Eco. Env. & Cons. 29 (January Suppl. Issue) : 2023; pp. (S12-S19)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2023.v29i01s.003

Detection of Azoxystrobin in Environmental Samples using FTIR Spectroscopic Method

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(Received 2 May, 2022; Accepted 4 July, 2022)

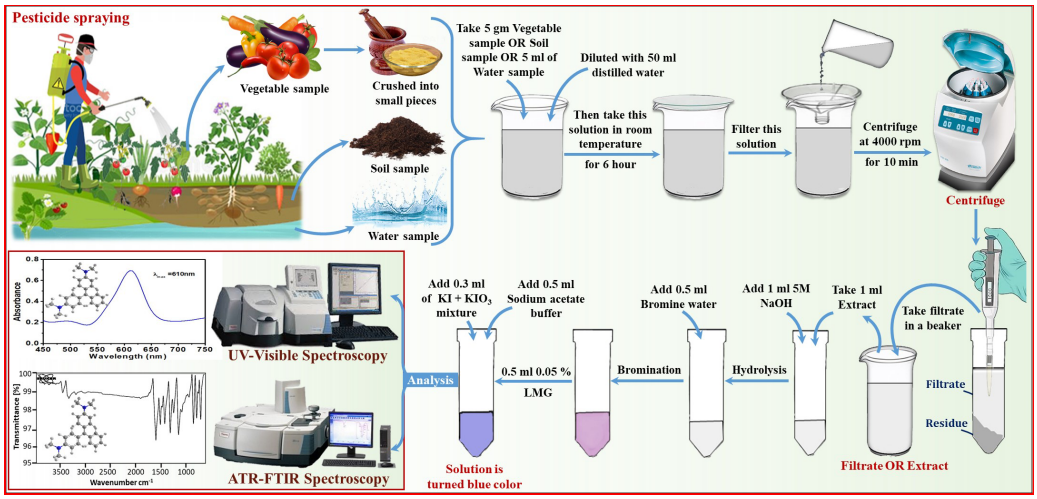
ABSTRACT

A new UV-Visible spectrophotometric method for determination of fungicide azoxysrtobin was developed. The method is based on the bromination of azoxystrobin to form dibromoazoxystrobin which react with Potassium iodide, Potassium iodate mixture in the presence of leucomalachite green (LMG) to form a bluish green colored complex. Characterization was done for the synthesis of bluish green colored complex by using UV-Vis spectrophotometer and FTIR methods. As a result, the UV-Visible absorption spectrum was observed at 615 nm. The limits of detection and limits of quantification were observed at 0.0019 µg ml^{-1} and 0.0059 µg ml^{-1} respectively. We have also studied the conformational and functional group (such as most characteristic band of O-H stretching frequency observed at 3346.49 cm^{-1}, Bonding N-H symmetrical is 1645.50 cm^{-1}, C=C bending is 691.07 cm^{-1}, Symmetrical stretching C-N is 1493.13 cm^{-1} and 1404.70 cm^{-1}, C-C stretching and other vibrational is 1059.74 cm^{-1}). Involved in the complexation between azoxystrobin and bromination by FTIR method. This developed method has been successfully applied for the detection of azoxystrobin in various environmental samples. Beer's law obeyed over the concentration range of 0.5-13 µg mL^{-1} in final solution volume of 10 ml. The reproducibility assessed by carrying out seven days replicate analysis of a solution containing 5 µg ml^{-1} of azoxystrobin in a final solution of 10 mL. The molar absorptivity of the colour system is 1.936×10^{6} L mol^{-1} cm^{-1} and Sandell's sensitivity is 0.800 $\times10^{-4}$ µg cm^{-2}. The relative standard deviation (RSD) for the absorbance value was found to be 1.9%. The suggested method is free from the interference of other toxicant agents. The analytical parameters were optimized and the method was applied to the determination of azoxystrobin in water, soil and food samples.

