CHAPTER-3

Ultrasound Assisted GC-NPD technique for determination of trace levels of tebuconazole residues in Garlic, Soil and Water Samples

3.1 Introduction

Tebuconazole, is widely used for controlling soil-borne and foliar diseases such as powdery mildew diseases in leaf spot, rust and root rot of crops acting as steroid demethylation (Sterol biosynthesis inhibitors) (Kwok, I. *et al.,* 1993). Garlic (Allium sativum L.) is a widely cultivated Allium species. Garlic production has been significantly reduced due to white rot disease caused by Sclerotium cepivorum. Sclerotium cepivorum is global importance, causes serious white rot disease on Allium species like onion, garlic, shallot and leek (Coley-Smith J. R. et al., 1987). Chemical control using fungicides is the most common method for white rot management. Several synthetic fungicides which belong to benzimidazoles and triazoles groups have been reported to be effective against this pathogen (Zewide, T. et al., 2007a). Avila De Moreno et al found that vinclozolin and carbendazim sowing the best control of the disease after application of 45 and 75 days (Avila de Moreno, C., 1991). Also earlier it has been reported that vinclozolin and iprodione (Utkhede R. S. et al., 1979), procymidone (Fullerton R.A., 1991) gave reduction of disease incidence up to 75–95%, applied as seed and soil treatment. Melero-Vara and Duff et al. also found that tebuconazole is effective in reducing the progress of the white rod disease and therefore, increases the yield when applied as a clove treatment (Melero-Vara J. M. et al., 2000; Duff A. A. et al. 2001). Many studies indicate tebuconazole (Folicur) is providing good protection against garlic rust disease (Koike S.T. *et al.,* 2001).

The presence of pesticides residues in food products, soil and ground water is extremely hazardous to human beings; as ground water is a major source of drinking water (Aprea, C. *et al.,* 1996 and Frenzel, T. *et al.,* 2000) and soil also an important component of the environment, acts as a sink for the pesticides used in agriculture.

Further its residue from soil can reach water bodies by leaching and runoff (Frank, R. *et al.,* 1987). Therefore, rapid, simple and efficient analytical method is needed for determining the residue of tebuconazole in food products and environment, in order to minimize the risk.

A variety of extraction techniques have been employed over the years to determine pesticides and their degradation products, including liquid–liquid extraction (LLE) (Sabik, H. *et al.,* 1997), solid-phase extraction (SPE) (Sabik, H. *et al.,* 1995 and Sabik, H. *et al.,* 1998), solid-phase microextraction (SPME) (Choudhury, T. K. *et al.,* 1996 and Boyd-Boland, A.A. *et al.,* 1996), semi-permeable membrane device (SPMD) (Ellis, G. S. *et al.,*1995 and Huckins, J. N. *et al.,* 1993) and supercritical fluid extraction (Papilloud, S. *et al.,* 1996) followed by various chromatographic techniques such as gas and liquid chromatography (GC and LC) coupled with nitrogen–phosphorus (NPD) (Bernal, J. L. *et al.,* 1997), electron-capture (ECD) (Aguilar, C. *et al.,* 1997), diode-array (Aguilar, F. *et al.,* 1996), fluorescence (Makela, M. *et al.,* 1995) and mass spectrometry (MS) detection systems (Verma, K. K. *et al.,* 1997). Most of the pre-concentration techniques are expensive, complicated, time consuming and have low extraction efficiency. The ultrasound assisted extraction (UAE) has high extraction efficiency, low amount of solvent consumption, lower equipment cost, ease to operate, no hassle related to sample preparation (e.g. partitioning and clean up procedure), is easy to perform and requires lower extraction temperature. This extraction process is fast in comparison with the traditional methods, because of the contact surface area between solid or liquid sample and liquid phase of extraction solvent is much greater, due to particle disruption taking place (Filgueiras A.V., *et al.,* 2000). These advantages make it a quick and efficient method for the extraction of analytes from complex matrices.

Tebuconazole is a triazole group organonitrogen pesticide containing three nitrogen atoms in ring and is polar in nature. Hence the separation of tebuconazole with low bleed mid-polar narrow bore column, DB-35(35% phenyl-methylpolysiloxane stationary phase) with nitrogen-phosphorous selective detector for low level detection of tebuconazole was adopted instead of HPLC

The aim of this work is to determine trace amounts of tebuconazole in garlic, soils and water samples by using capillary GC-NPD followed by Ultrasound assisted extraction (UAE) technique. The UAE accelerates the emulsification of ethyl acetate thus enhancing the extraction efficiency of tebuconazole followed by centrifugation for layer separation prior to GC separation. The UAE is an alternative extraction technique for common Soxhlet, QuEChERS (quick, easy, cheap, effective, rugged and safe), SPE and shaking flask extraction (Sporring, S., *et al.,* 2005, Aydin, M. E., *et al.,* 2006, Tor, A. *et al.,* 2006, and Tor, A., *et al*.,2006a) for pesticides from complex matrices.

3.2 Materials and Methods

3.2.1 Reagents and standards

A reference standard of tebuconazole (purity 99.24%) and formulation of tebuconazole, Folicur 25.9% EW (source, Bayer Crop Science, India). Ethyl acetate and anhydrous sodium sulfate (AR grade) were purchased from Merck India. The stock solutions of tebuconazole (500 mg/L) were prepared in ethyl acetate.

