Ltd. 2010. Zest for life: specialized for vitamins. www.anyvitamins.com/centella-asiatica-info.htm. access on 22th February 2010.
- Sanders, J., (1993). *Executive Summary: Assessment of Malathion and Malaoxon Concentrations and Persistence in, Water, Sand, Soil and Plant Matrices Under Controlled Exposure Conditions*. Environmental Monitoring and Pest Management Branch Department of Pesticide Regulation. California Environmental Protection Agency. State of California.
- Subathra, M., Shila, S., Devi, M.A., and Panneerselvam, C., (2005). Emerging role of *Centella asiatica* in improving age-related neurological antioxidant status. *Experimental Gerontology*, **40**: 707–715.
- Tariq, M.I., Afzal, S., Hussain, I., and Sultana, N., (2007). Pesticides exposure in Pakistan: a review. *Environment International*, **33**: 1107 – 1122.
- Wikipedia. (2007). *Centella asiatica*. http://en.wikipedia.org/wiki/Centella_asiatica. Accessed on 12th September 2007.
- Zambonin, C.G., Quinto, M., De Vietro, N., and Palmisano, F., (2004). Solid-phase microextraction – gas chromatography mass spectrometry: A fast and simple screening method for the assessment of organophosphorus pesticides residues in wine and fruit juices. *Food Chemistry*, **86**: 269 – 274.
- Zhang, Z.Y., Liu, X.J., Yu, X.Y., Zhang, C.Z., and Hong, X.Y., (2007). Pesticide residues in spring gabbage (*Brassica oleracea L. var. capitata*) grown in open field. *Food Control*, **18**: 723-730.