



FLOTATION- DISSOLUTION- SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF QUINALPHOS IN VARIOUS ENVIRONMENTAL SAMPLES

¹Ajay Kumar Sahu, ²Yaman Kumar Sahu, ^{3*}Manish Kumar Rai, ⁴Joyce Rai, and ⁵Shraddha Ganesh Pandey

^{1, 2, 5} Research Scholar, SOS in Chemistry, Pt. Ravishankar Shukla University Raipur, Chhattisgarh, India,

^{3*} Associate Professor, SOS in chemistry, Pt. Ravishankar Shukla University Raipur, Chhattisgarh, India,

⁴Scientist- D, Chhattisgarh Council of Science and Technology(C-COST) Raipur, Chhattisgarh, India.

Abstract: The proposed method is based on flotation – dissolution an easy, impressible, extractive spectrophotometric determination, explained for easy investigation of the organothiophosphate pesticide quinalphos (O, O-diethyl O-quinoxalin-2-yl phosphorothioate) on trace levels. A molybdophospho complex is generated when quinalphos is treated with ammonium molybdate in an acidic medium. As an ion associate complex with methylene blue, the complex is present in between the water and organic layers which is extracted and then dissolved with acetone. The greenish-blue complex produced shows absorption maxima at 660 nm. Beer's law range is found to be 10 to 80µg per 10 mL for quinalphos. The molar absorptivity is $1.2 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ and Sandel sensitivity is 1.01×10^{-3} . Also calculated the standard deviation and relative standard deviation for the above method were ± 0.0005 and 1.14% respectively. The method has been applied and checked for the determination of quinalphos in water, soil, and vegetables.

Keywords: Quinalphos, Ammonium molybdate, Methylene blue, Spectrophotometric determination.

I. INTRODUCTION

Among synthetic pesticides, organophosphates are widely used in agriculture and in health and hygiene programs due to their high effectiveness as insecticide but less persistence in the environment (Sadique et al., 2016). Quinalphos (O, O-diethyl O-quinoxalin-2-yl phosphorothioate), (Rohit et al., 2016) an organophosphate (OP) pesticide, is used in controlling the pests of a variety of crops. (Eid and Abha, 2017). Quinalphos is a synthetic OP, non-systemic, broad-spectrum insecticide, and acaricide extensively used in India owing to its action on inhibition of acetylcholinesterase in target pests. Being ranked as moderately hazardous by the World Health Organization (WHO) and classified as a yellow label (highly toxic) pesticide in India, quinalphos is either banned or restricted in its usage in most of the nations. Nevertheless, quinalphos is still being used to treat the following crops: wheat, rice, groundnut, cotton, sugarcane, coffee and other ornamental crops. Only 1% of the pesticides applied to make contact with the target pest, while the remaining 99% of the pesticide drifts into the environment contaminating soil, water and biota. It may be an undesirable and persistent pollutant to the non-target environment like river and other ecosystems. (Gangireddygaru et al., 2017). There is, therefore, the need to continuously monitor the quality of the environmental samples available in the field.

A review of existing literature reveals that several unofficial assay methods have been developed and deployed for the determination of the quinalphos and Because of its toxicity and Implication a, number of instrumental sophisticated method such as atmospheric pressure matrix-assisted laser desorption/ionization high-resolution mass spectrometry (Mahale et al., 2017), headspace–solid-phase microextraction–gas chromatography, (Abdulra and Tan, 2015), Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization High-Resolution Mass Spectrometry. (Mahale et al., 2017), Yeast-Based Bioassays, (Westlund and Yargeau, 2017), Headspace–Solid Phase Microextraction–Gas Chromatography, (Abdulra and Tan, 2015), Quenchers Sample Preparation Procedure And Gas Chromatography – Flame Photometric Detector, (Yang et al., 2015), Direct Gas Purge Microsyringe Extraction Coupled On-Line With Gas Chromatography–Mass Spectrometry (Nan et al., 2015), Dispersive Solid Phase Extraction Gas Chromatography-Mass Spectrometry (Su et al., 2011), Direct Solid-Phase Microextraction Combined With Gas Chromatography-Mass Spectrometry, (Gallardo et al., 2006), sample preparation procedure and gas chromatography – flame photometric detector, (Yang et al., 2015), The aforementioned methods each have their unique strengths and advantages, but many of them are generally complex in nature and due to less sensitive of some of these method and may require the use of expensive instruments as well as require regular maintenance to overcome of these drawback a low cost and sensitive method is required.