3.2.2 GC and operating conditions

The capillary gas chromatography was equipped with a nitrogen-phosphorous selective detector and spit mode injector (Perkin Elmer, USA, and Model, Clarus 500). The Dura Bond mid-polar fused-silica capillary column with low bleed (DB-35

column: packing with 35% Phenyl- methylpolysiloxane, dimension: 30 m×0.25 mm I.D., 0.25 micron film thickness) was connected with GC and the instrument was controlled by Total Chrom software for data integration. The optimum temperature parameters were 200 to 275°C@25°C/min (hold for 8.0 min), 260 and 290°C for column oven, injector port and detector respectively. A 2µL volume of aliquot test solutions were injected into the split mode injector (split ratio, vent: column, 2:1). At optimum conditions of carrier gas–helium and flow rate, 1.0 ml/min, hydrogen gas rate 1.5 ml/min and air 95 ml/min, attenuation 32, range 1, bead background 0.5 mV, and time constant 200. At this optimum condition, an excellent linear relationship was observed between detector response and concentration. The method was found to have good sensitivity and precision. Ultrasonic water bath (Fixed Frequency 35 kHz, Spectra lab, India) was used for extraction; the round type bottom glass tube of 15 ml capacity with interchangeable polypropylene cap (Borosil, India) was used as sample container for extraction and refrigerated centrifuge (Rota 4RV/FM, Plasto Crafts, India) was used for centrifugation of samples.

3.2.3 Optimization of GC-NPD operation condition

The GC-NPD temperature parameters have been optimized by changing column oven, injector and detector temperature separately (Table 3.1). The optimum temperature parameters for column, injector and detector were found to be 200 to 270°C@25°C/min (8.0min hold), 260 °C and 290°C respectively. At this operation condition, detector showed maximum and repeatable response for tebuconazole. The details of optimization sequence are given in table 3.1:

Table 3.1: Optimization of GC-NPD Temperature Parameters

A (Variation in GC Oven Temperature)							
Column : DB-35[30 m (length) x 0.25 mm (i.d) x0.25 µm (film thickness)]							
Detector : NPD	Carrier Flow : 1.5 ml/ min						
A (Variation in Column Temperature)							
Variation-(1) In Column Temp.	Column Temperature : 200 to 270°C@25°C/min(8.0 min hold)						
	Injector Temperature : 260 °C						
	Detector Temperature : 290 °C						
Compound	Conc. (ppm)	Peak Area (R1)	Peak Area (R2)	Mean Peak Area	Average Noise	Signal to Noise ratio (S/N)	% Variation in replication
Tebuconazole	0.52	9152	9193	9172.5	75.60	121.33	0.45
	0.10	1775	1745	1760.0		23.28	1.69
	0.05	850	863	856.5		11.33	1.51
Remark: Response increases relative proportion to concentration. Found best response and repeatability in area of replication.							
Variation-(2) In Column Temp.	Column Temperature : 180 to 260°C@25°C/min(8.0min hold)						
	Injector Temperature : 260 °C						
	Detector Temperature : 290 °C						
Compound	Conc. (ppm)	Peak Area (R1)	Peak Area (R2)	Mean Peak Area	Average Noise	Signal to Noise ratio (S/N)	% Variation in replication
Tebuconazole	0.52	1613	1591	1602.0	107.83	14.86	1.36
	0.10	396	417	406.5		3.77	5.04
	0.05	183	195	189.0		1.75	6.15

Remark: Response increases not relative proportion to concentration. The repeatability in area of replication but poor in response							
Variation-(3) In Column Temp.	Column Temperature : 240°C (15.0 min hold)						
	Injector Temperature : 260°C						
	Detector Temperature : 290 °C						
Compound	Conc. (ppm)	Peak Area (R1)	Peak Area (R2)	Mean Peak Area	Average Noise	Signal to Noise ratio (S/N)	% Variation in replication
Tebuconazole	0.52	5478	5407	5442.5	72.34	75.24	1.30
	0.10	724	725	724.5		10.02	0.14
	0.05	662	653	657.5		9.09	1.36
Remark: Response increases not relative proportion to concentration. The repeatability in area of replication but poor in response.							
B (Variation in Injector Temperature)							
Variation-(1) in Injector Temp.	Column Temperature : 200 to 270°C@25°C/min(8.0 min hold)						
	Injector Temperature : 250 °C						
	Detector Temperature : 290 °C						
Compound	Conc. (ppm)	Peak Area (R1)	Peak Area (R2)	Mean Peak Area	Average Noise	Signal to Noise ratio (S/N)	% Variation in replication
Tebuconazole	0.52	7807	7709	7758.0	68.25	113.67	1.26
	0.10	725	741	733.0		10.74	2.16
	0.05	644	642	643.0		9.42	0.31
Remark: Response increases not relative proportion to concentration. The repeatability in area of replication but poor in res ponse.							