Key words : *UV-Visible Spectrophotometer, FTIR, Azoxystrobin, Bromination and Leucomalachite green (LMG).*

Introduction

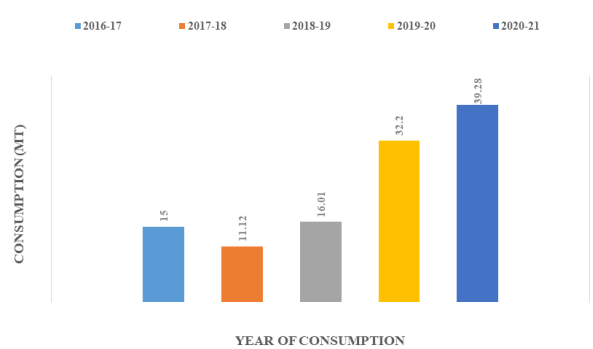
Pesticides are the major basis with signicant role in ensuring safety from the destruction caused by many pests. At present time, the viable food production can't achieved without the vital role of pesticides. Pesticides are applied directly on the plants which are able to determine for long time in vegetables. When pesticides are use in the field, it is assessed that only about 1% of the pesticide is able to



Scheme 1. Graphical abstract of the present investigation

touch its targeted pathogen, while the rest is unused, with a negative effect on water, soil and air environments. However, public exposure to pesticides residues via food chain is posing possible risks to human health and wellbeing. One major means for ensuring human and environmental is the study of pesticides dissipation pattern and spreading of pesticides in agricultural commodity after pesticides application. It is well identified that pesticides are potentially injurious to the environment, and consequently to human beings by direct contact and also through the consumption of pesticide contaminated food.

Strobilurins have been main classes of fungicides since their commercial introduction in 1996. Now, over ten strobilurin fungicides are available. The massive influence of this new class of pesticides in agriculture is well represented by the rapid success



Scheme 2. Consumption of azoxystrobin fungicide in India during 2016-2021. Graph shows that it is gradually increases every year (Source: http://ppqs.gov.in).

of azoxystrobin. After few years of launching on the market, azoxystrobin was registered for use on over 400 crop/disease systems, making it the world's highest-selling fungicide. Azoxystrobin is a systemic fungicide and chemically known as (methyl (E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxy-propenoate) is a fungicide used to control a wide selection of fungal diseases (Feng *et al.*, 2020, Augusto *et al.*, 2020). Many literatures and survey shows that azoxystrobin is extensively used to kill fungi in various fruits and vegetables which are commonly used in our day to day life.

In the recent years many methods and techniques have been established for detection of various pesticides but they all are costly and lengthy. To eradicate the problems due to the pesticides and to defend the bionetwork, many cost effective, sensitive and simple methods have been settled for the detection of pesticides such as Liquid Chromatography, high performance-liquid chromatography, QuEChERS Method Combined with GC-MS (Abdel Ghani *et al.*, 2016), Ultra HPLC–High-Resolution Quadrupole Time-of-Flight Mass Spectrometry (Amelin *et al.*, 2018), Dispersive Liquid–Liquid Micro-extraction of Pesticides Using Ionic Liquids as Extractants (Bessonova *et al.*, 2020), Gas Chromatography-Mass Spectrometry/Mass Spectrometry, Near-Infrared Spectroscopy.

In this work, we aimed to develop simple, sensitive, selective and low cost method. Present method is established on the hydrolysis of azoxystrobin in alkaline medium and bromination reaction was followed by reaction of KI and KIO_3 and

leucomalachite green. In this study, we determined azoxystrobin in different vegetable, fruits and other environmental samples through UV-Visible and FTIR spectroscopy. Primarily we have collected samples and executed hydrolysis of azoxystrobin using sodium hydroxide and further it reacts with bromine water, KI and KIO_3 and leucomalachite green to obtained bluish green colour complex.