Due to its acaricidal and insecticidal properties, the ever-increasing use of quinalphos as a first-line treatment in agriculture demands the development of new and alternative methods which are simple and easy to carry out to enable the successful assay of the quinalphos. The aim of the present work is, therefore, to develop a validated, rapid, reliable, and sensitive analytical method for the quantification of quinalphos in environmental samples.

II. MATERIALS AND METHOD:

II.1. Materials

An analytical pesticide sample of quinalphos was obtained from Swal Corporation Ltd. Mumbai India. All solvents and chemicals were of analytical grade. Demineralized water was used all over the experiment.

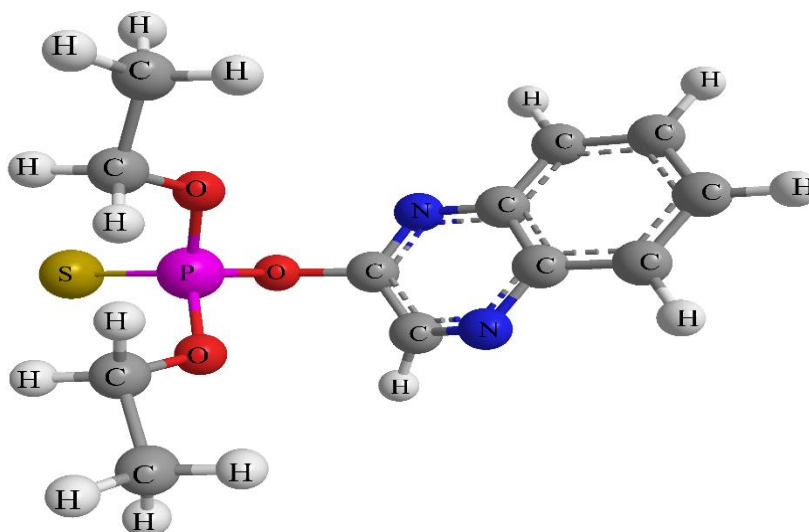


Figure 1. Molecular structure of quinalphos.

II.2. Instruments

For all spectral analysis, Systronics UV-Visible spectrophotometer (Model Visiscan 167) having two matched silica cell with 1cm optical pathlength in the wavelength range from 600-700nm. Other equipment used including electronic analytical balance (Mettler Toledo,UK). pH meter model Systronica digital pH meter 335 for pH determination. For centrifugation Remi C-854/4 clinical centrifuge force of 1850rpm with fixed swing-out rotors was used.

II.3. Preparation of quinalphos standard solution

An accurately measured 4mL of pesticide in which quinalphos was present was transferred into a 1000mL volumetric flask, dissolved in demineralized water and the volume was made up to the mark with water to obtain a 1000 μ g/mL stock solution. This stock solution was subsequently diluted to obtain a working standard solution and was used for optimization experiments.

II.4. Preparation of chemical solutions and reagent

Ammonium Molybdate- A 0.05mol L⁻¹ was prepared in dilute (1.5mol L⁻¹) Sulfuric acid.

Oxalic acid- Solution of 0.10mol L⁻¹ Oxalic acid was used.

Methylene Blue- 4 x 10⁻⁵ mole L⁻¹ solution was prepared by dissolving 0.013g of methylene blue in 100mL of water.

II.5. Procedure

In 250mL Erlenmeyer flask, 10mL of an aqueous solution consisting of 0.5-16 μ g per 10 mL of quinalphos was taken and 1mL of 1.5 mol L⁻¹ sulfuric acid was added in each concentration of the solution and then ammonium molybdate solution 0.5 mL of 0.05mol L⁻¹ were added. Kept for 20min and then the solution was brought to room temperature. In order to eliminate the excess molybdate, the mix is treated with 0.5 mL volume of Oxalic acid and the solution was placed into a 250mL separating funnel. (Patel et al., 2016). Add methylene Blue solution 0.2mL in volume and then extraction was carried out with 5mL of Butanol. The lower water-soluble layer was discarded and the floating ion-containing layer with 2 mL of the organic phase was transposed to a graduated tube and to which 5 mL of acetone was added and dissolved and then the absorbance was measured at 660nm against a reagent blank (Fig. 2).

II.6. Optimization of the reaction condition

The optimal conditions of the complex reaction between the compound and the reagent were determined using the single factor test method in which a single analytical condition is varied while keeping others constant. The conditions studied include investigating the effect of varying concentrations of Ammonium molybdate, oxalic acid, and methylene blue.