Table 3.1: Optimization of GC-NPD Temperature Parameters (Continued…)

Variation-(2) in Injector Temp.	Column Temperature : 200 to 270°C@25°C/min(8.0 min hold)						
	Injector Temperature : 270 °C						
	Detector Temperature : 290 °C						
Compound	Conc. (ppm)	Peak Area (R1)	Peak Area (R2)	Mean Peak Area	Average Noise	Signal to Noise ratio (S/N)	% Variation in replication
Tebuconazole	0.52	5125	5159	5142.0	75.16	68.41	0.66
	0.10	958	430	694.0		9.23	55.11
	0.05	890	255	572.5		7.62	71.35
Remark: Response increases not relative proportion to concentration. The response is poor and also not repeatability in area of replication.							
C (Variation in Detector Temperature)							
Variation-(1) in Detector Temp.	Injector Temperature : 260 °C						
	Detector Temperature : 300 °C						
Compound	Conc. (ppm)	Peak Area (R1)	Peak Area (R2)	Mean Peak Area	Average Noise	Signal to Noise ratio (S/N)	% Variation in replication
Tebuconazole	0.52	4541	4581	4561.0	74.00	61.64	0.87
	0.10	472	468	470.0		6.35	0.85
	0.05	989	962	975.5		13.18	2.73
Remark: Response increases, but not relative proportion to concentration. The repeatability in area of replication but response is poor.							

Table 3.1: Optimization of GC-NPD Temperature Parameters (Continued…)

Variation-(2) In Detector Temp.	Column Temperature : 200 to 270°C@25°C/min(8.0 min hold)						
	Injector Temperature : 260°C						
	Detector Temperature : 280 °C						
Compound	Conc. (ppm)	Peak Area (R1)	Peak Area (R2)	Mean Peak Area	Average Noise	Signal to Noise ratio (S/N)	% Variation in replication
Tebuconazole	0.52	4255	4307	4281.0	63.83	67.07	1.21
	0.10	609	605	607.0		9.51	0.66
	0.05	717	738	727.5		11.40	2.85
Remark: Response increases, but not relative proportion to concentration. The repeatability in area of replication but respon se is poor.							

3.2.4 Sample preparation of garlic, soil and water for recovery studies

The white variety of garlic was purchased from the Vapi, Valsad. Whole garlic bulbs were blended into paste. About 5 g of accurately weighed homogenized garlic paste, Nanivaliyal soil samples and 10 ml River water sample were taken into 15-ml screw-capped round type bottom glass tubes separately. No any pesticide was applied in selected field plat since last six month. Each sample was separately spiked with 5 and 20 µg/kg of tebuconazole for soil and garlic paste samples or 5 and 20 µg/L of tebuconazole in river water sample. A volume of 2 ml distilled water and 5 ml ethyl acetate were added into garlic and Nanivaliyal soil samples tubes separately and 2 ml ethyl acetate was added into River water sample tubes . The tubes were placed on ultrasonic bath (Frequency of 35 kHz, Spectra lab, India) at 30 °C temperature for 15 min then centrifuged for 10 min at 4000 rpm. The organic layer was separated; moisture from the extracts was removed by passing through sufficient amount of sodium sulfate and extracts dried under gentle stream of nitrogen gas. The residues were re-concentrated with 1 ml ethyl acetate and then 2 µL of aliquot was injected in optimum condition into capillary GC-NPD for recovery analysis. Five replicate experiments were performed for each sample.

3.2.5 Pesticide application and sampling

Field experiments were conducted to determine the harvest time residue of tebuconazole in garlic, Nanivaliyal field soil and expected contamination in River water because the garlic was planted 4 by 4 inches apart in triple rows on 25 October 2008. Clove tops was covered with 1 to 1½ inches of soil. After plantation 9 time irrigations was given i.e., first irrigation just after sowing, second at 15 days after sowing and remaining 7 irrigations at an interval of 10-15 days and applied 50% Ruminal Degradable Nitrogen (RDN) as urea + 50% N through bio compost for

achieving higher bulb yield. The formulation of tebuconazole, Folicur 25.9% EW was applied 65 days after planting garlic and second spray was applied after 15 days of first application. The fall-planted garlic was harvested in month of July 2009. 15 garlic bulbs were selected randomly and soil samples were ploughed and collected from 4–5 spots of the plot for residue analysis. The soil samples were mixed thoroughly, air dried, and passed through a 2 mm sieve. The sample was spread on a glass plate and divided into four parts (quarters). Soil from two opposite quarters was retained, rejecting the remaining two. The process were used to obtain 500 g of representative soil sample and the moisture content was maintained approximately at 60% of the maximum water holding capacity, using distilled water. The details of soil characterization were given in Chapter **2**. Water samples were collected in triplicate into amber colored glass bottles from Par River flowing approximately 30-50 meter far from the selected plot (5 × 5 m) expecting contamination

3.3 Results and Discussion

3.3.1 Effect of the solvents an extraction

For the ultrasound extraction method, the selection of extraction solvents should obey the following principles: the target analytes must be dissolved in the extraction solvents, solvent must have good chromatography behavior and solvents must be sufficiently immiscible in aqueous phase, hence after extraction no extra partition step would be required. Keeping these criteria in mind, ethyl acetate was tested and the effect of this solvent on the performance of UAE was investigated. It was observed that good recovery of tebuconazole was found in ethyl acetate (see Table 3.2) as its polarity was suitable for extraction of tebuconazole from garlic, soil and water samples. The clean-up steps were omitted to avert the loss of the tebuconazole so it was detected sensitively.