Materials and Methods

Chemicals and reagents

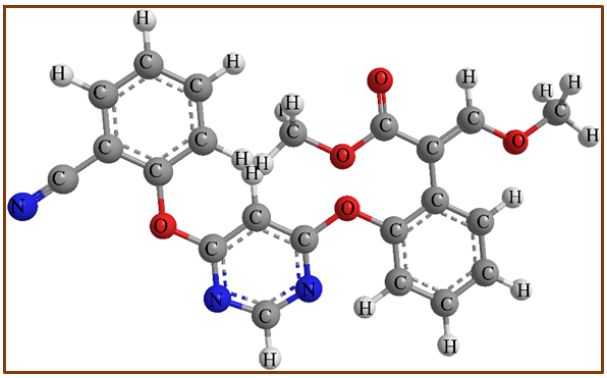
This active ingredient azoxystrobin was selected based on their current use to control fungal diseases in crops in India. Azoxystrobin was purchased from Swal Corporation Ltd., Mumbai which is shown in scheme 1.3. All others reagents which was AR grade (Bromine, potassium iodide , potassium iodate, formic acid, leucomalachite green) was purchased from Sigma Aldrich Private Limited. Bangalore.

Apparatus

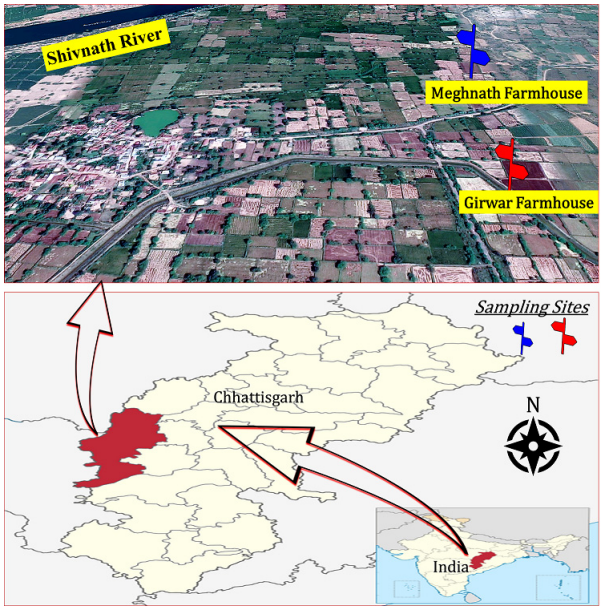
UV-Visible absorption spectrum was achieved on double beam spectrophotometer make Cary -60 UV-Visible Spectrophotometer (Agilent technologies) with quartz glass. ATR-FTIR method was verified on Nicolet iS10 Spectrophotometer (Thermo fisher) using potassium bromide (KBr) pellets. Basic pH meter pH700 EUTECH instrument was used for pH measurements. For centrifugation process of extracted samples, a REMI C-854/4 clinical centrifuge was used having a maximum speed of 3600 rev min^{-1} and a maximum centrifugal force of 1850 g with fixed swing out rotors.

Sample collection

Rajnandgaon is a major district of Chhattisgarh state, beside on the bank of Shivnath River being located at Latitude-21.0, Longitude-81.0. The sample was taken from Meghnath farmhouse and Girwar farmhouse based beside Shivnath River, near Sukhari and Mokhali village respectively. Various vegetables like potato, tomato, Brinjal were taken from Meghnath farmhouse. Soil sample were taken from the field of both the farmhouse whereas contaminated water samples were collected from various discharge channels of excess water from agricultural field and stored in refrigerator in isolated containers (Scheme 4).



Scheme 3. Chemical structure of azoxystrobin.



Scheme 4. Location map of the sampling site for real sample collection for analysis.