II.7. Method validation

The method was validated with respect to linearity, sensitivity, precision, and accuracy.

II.7.1. Linearity

The linear relationship between concentration and absorbance for the complex was evaluated over the concentration range of 0.5-16 μ g/mL. The linearity range for quinalphos was replicated 3 times.

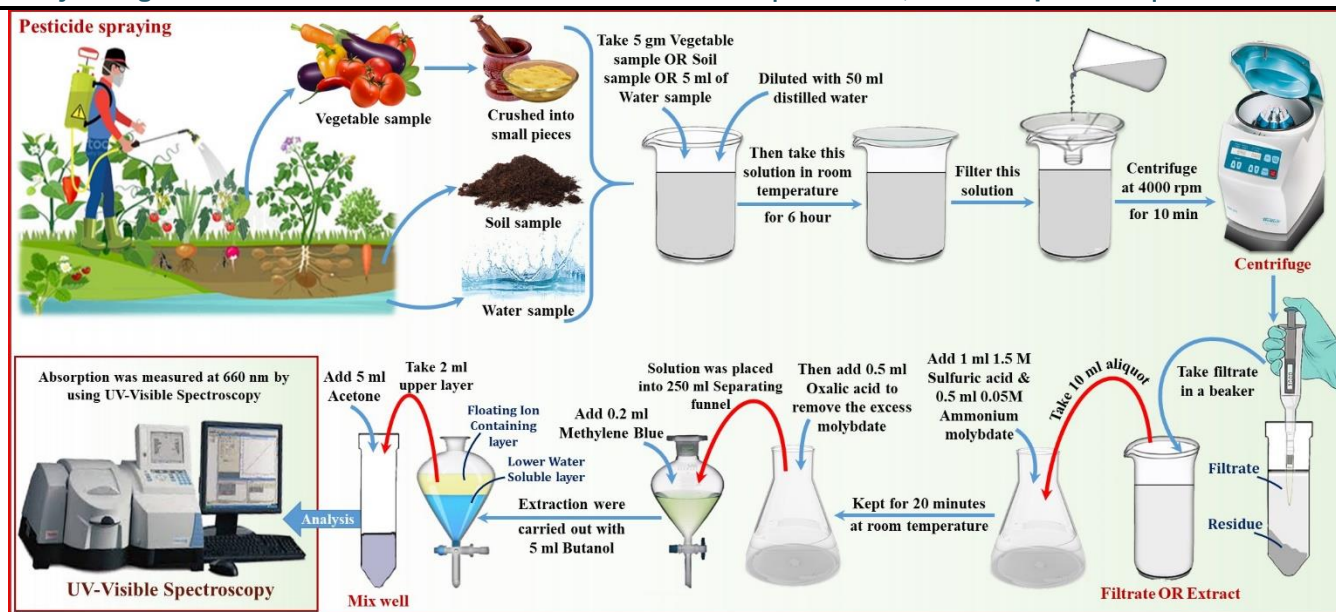


Figure 2. Flow diagram of sample collection, pre-treatment, and analysis technique for the detection of quinalphos in collected samples such as vegetables, soil, and water.

II.7.2. Sensitivity

The sensitivity of the method was measured in terms of limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ of the developed method were calculated from the standard deviation of the response (s) and slope of the calibration curve (S) of each drug using the formula, limit of detection = $3.3*s/S$; Limit of quantitation = $10*s/S$.

II.7.3. Precision

The precision of the developed method was evaluated by performing intraday and inter-day precision studies. Intraday precision was carried out by performing three replicates (at morning, afternoon, and evening) at three different concentrations (5, 10, and 15 $\mu\text{g/mL}$ for quinalphos complex) on the same day, and percent relative standard deviation (%RSD) was calculated. The interday precision study was assessed by analysis of the mentioned concentrations of the complex on seven days in triplicate, and % RSD was calculated.

II.7.4. Accuracy

To ascertain the accuracy of the proposed method, recovery studies were carried out using a standard addition method by adding a known amount of standard (10 $\mu\text{g/mL}$) solution of quinalphos. The mean percentage recovery was calculated. Recovery studies were performed in triplicate.