Table 3.2: Recoveries, precision (% RSD, n = 5), linear range, correlation coefficient, limits of quanitation (S/N = 7.5 ± 2.5) and limits of detection (S/N = 3 ± 0.5) of UAE technique

Sample	Blank	Fortification Level (µg/kg or µg/L)	Recovery (%) in Sample	RSD(%, n = 5)	Range of Concentr-ation (µg/kg or µg/L)	LOQ (µg/kg or µg/L)	LOD (µg/kg or µg/L)
Nanivahiyal Soil	-	5	99.33	4.85	1-50	10	3
		20	96.28	3.40			
River Water	-	5	105.15	3.78	1-50	1	0.2
		20	95.04	1.33			
Garlic Vegetable	-	5	101.26	4.58	1-50	5	2
		20	95.55	2.71			

3.3.2 Influence of samples matrix on recovery

The fungicide recovery in soil may be affected by the physico-chemical properties of soils (Perez, R.A. *et al.,* 1998), in particular, the soil pH, cation exchange capacity, water contents and characteristics of fungicides. In present study, distilled water was used for extraction as aqueous extractant. The pH values and cation exchange capacity of soil were 7.02, 10.57 meq/100 g soil respectively and pH value of garlic paste and water samples were 5.97, 7.19 respectively, of tebuconazole. The pH value of soil, garlic paste and water samples did not affect ionization (degradation) of tebuconazole in extraction samples as tebuconazole is weakly acidic (*pK*a, 5 ± 0.1) (Cadkova E., et al. 2013) **(**Table 3.2**).**

3.3.3 Effect of sonication and centrifugation time

During UAE, the effect of sonication time on quantitative recoveries of tebuconazole was investigated. Different extractions were done using increasing sonication times (5, 10, 15, 20 and 25 min), to establish the kinetic of the extraction.

Figure 3.1 shows that the sonication time played a significant role in extraction efficiency of tebuconazole The extraction efficiency was maximum and remained constant after 15 min sonication The transfer of analytes from aqueous phase to extraction solvents phase could be taking 15 minutes. Then to achieve the equilibrium state, the centrifuging time was also studied from 5 min to 20 min with a rotation speed of 4000 rpm. Figure 3.2 shows that the extraction efficiency of tebuconazole reached maximum at 10 min centrifugation and remained unchanged with further increase in centrifuging time up to 20 min. Therefore the sonication time and centrifugation time for subsequent analysis were decided to be 15 min and 10 min.

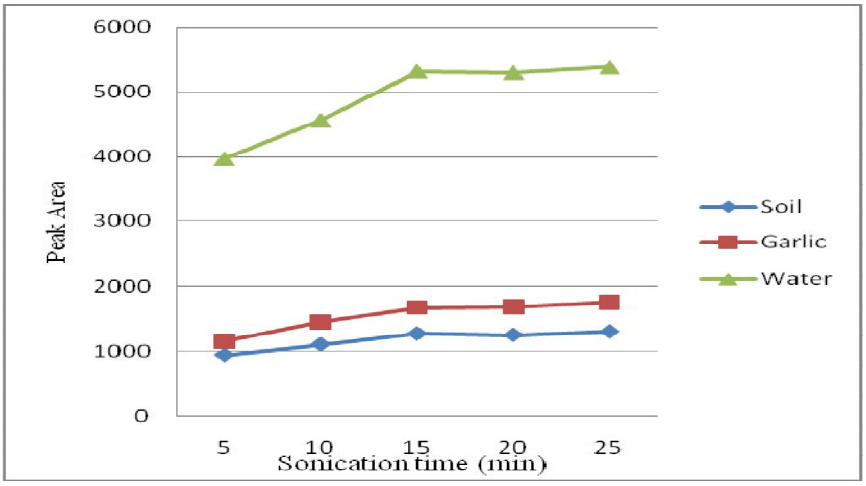


Figure 3.1: Effect of sonication time on extraction efficiency: Organic solvent (ethyl acetate) volume added 1 ml; constant centrifugation time 5 min; spiked sample concentration 20 µg/kg or L^{-1} for garlic, soil and water samples separately.

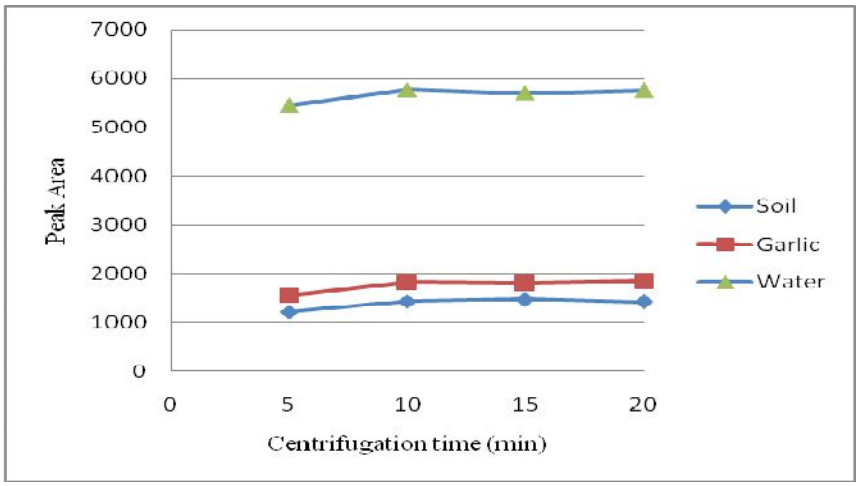


Figure 3.2: Effect of centrifugation time on extraction efficiency: Organic solvent (ethyl acetate) volume added 1 ml; constant sonication time 15 min; spiked sample concentration 20 µg/kg or L^{-1} for garlic, soil and water samples separately.