Sample preparation for UV-Visible and FTIR Spectroscopy

The different vegetables and grain samples (i.e. cabbage, potato, brinjal, tomato, bean, and paddy) were collected and cut into fine pieces and were permitted to dry at normal temperature. Then 1 ml of 2 µg ml^{-1} azoxystrobin was added and mixture was centrifuged. Further an aliquot was hydrolysed in alkaline medium with sodium hydroxide and in the filtrate bromine water was added for bromination reaction. Then mixture was mixed thoroughly for 2-3 minutes. After those few drops of formic acid, 0.5 ml of potassium iodide and potassium iodate solution and 0.5 ml of leucomalachite green was added. The above resultant solution was allowed to stand for 15 minutes for full colour development. The solution

was makeup with 25 ml of distilled water. As a final point 3 ml of the overhead mixture was taken in a cuvette for UV-Visible and FTIR spectroscopy analysis.

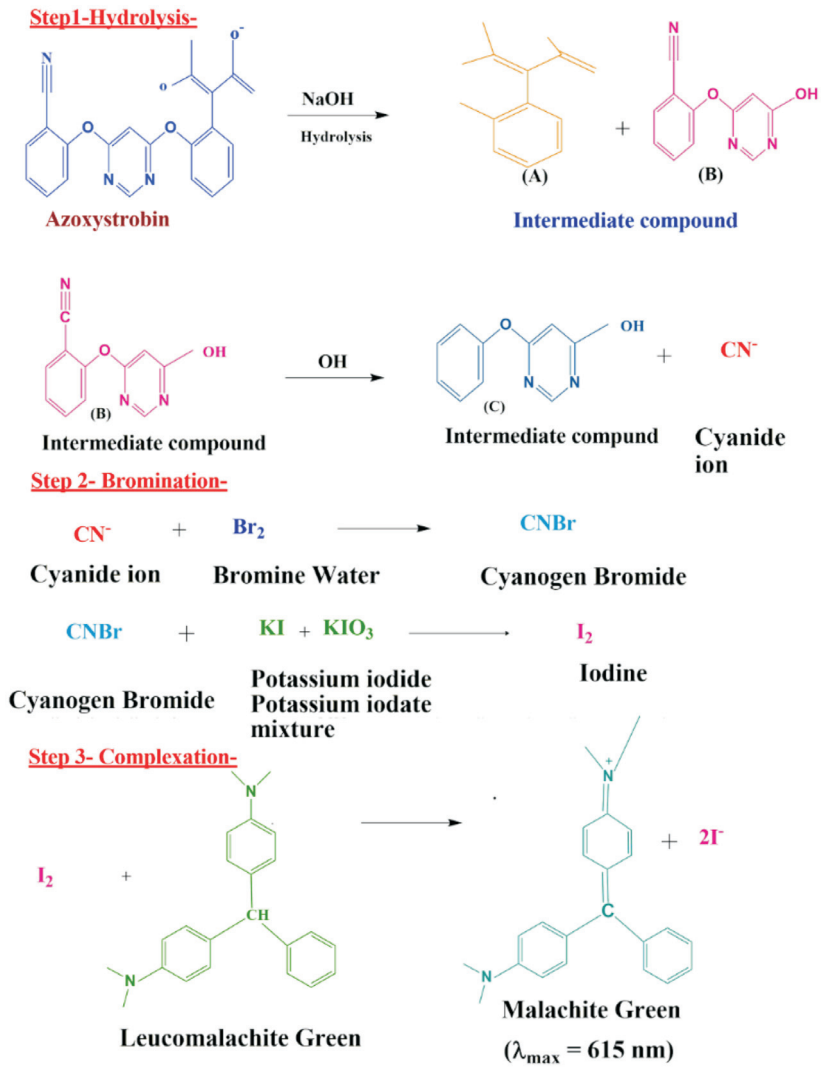
UV–Visible and ATR-FTIR spectroscopic analysis

UV-Visible absorption spectrum of azoxystrobin dye was observed at 615 nm at room temperature using a UV-Visible spectrophotometer. The concentration of azoxystrobin solution was set for the calibration. Double distilled water is used as references for the analysis. FTIR spectra were verified by the attenuated total reflection method by using Potassium bromide pellets as diluents. The FTIR spectra of azoxystrobin systems were recorded at 400-4000 cm^{-1} at room temperature.