II.8. Analysis of quinalphos in environmental samples

II.8.1. Determination of quinalphos in the soil sample

Soil sample 5g was taken in an Erlenmeyer flask of 250mL. 20mL of 0.3% sulfuric acid was added to this flask along with 10mL of 6% m/v hydrogen peroxide plus glycerin 0.5mL. The obtained mixture is boiled at 160-180°C for 20min on a sand – bath, then again add 2mL of hydrogen peroxide and further boil it for 10min more. After this bring the mixture to room temperature and add 50mL of deionized water.

II.8.2. Determination of quinalphos in polluted water

Water samples from the pond near an agricultural field were collected. Soil sample (5g) was taken in Erlenmeyer flask of 250mL. This sample was extracted with a 2×25 mL portion of diethyl ether. The ether solution was evaporated to dryness, and the residue was dissolved in 50 mL of ethanol. Aliquots were then analyzed as the proposed above method.

II.8.3. Determination of quinalphos in different fruit and vegetables

Different fruits and vegetable samples were weighed, mashed along with acetone de-ionized water (1:1), and then strained and passed from a thin cotton cloth. The strained solution is then preceded with centrifugation at 1850 rpm for 10 min. than 10mL of sample, aliquot were treated with the proposed method.

III. RESULT AND DISCUSSION

Absorption maxima by the colour system at 660 nm is presented in Fig.3. All the spectral determination was carried out against deionized water as the blank showed negligible absorbance at this wavelength. Beer's law followed at the range of 0.5 μg to 16 μg for colour system of quinalphos per 10mL of final solution at 660nm (Fig.4). In terms of quinalphos, the molar absorptivity is found to be $1.2 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ and Sandell's sensitivity is 1.01×10^{-3} .

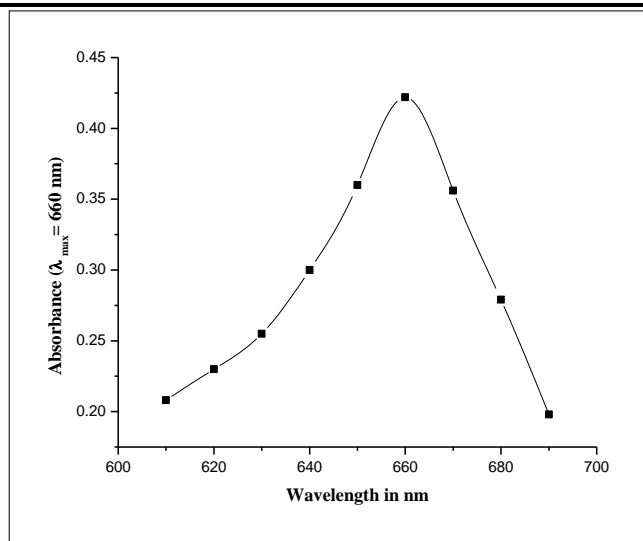


Figure 3. Absorption spectra of the colored complex

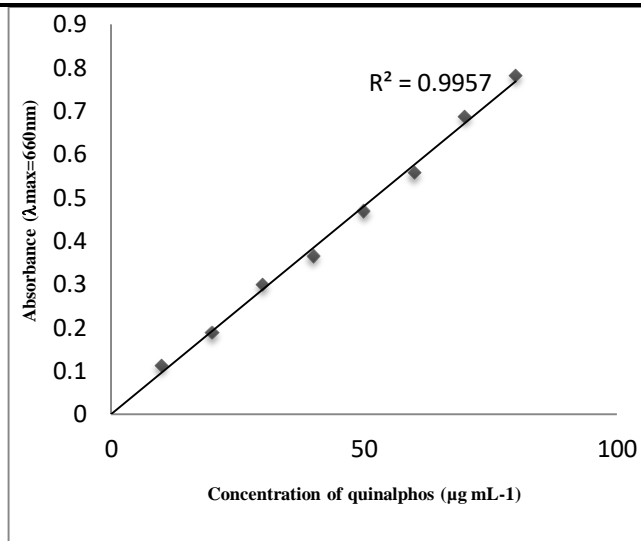


Figure 4. Calibration curve of the colored complex

III.1. Optimization of the reaction condition

III.1.1. Effect of reagent volume

Also 0.2 mL volume of 4×10^{-5} mol L⁻¹ methylene blue solution was found to be enough for complete interaction so that color complex could be formed. If more than 0.2mL was used it was seen that the blank also produce colour (Fig.5).