3.3.4 Effect of solvent volume

In order to examine the effect of extraction solvent volume for extraction of tebuconazole from garlic, soil and water samples, different volumes of ethyl acetate ranging from 1 to 6 ml were investigated for extraction. It is observed from Figure 3.3 that the extraction efficiency increased when the volume of ethyl acetate was increased and then remained almost constant at 5 ml in garlic and soil and 2 ml in water samples. Therefore, 5 and 2 ml ethyl acetate were selected for garlic, soil and water respectively for further studies.

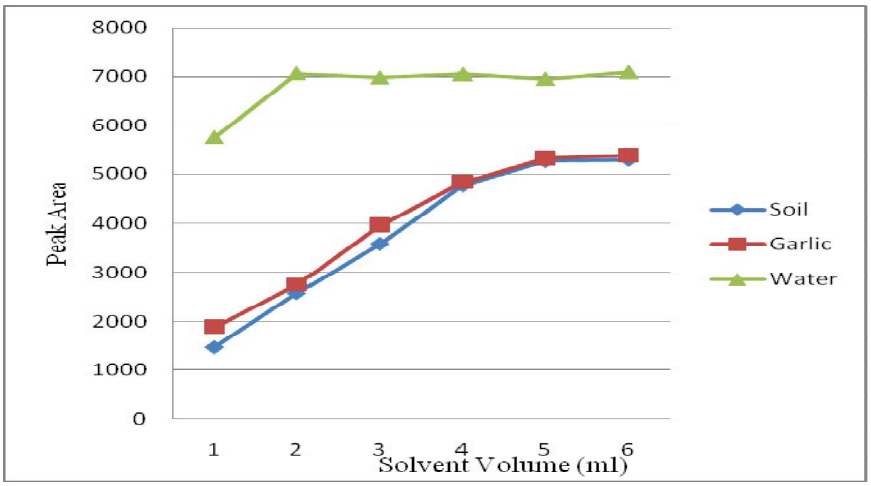


Figure 3.3: Effect of organic solvent (ethyl acetate) volume on extraction recoveries: Sonication time 15 min; Centrifugation time 10 min; spiked sample concentration 20 µg/kg or µg L^{-1} for garlic, soil and water separately.

3.3.5 Feature of the method

The optimization of experimental condition is given in Table 3.1. The combined capillary GC-NPD and UAE procedure for determining tebuconazole residues in soil, water and garlic samples were validated by evaluating the parameters such as linear range, correlation coefficients, detection limits (LODs), quantification limits (LOQs) and percentage relative standard deviation (% RSD) as results listed in Table 3.2. The method had excellent linearity between the peak area and concentration of tebuconazole in the range between 1 and 50 µg kg^{-1} or µg L^{-1} in garlic, Nanivaliyal soil and River water samples. The linearity graph plots (water, soil and garlic) are shown in Figure 3.4 (A, B and C). The detection limits of method, calculated on the basis of signal to noise ratio of 3 ± 0.5 (S/ N = 3 ± 0.5) of GC-NPD with UAE were in the range of 0.2 to 3 µgkg^{-1} or µg L^{-1}. The quantification limits, calculated on the basis of signal to noise ratio of 7.5 ± 2.5 (S/ N = 7.5 ± 2.5) of GC-NPD with UAE was in the range between 1 to 10 µgkg^{-1} or µg L^{-1}.The method precision was investigated

for tebuconazole by performing five replicate experiments for each sample separately after spiking 5 and 20 µgkg^{-1} or µg L^{-1} (in soil, garlic, water samples) and the relative standard deviation (RSD) was ≤ 5%.