Results and Discussion

Reaction mechanism

Azoxystrobin was hydrolysed with basic medium (OH^-) and dissociated into two intermediate compounds (A&B) on further hydrolysis compound B give another intermediate and release hydrogen cyanide (HCN). Then bromination of HCN was carried out forming cyanogen bromide (CNBr). The cyanogen bromide reacted with potassium iodide and potassium iodate mixture (1:5) with few drops of formic acid iodine was released. This iodine finally reacts with leucomalachite green reagent and form bluish green colour dye (Scheme 5).



Scheme 5. Representation of reaction mechanism for proposed method.

UV-Visible spectroscopic method

Azoxystrobin is a pesticide having chemical structure with cyanide group. For the bromination, first of all, hydrolysed azoxystrobin with alkaline medium was done subsequent in the formation of Methyl (E)-2-{2-[(6-hydroxy) pyrimidin4-yloxy] phenyl}-3-methoxyacrylate and 2-Hydroxybenzonitrile. When this solution again treated with sodium hydroxide then it form hydrogen Cyanide (HCN). The follow-on solution was treated with bromine water to form cyanogen bromide (CNBr) and then cyanogen bromide reacted with a mixture of KI and KIO3 and few drops of formic acid resulting in the formation of iodine releasing extra bromine. Now leucomalachite green (LMG), which has bluish green reagent is oxidized by iodine liberate from KI and KIO3 consequentially in the formation of malachite green.

In this process, liberated iodine reacted with leucomalachite green to form dark bluish green colour dye due to complex formation and the maximum absorption spectra recorded was at 615 nm shown in Figure 1(A). The found colour system obeys Beer's law and the concentration range of 0.5-13 µg ml^{-1} of azoxystrobin in 25 ml of final solution at 615 nm shown in Figure 1 (B). RSD value was calculated as 0.509%. LOD is a statistical methodology in which investigation is possible to do and LOQ in which quantitative results described. For azoxystrobin LOD and LOQ determination was repeated three times and LOD and LOQ calculated 0.0019 µg ml^{-1} and 0.0059 µg ml^{-1} respectively. The molar absorptivity and Sandell's sensitivity 1.936×10^{6} L $mol^{-1}cm^{-1}$ and 0.800×10^{-4} µg cm^{-2} respectively was also calculated. This bluish green colour dye showed maximum absorbance at 615 nm and the blank reagent showed negligible absorption at this wavelength. UV-Visible absorption spectra at different concentration are shown in Figure 2.

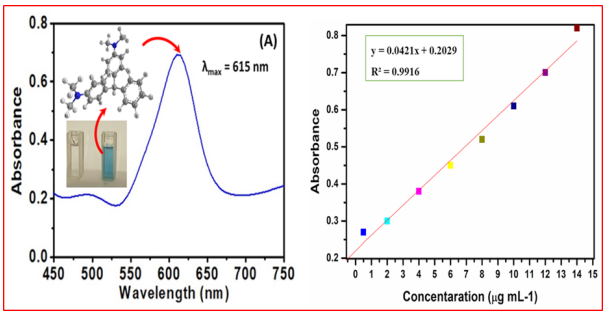


Fig. 1. (A) UV–Visible Absorbance spectra of coloured complex at room temperature; (B) Calibration curve of Azoxystrobin at room temperature.

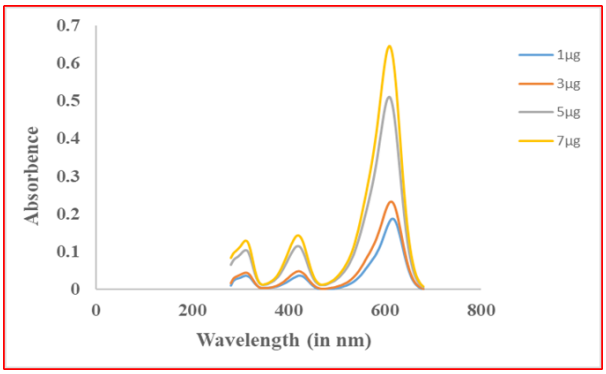


Fig. 2. UV-Vis Absorption spectra of dye at different concentration.