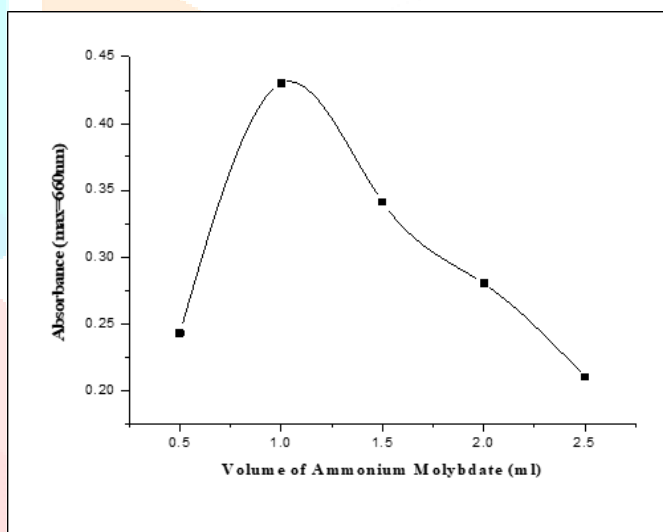


Figure 5. Effect of reagent (Methylene blue) volume on complex formation

III.1.2. Effect of foreign ions and Species

Effects of Foreign ions and Species For the determination of quinalphos the effects of common foreign species and other pesticides were studied to check the validity of the method. A known amount of pesticides and foreign species were added to a standard solution containing 10μg of quinalphos per 10mL before the determination and the solution was carried out by the above-narrated method.

It was found that the foreign species are not interfering in the condition as described in the present method, as they degrade and release phosphorous at a very higher concentration. Table 1 shows the limit acceptable for various other pesticides and ions.

Table-1 Effect of foreign ion and species i.e. metal ion and other pesticides (Concentration of Quinalphos 10μg/10mL)

Foreign Species	Tolerance limit* (μg/10mL)	Foreign ions	Tolerance limit* (μg/10mL)
Lamda-cylothrin	800	Al ³⁺	800
Buprofezin	800	Ba ²⁺ , Cl ⁻	950
Metsulfuron methyl	900	Ca ²⁺ , SO ₄ ²⁻	900
Azoxystrobin	500	Zn ²⁺	500
Carbendazim	750	Pb ²⁺ , NO ₃ ⁻	600

*Amount causing an error of ±2% in absorbance value

III.2 Method validation

III.2.1. Precision

By determining 10 μ g of quinalphos in 10mL final solution the experiment was repeated 3 times in a day (intraday precision), and the average %RSD value of the result was calculated. Similarly, the experiment was repeated over a period of 7days (interday precision) and the average %RSD value was calculated. The interday variation range from $\pm 0.005\mu$ mL and 1.4%, while intraday variation range ± 0.010 and 1.38% for the method. The developed method was found to be highly precise as the %RSD value for intraday and interday precision were all <2%.

III.2.2. Accuracy

The accuracy of the method was evaluated by applying the standard addition method where good mean recoveries were obtained ranging from 96.7 to 98.7 for the method. %RSD value was found to be 2% confirming the accuracy of the proposed method.

Table-2 Determination of Quinalphos in various environmental and agricultural samples

Samples	Quinalphos originally found (μ g)	Quinalphos Added (μ g)	Total quinalphos Found*(μ g)	Difference	Recovery (%)
Water**	1.07	10	10.94	9.87	98.7
Soil***	1.58	10	11.40	9.82	98.2
Paddy***	1.74	10	11.52	9.78	97.8
Potato***	2.25	10	12.08	9.83	98.3
Ladyfinger****	1.64	10	11.31	9.67	96.7
*Mean of three replicate analyses.					
**Water sample 10mL after treatment 10mL aliquot was analysed.					
***Sample 5g (taken from the agricultural field, 10mL aliquot of sample was analysed after treatment).					
**** Sample 5g (taken from local market, 10mL aliquot of sample was analysed after treatment).					

III.2.3. Application

For the determination of quinalphos in different samples of soil, agricultural products, and polluted water gathered from a crop field where quinalphos was used as pesticide the proposed method was applied most efficiently. The outcome observed was far better than those of the previously determined spectrophotometric method.

Known amounts of quinalphos were mixed with different samples of soil, agricultural product, etc., and then analyzed by the present method for the determination of quinalphos. Table-2 shows the recovery percentage of the sample analyzed.