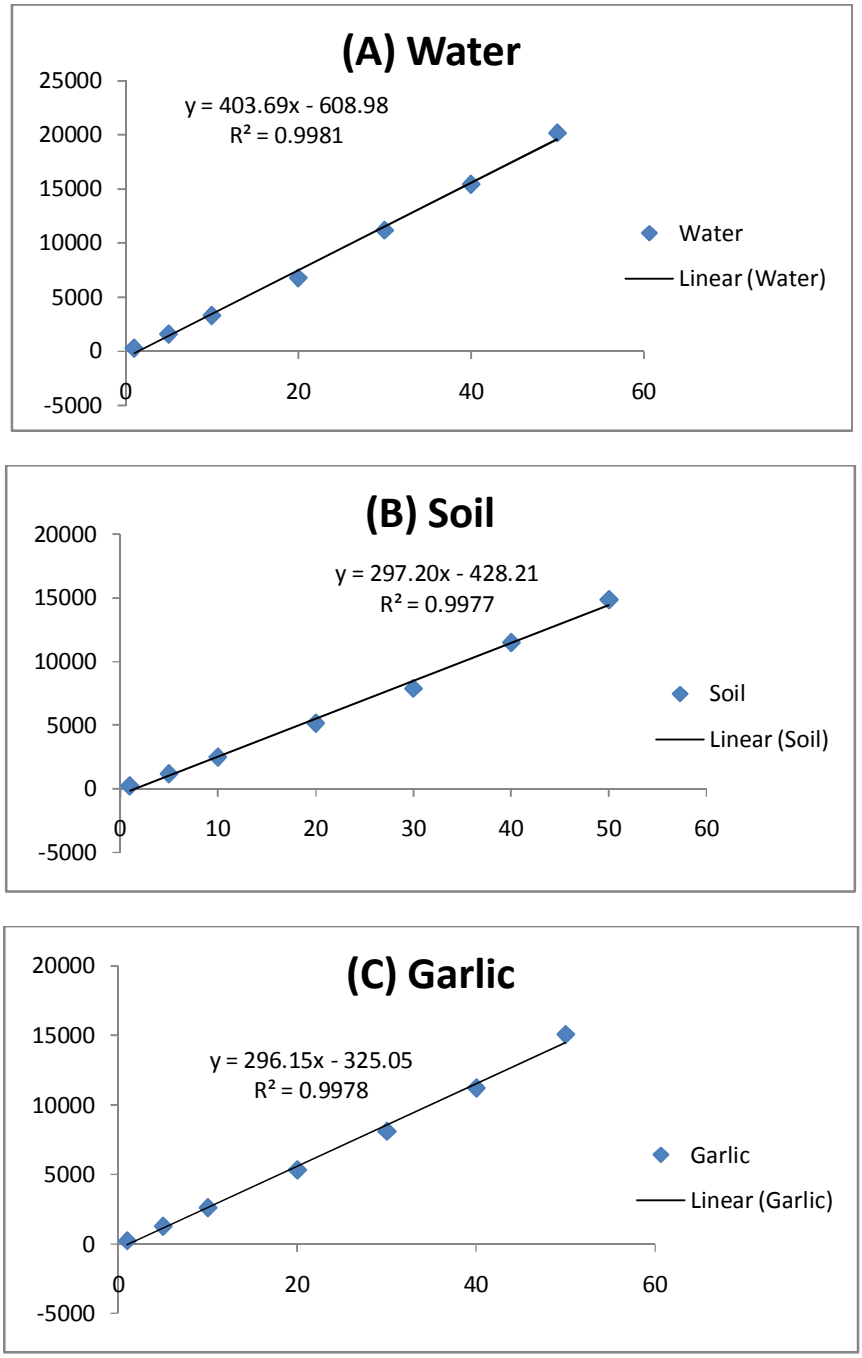
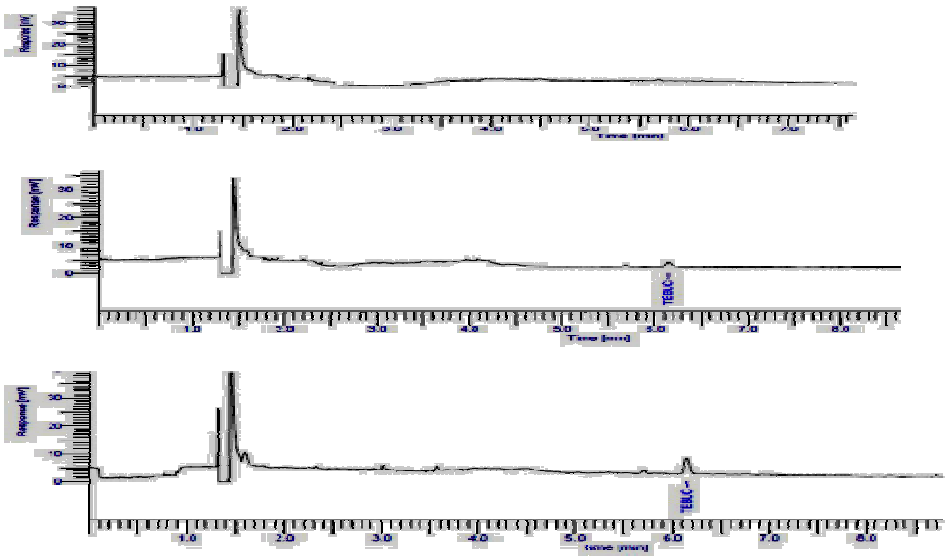


Figure 3.4: (A) Linearity graph plot in (A) water, (B) soil and (C) garlic vegetable between 1 and 50 µg kg^{-1} or µg L^{-1}.

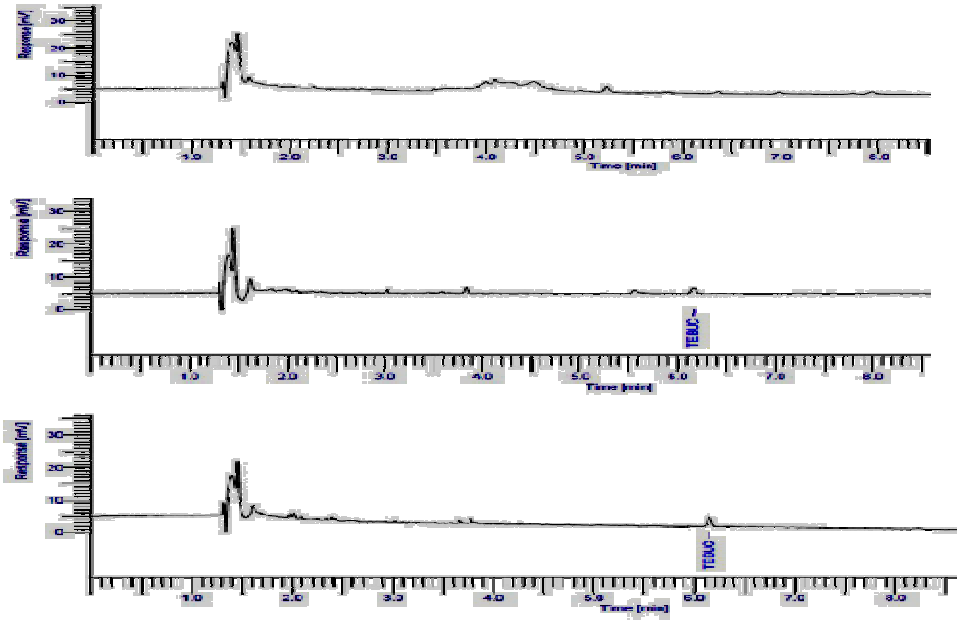
3.3.6 Analysis of garlic, soil and water during harvesting of garlic