FTIR spectroscopic study

Pure pesticides

According to WHO, pesticides are chemical compounds that are numerous used to kill pests, *i.e.*, insects, fungi and weeds. Pesticides are used in communal health to kill carrier of disease, *i.e.*, mosquitoes, and in agriculture, to kill pests that damage crops. In nature, pesticides are potentially toxic to other organisms, including humans and need to be used carefully and disposed accurately. FTIR spectra (spectral range: 4000–500cm^{-1}) showed that variety of pesticide groups could be distinguished. The most characteristic band of O-H stretching frequency observed is 3346.49cm^{-1} is board band, and second sharp peak of Bonding N-H symmetrical is 1645.50cm^{-1}, C=C bending is 691.07 cm^{-1} (Table 1).

FTIR study of dye

IR spectroscopy can reveal the change of the frame-

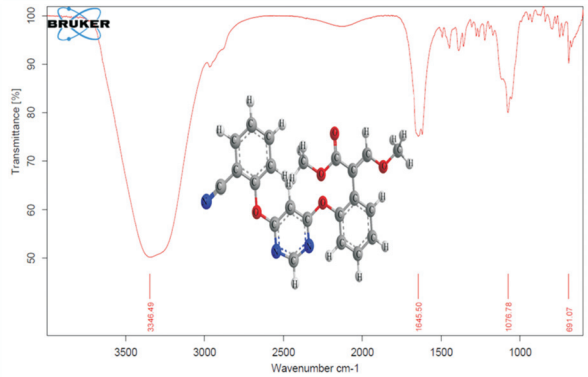


Fig. 3. ATR-FTIR spectra of azoxystrobin.

Table 1. Characteristic absorption band (cm^{-1}) of azoxystrobin and reaction mixture and various sample

Assignment	Characteristic absorption band (cm^{-1})				
	Pure azoxystrobin	LMG	LMG + I_2	Potato	Beans
Symmetrical stretching OH	3346.49	3463.87	3423.66	3407.10	3406.90
CH Stretching				~2900	
Bonding N-H symmetrical	1645.50		1622.07	1631.71	1629.12
Symmetrical stretching C-N		1493.13	1466.33		
N-H, C-N, C-O-C bond				~1150, 700-900	
C=C stretching + other vibrational	691.07	1059.74	707.20		
Wagging CO, wagging NH_2 + CO out of phase				~1000-1100	
C-N stretching band, rocking mode of CH_3 chain		825.97			

work configuration of the dyes after the incorporation of the pesticides. In Figure 2, the FTIR spectrum of color dye shows a distortion of the spectrum between 3500 and 600cm^{-1}. This region of the spectrum was assigned to O-H stretching is 3463.87 cm^{-1}, 3339.46 cm^{-1}, C-H bending aromatic ring is 1892.76 cm^{-1}, Bonding N-H symmetrical is 1582.74 cm^{-1}, Symmetrical stretching C-N is 1493.13 cm^{-1}, 1404.70 cm^{-1}, C-C stretching and other vibrational is 1059.74 cm^{-1}, C-N stretching band, rocking mode of CH3 chain is 825.97 cm^{-1}, C=C bending (trisubstituted) is 760.83 cm^{-1}, C=C bending (disubstituted (cis)) is 679.17 cm^{-1}.

Further, the characteristic IR stretching frequency of synthesized mixed dye is observed of O-H stretching is 3423.66 cm^{-1}, Bonding N-H symmetrical is 1622.07 cm^{-1}, Symmetrical stretching C-N is 1466.33 cm^{-1}, and C=C bending is 707.20 cm^{-1}.