IV. CONCLUSION

This proposed method is found to be a simple, sensitive, and rapid spectrophotometric method for the analysis of quinalphos. Also, it used the less toxic substance as reagents for the analysis. This method can be considered as one of the good alternatives to most of the high costing, delicate apparatus which need much more maintenance. It can be very efficiently applied for the determination of quinalphos in water, fruits, and vegetable samples, which is yet not been determined with a spectrophotometer.

V. ACKNOWLEDGMENT

The authors are thankful to the SOS in Chemistry, Pt. Ravishankar Shukla University Raipur, for providing necessary facilities and laboratory facilities and I am also thankful to the Chhattisgarh council of Science and Technology (C-COST) for providing me grant facilities as a project fellow.

REFERENCES

- [1]. Abdulra, B. L., and Tan, H. G. (2015). Chemometric approach to the optimization of HS-SPME/GC-MS for the determination of multiclass pesticide residues in fruits and vegetables *Headspace-Solid Phase Microextraction-Gas Chromatography, Food Chemistry*, 177(15): 267-273.
- [2]. Eid, R. and Abha, R. (2017). Apoptosis of rat renal cells by organophosphate pesticide, quinalphos-Ultra structural study, *Saudi Journal of Kidney Diseases and Transplantation*, 28(4): 725-736.
- [3]. Gallardo, E., Barroso, M., Margalho, A., Cruz, D, N., Vieira, M. and Revadulla, L. (2006). Direct Solid-Phase Microextraction Combined with Gas Chromatography-Mass Spectrometry, *Journal of Chromatography B*, 832 (1): 162-168.
- [4]. Gangireddygar, R.S.V., Kamderi, D., Golla, R., Bangeppagari, M., Anad, V., Gundi, B. K., Ntushelo, K. and Bontha, R. R. (2017). Biodegradation of Quinalphos by a soil Bacterium- *Bacillus subtilis*, *Pakistan Journal of Biological Science*, 20(8): 410-422.
- [5]. Mahale, V., Singh, A., Phadke, S. G., Ghanate, D. A., Olukar, P. D. Banarjee, K. and Panchagunla, V. (2017), Determination of triazine and triazoles in grapes using Atmospheric Pressure Matrix- Assisted Laser Desorption/Ionization High Resolution Mass Spectrometry, *Journal of AOAC International*, 100(3): 640-646.
- [6]. Nan, J., Wang, J., Piao, X., Yang, C., Wu, X., Quinto, M. and Li, D. (2015). Novel and rapid method for determination of organophosphorus pesticide residues in edible fungus using Direct Gas Purge Microsyringe Extraction Coupled On-Line with Gas Chromatography-Mass Spectrometry, *Talanta*, 142: 64-71.
- [7]. Patel, V., Khatoon, R., Nirmal, M., Wani, K., Rai, M.K. and Rai, J. (2016). Flotation-Dissolution based spectrophotometric determination of ethion, *Asian Journal of Chemistry*, 28(5): 957-959.
- [8]. Rohit, V. J., Basu, H., Singhal, K. R., and Kailasa, K. S. (2016). Development of *p*-nitroaniline dithiocarbamate capped gold nanoparticles-based microvolume UV-vis spectrometric method for facile and selective detection of quinalphos insecticide in environmental samples, *Sensors and Actuators B: Chemical*, 237: 826-835.
- [9]. Sadique, M. I., Ferdour, Z., Nannu, A. T., Mostakim, M. G., and Rahman, K. M. (2016). Acute exposure to a quinalphos containing insecticide (convoy) causes genetic damage and nuclear changes in peripheral erythrocytes of silver barb, *Barbonymus gonionotus*, *Environmental Pollution*, 219: 949-956.
- [10]. Su, R., Xu, X., Wang, X., Li, D., Li, X., Zhang, H. and Yu, A. (2011). Determination of organophosphorus phase pesticides in peanut oil by dispersive solid extraction gas chromatography-mass spectrometry, *journal of Chromatography B*, 879: 3423-3428.
- [11]. Westlund, P., and Yargeau, V. (2017). Investigation of the presence and endocrine activities of pesticides found in wastewater effluent using yeast-based bioassays, *Science of the total environment*, 607-608: 744-751.
- [12]. Yang, Y., Kong, W., Zhao, W., Xiao, Q., Liu, H., Zhao, X. and Yang, M. (2015). A multi residue method for simultaneous determination of 44 organophosphorous pesticides in *Pogostemon cablin* and related products using modified, Quechers Sample Preparation Procedure And Gas Chromatography - Flame Photometric Detector, *Journal of Chromatography- B*, 974(1): 118-125.