The extraction and determination of tebuconazole residue at harvest time in garlic and Nanivaliyal soil samples and expected contamination in Par River water samples were performed according to the procedures described herein. It was observed that there were no harvest time residues of the tebuconazole in garlic and Nanivaliyal soil and further tebuconazole was not detected in River water. Thus, to assess matrix effects, the Garlic paste, Nanivaliyal soil and river water samples were spiked with standard solution of tebuconazole at concentration levels of 5 and 20 µg/kg or µg/L. For each concentration level, five replicate experiments for the whole analysis process were made. The recoveries of the method were expressed as the mean percentage between the amounts found and the ones added. The extraction performance was evaluated and the results are given in Table 3.2. The mean recoveries of the tebuconazole in garlic, Nanivaliyal soil and River water samples at spiked concentration levels 5 and 20 μgkg^{-1} or μgL^{-1} were in the range 95.55 to 101.26, 96.28 to 99.33, and 95.04 to 105.15 %.

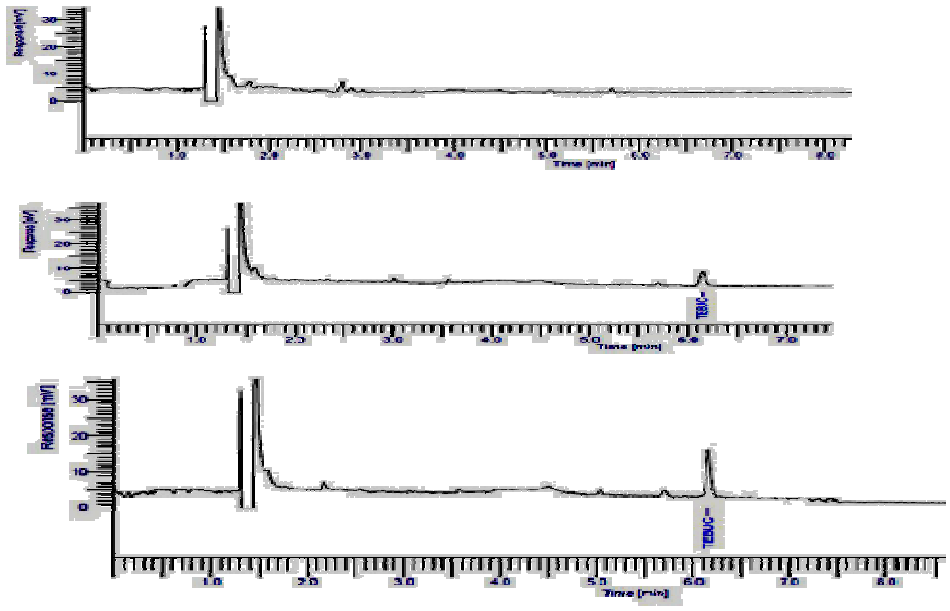
Figure 3.5 (A, B and C) shows the typical chromatograms of the garlic paste sample, Nanivaliyal soil and Par River water samples before and after being spiked of the tebuconazole standard solution at level 5 and 20 μgkg^{-1} or μgL^{-1} for each separately. The retention time (RT) of tebuconazole in spiked samples is at 6.16 minutes.



A)



B)



C)

Figure 3.5: (A) Typical chromatograms of the real garlic vegetable (B) Nanivaliyal soil and (C) Par River water samples before and after being spiked of the tebuconazole standard solution at concentration level 5 and 20 μgkg^{-1} or μgL^{-1} for each respectively, The retention time (RT) of tebuconazole in spiked samples is found at 6.16 minutes.

The method under study was compared with other literature reported methods for determination of tebuconazole in water, soil and vegetables (Table 3.3). The proposed method was found to have comparable sensitivity with good reproducibility and recovery from soil, garlic and water samples as seen from Table 3.2.

Table 3.3: Comparison of method sensitivity with literature reported methods

Method	Matrix	LOD	LOQ	Reference
Titanate array µSPE-HPLC-UV	Water	0.016–0.086 µg L^{-1}	-	Huang Y. *et al.,* 2013
LC-MS/MS	Vegetables, fruits, soil and water	less than 0.6 µg/kg	less than 2.0 µg/kg	Li. Y. *et al.,* 2011
GC-NPD, GC-IT-MS	Vegetables, fruits, soil and water	0.4-7 µg/kg	1.2-20 µg/kg	Wang, X. *et al.,* 2011
GC-MS	Plant material, water and soil	0.001-0.01 mg gk^{-1}	0.01 mg kg^{-1}	Lee, P. W. etal., 2003
LC-MS/MS	Soya products	-	100 µg kg^{-1}	Ionara R. *et al*., 2009
UAE- GC-NPD	Soil	3.0 µg/kg	10.0 µg/kg	Present Study
	Water	0.2 µg/L	1.0 µg/L	
	Garlic	2.0 µg/kg	5.0 µg/kg	

3.4 Conclusion

It is concluded that the proposed method would have good selectivity compared with some of the most recent, and efficient extraction techniques, such as µSPE. The results demonstrate that the proposed method can consume low amount of organic solvent and is faster in comparison with the traditional methods, as the contact surface area between solid or liquid sample and liquid phase of extraction solvent is much greater, easy to operate and more efficient, because the ultrasound radiation applied accelerates the emulsification of the ethyl acetate in aqueous samples to enhance the extraction efficiency of tebuconazole without requiring extra partitioning or cleaning. The proposed method was found to be sensitive with good reproducibility and recovery from soil, garlic and water samples. Results demonstrate the proposed technique was a viable alternative for determination of tebuconazole in complex samples.