Effect of different reaction conditions

The reaction was completed at the pH 8.0 it was found suitable for complete hydrolysis of the pesticide (Figure 5 A). The result of temperature on the reaction was observed in the 30 °C with the optimum of the reagent concentration. The reaction required at room temperature and does not complete when it was changed from it optimum temperature (Figure 5 B) 10-15 minutes time required for completing the reaction and full colour development of dye and the colour was stable for many days. It was observed that 0.5 ml bromine water, 0.5 ml KI and KIO_3 and leucomalachite green was used the reaction was completed. But when the volume of the components was changed when different volume of leucomalachite green was added the absorbance becomes stable after maximum absorbance point (Figure 6)

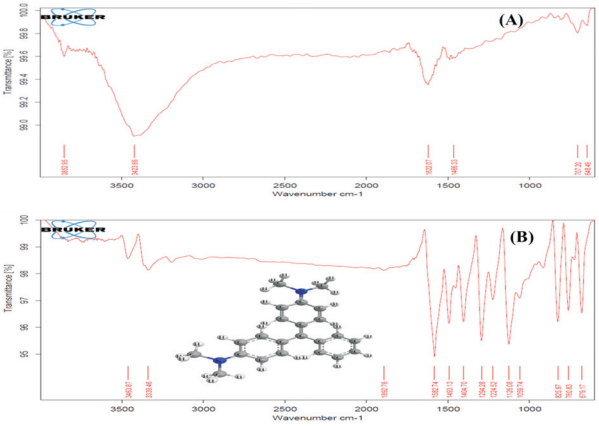


Fig. 4. (A) ATR-FTIR spectra of leucomalachite green (LMG). (B) Complex of azoxystrobin after complex formation.

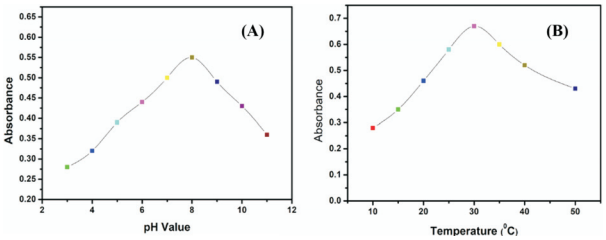


Fig. 5. (A) Graph plotted between absorbance verses different pH values, (B) Graph plotted between different temperature and absorbance.

Selectivity is basic phenomenon the interference effect of the proposed protocol is resolute and tested by Ultraviolet–Visible and FTIR techniques. We estimated that various chemical agents are present in different environmental and vegetable samples. The spectroscopic evaluation of the effect of interfering agents, i.e. pesticides has been treated.

Linearity, accuracy and precision

In this UV-Visible spectrophotometric method linear

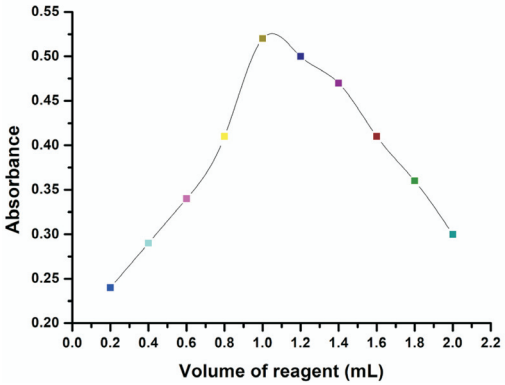


Fig. 6. Effect of volume of reagent on reaction.

correlation was observed in between the range of 0.5 –13 µg ml^{-1}. The linearity for the method was obtained through high value of correlation coefficient (R^2=0.991) and minor value of intercept of the regression equation (0.0421x + 0.2029).

The recovery percentage of azoxystrobin was obtained in the range of 96 to 103%. Good accuracy and precision value was calculated by UV–Visible spectrophotometric method and analysing three replicate samples of each concentration. The RSD value of established method was 1.9%. Table 2 shows the different optical parameters of the method.