3.5 References

- Aguilar C., Borrull F., Marce´ R.M., *J. Chromatogr. A*, **1997**, *771*, 221.
- Aguilar F. B., Marce´ R.M., *Chromatographia*. **1996**, *43*, 592.
- Aprea C., Sciarra G., Lunghini L., *J. Anal. Toxicol*. **1996**, *20*, 559.
- Avila De Moreno, C., *Fitopatol. Colombiana* **1991**, *15*, 62.
- Aydin, M. E., Tor, A., Ozcan, S., *Analytica Chimica Acta*. **2006**, *577*, 232.
- Bernal J. L., Nozal M. J., Jime´nez J. J., Rivera J. M., *J. Chromatogr. A*. **1997**, *778*, 111.
- Boyd-Boland A.A., Magdic S., Pawliszyn J.B., *Analyst*, **1996**, *121,* 929.
- Cadkova E., Komarek M., Debord J., PuppaLoic D., Bordas F., Bollinger J, *J. of Solution Chem*., **2013**, *42*, 1075-1082.
- Choudhury T. K., Gerhardt K. O., Mawhinney T. P., *Environ. Sci. Technol*.**1996**, *30*, 3259.
- Coley-Smith J. R., Parfitt D., Taylor I. M., *Plant Pathol.*, **1987**, *36*, 594.
- Duff A. A., Jackson K. J., O'Donnell W. E., *Acta Horticult*. **2001**, *555*, 247–250.
- Ellis G. S., Huckins J. N., Rostad C. E., Schmitt C. J., Petty J. D., Carthy P. M., *Environ. Toxicol. Chem.* **1995**, *14*, 1875.
- Filgueiras A. V., Capelo J. L., Lavilla I., Bendicho C., *Talanta*. **2000**, *53*, 433.
- Frank R., Clegg B. S., Ripley B. D., Braun H. E., *Arch. Environ. Contam. Toxicol.* **1987**, *16*, 9.
- Frenzel T., Sochor H., Speer K., Uihlein M., *J. Anal. Toxicol*. **2000**, *24*, 365.
- Fullerton R. A., Stewart, A, *J. Crop Horticult. Sci.* **1991**, *19*, 121.
- Huang Y., Zhou Q., Xie G., *Chemosphere*, **2013,** *90*, 338.
- Huckins J. N., Manuweera G. K., Petty J. D., Mackay D., Lebo J. A., *Environ. Sci. Technol*. **1993**, *27*, 2489.

- Koike S. T., Davis S. R. F., *Plant Dis*. **2001**, *85*, 585.
- Kwok I., Loeffler R., *Pestic. Sci.* **1993**, *39*, 1.
- Lee P. W., (2003). DuPont Crop Protection USA, Handbook of Residue Analytical Methods for Agrochemicals VOLUME 1 and VOLUME 2. John Wiley & Sons Ltd. The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England.
- Li Y. , Dong F., Liu X., Xu J., Li J., Kong Z., Chen X., Zheng Y., *J Sep Sci.* **2012,** 35 (2), 206.
- Makela M., Py. L., *J. Chromatogr. A*. **1995**, *49*, 699.
- Melero-Vara, J. M., Parados-Ligero A. M., Basallote U.M.S., *Eur. J. Plant Pathol*. **2000**, *166*, 581.
- Papilloud S., Haerdi W., Chiron S., Barcelo D., *Environ. Sci. Technol*. **1996**, *30*, 1822.
- Perez R.A., Sa´nchez-Brunete C., Miguel E., Tadeo J. L., *J. Agric. Food Chem*. **1998**, *46* 1864.
- Ionara R. P., Kok A., Hiemstra M., Wickert C., Prestes O. D., *Journal of Chromatography A,* **2009**, *1216*, 4539.
- Sabik H., Cooper S., Lafrance P., Fournier J., *Talanta* **1995**, *42*, 717.
- Sabik H., Fouquet A., Proulx S., *Analysis* **1997**, *25*, 267.
- Sabik H., *Int. J. Environ. Anal. Chem*. **1998,** *71*, 87.
- Sporring S., Bøwadt S., Swensmark B., Björklund, E., *J. Chromatogr. A*. **2005**, *1090*, 1.
- Tor A., Aydin M. E., *Analytica Chimica Acta*, **2006**, *575 (1)*, 138.
- Tor A., Aydin M. E., Ozcan S., *Analytica Chimica Acta*. **2006a**, *559*, 173.
- Utkhede R. S., Rahe J. E., Pesti. Sci. **1979**, *10*, 414.

- Verma K. K., Louter A. J. H., Jain A., Pocurull E., Vreuls J. J., Brinkman U. A. T., *Chromatographia,* **1997**, *44*, 372.
- Wang X., Xu J., Dong F., Song W., Zheng Y., *Biomed Chromatogr.* **2011,** *25(10)*, 1081.
- Zewide T., Fininsa C., Sakhuja P. K., *Crop Protection,* **2007a**, *26(6),* 856.