Table 2. Optical parameters and statistical data of regression equation for reaction of azoxystrobin with Leucomalachite green reagent

Parameter	Values for reaction
Absorbance of dye (λmax in nm)	615
Colour	Bluish Green dye
Beer's law limit (µg mL^{-1})	0.5-13.0
Molar Absorptivity (L mol^{-1}cm^{-1})	1.936×10^{6}
Sandell's sensitivity (µg cm^{-2})	0.800×10^{-4}
Limit of detection LOD (µg ml^{-1})	0.0019
Limit of quantification LOQ (µg ml^{-1})	0.0059
Regression equation	y = 0.0421x + 0.2029
Relative standard deviation (%)	1.9
Correlation coefficient (R^2)	0.991

Real Sample analysis

To examine appropriateness of proposed method, different environmental samples from soil, vegetable and water have been analysed and calculated. To avoid any matrix effect, standard addition method was used to determine azoxystrobin in real samples. The recovery of azoxystrobin fungicide was also calculated from the samples spiked with known amount of pesticide. The results showed that, the added azoxystrobin was quantitively recovered from the real samples. The reproducibility of the suggested method was found through the RSD. The recoveries ranges have found from 96-103% and the results are summarized in Table 3.

Comparison with other methods for analysis of azoxystrobin

The comparison between present established methods with other reported analytical methods for analysis of azoxystrobin fungicide are shown in Table 4. The present method affords greater recovery percentage and minimum RSD value. This method provide, high value of accuracy and precision in the detection and quantiûcation limit of azoxystrobin. The spectrophotometer determination of azoxystrobin is cost effective, selective and easy to handle method as compared to other different techniques for the determination of azoxystrobin.

Table 3 Application to the determination of azoxystrobin by various environmental samples

Sample	Azoxystrobin original found[a]	Azoxystrobin added µg ml^{-1}	Total azoxystrobin Found	Difference (µg ml^{-1})	Recovery (%)	RSD (%)
Water[b]	2.15	2	4.20	1.90	103	0.81
Cabbage[c]	1.89	2	3.83	0.94	97	0.87
Paddy[c]	2.86	2	4.80	1.94	97	0.70
Potato[c]	2.63	2	4.58	1.95	98	0.94
Beans[c]	3.47	2	5.40	1.93	97	1.40
Tomato[c]	0.96	2	2.96	1.96	100	0.96
Brinjal[c]	1.78	2	3.70	1.92	96	1.29
Soil[c]	3.60	2	5.52	1.92	97	0.92

[a]Mean of three replicate analyses. [b]Water sample 50 ml after treatment 50 ml of aliquot was analyzed. [c]Sample 5g (Taken from the agricultural field, 10 ml aliquot was analyzed after treatment as described in the currently proposed method).

Table 4. Comparison of present spectrophotometric method with other methods for analysis of azoxystrobin

S. No.	Methods/techniques	RSD (%)	LOD (mg kg^{-1})	Recovery (%)	References
1.	Liquid Chromatography Tandem Mass Spectrometry	-	5.00	79-93	[11]
2.	Liquid Chromatography	<1.0	130.00	97-103	[19]
3.	High Performance Liquid Chromatography-UV-Visible Spectrophotometry	4.55	12.88	83-91	[24]
4	Spectrophotometer method	0.509	1.90	97-103	Present method

Conclusion

At present environment pesticide contamination in our food chain and other environment like aquatic ecosystem and soil system is our main challenge. To overcome of this problem and awareness to public we have successfully established a simple procedure for the detection of azoxystrobin fungicide in trace amounts in contaminated samples, i.e. vegetables, soil and water samples by advance techniques viz., Ultraviolet–Visible and ATR-FTIR techniques. The advantage of this method is its simplicity and sensitivity.

Declaration of competing interest

All authors declare no conict of interest.

Acknowledgements

The authors are graceful to the Head, School of Studies in chemistry, Pt. Ravishankar Shukla University Raipur and Director General CCOST Raipur for providing a grant facility. I am also great thankful to NCNR, Pt. Ravishankar Shukla University Raipur for providing me instrumental facilities for FTIR sample analysis.

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