

**Construction of coumarin-appended calix[4]arene-based fluorescence sensor for
detection of carbofuran from cabbage**

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Materials

All chemicals such as, K_2CO_3 (Potassium carbonate), KI (Potassium iodide), ethyl bromoacetate, KOH (Potassium hydroxide), cystamine dichloride, DCC (N,N'-Dicyclohexylcarbodiimide), and DMAP (4-Dimethylaminopyridine), Various standard pesticides were purchased from Sigma Aldrich. Stock solutions of pesticides and proposed fluorescence probe CouC4S were prepared in DCM (Dichloromethane): methanol (70:30). Further dilutions are completed as per requirement. Spectroscopic properties of CouC4S were investigated in mixed aqueous organic medium [DCM:Methanol/Carbonate-Bicarbonate Buffer (pH 9.2-10.6; 6:4, v/v)]. Cabbages were bought from a local market in Dakor, Gujarat, India.

Apparatus

Melting points were taken on Opti-Melt (Automated melting point system). FT-IR spectra were recorded as KBr pellet on Bruker TENSOR-27 in the range of 4000-400 cm^{-1} . Discover Bench Mate system-240 V (CEM Corporation) microwave synthesizer was used for synthesis of p-tertbutylcalix[4]arene. GmbH Vario Micro cube elemental analyser was used for elemental analysis. 1H NMR spectra was scanned on 600 MHz FT-NMR JEOL in the range of 0.5 - 15 ppm using internal standard tetramethylsilane (TMS) and deuterated $CDCl_3$ as a solvent. ESI Mass spectra were taken on a Shimadzu GCMS-QP 2000A. MALDI-TOF was taken on Bruker autoflex[®] maX model. The emission spectrum was recorded on Horiba Fluoromax Plus model. UV-Vis absorption spectra were acquired on a Jasco V-730. PXRD diffractograms has been taken on Panlytical X`pert Pro model.

Experimental

Method development and validation

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated by the minimum level at which the solution of CBF can be readily quantified with accuracy. LOD and LOQ were calculated according to the $3\sigma/s$ and $10\sigma/s$ criteria respectively where “ σ ” is the standard deviation of intercept of regression equation and “ s ” is the slope of the corresponding calibration curve. Precision was determined by calculating intra- day and inter-day variations of the developed method in 3 replicates at their different concentrations of CBF ($5\ \mu\text{M}$ - $25\ \mu\text{M}$). For intra-day precision, analyzed these CBF– CouC4S complex solutions in triplicates on same day. To determine inter-day precision each of three samples was analyzed on different day. Accuracy was determined by executing recovery studies by standard addition method. In this method, standard solution of CBF was added at three different levels i.e. 50%, 100% and 150% to known pre-analyzed sample solution. By using the proposed method, the total concentrations were determined. The % recovery of added ions were calculated as:

$$\% \text{ Recovery} = \frac{\text{Amount of CBF found}}{\text{Amount of CBF added}} \times 100$$

Electrochemical measurements:

The electrochemical behavior of modified screen-printed electrodes was initially examined by using cyclic voltammetry and differential pulse voltammetry. All electrochemical measurements were performed with using a 910 PSTAT mini (Metrohm Company Ltd.) controlled with pSTAT software. Carbon electrode was used as a working electrode. In cyclic voltammetry (CV), potential was scanned from -0.4 to 1 V with a scan rate 0.1 V/s. Differential pulse voltammetry (DPV) was performed with a potential from 0.0 - 0.6 V with an Estep potential of 0.01 V, t_{puls} and E_{puls} of 0.1 s and 0.2 V respectively with a scan rate of 0.02

V/s. All the reactions were recorded using $K_4[Fe(CN)_6] \cdot 3H_2O$ in 1M KCl as electrolyte solution.

Calculation of analytical parameters

We have calculated the limit of detection (LOD) for our fluorescence probe CouC4S from the fluorescence analysis and found to be 5.55 μM for CBF. The limit of quantification (LOQ) for CouC4S was found to be 18.52 μM for CBF. As depicted in [Table S1-S2, ESI†], the % RSD values are found between the ranges of 0.95 and 0.99 for the inter-day as well as intra-day precision, and the similar type of results were got when this investigation was carried out by different analysts. As we can see from the results, the % RSD values are not more than 2% in intra- and inter-day precision study, which is a sign of good precision of the developed technique. To determine the accuracy of our method, we have prepared different sets of CBF concentration solutions with CouC4S and recorded their fluorescence intensities, which further were compared to standard results of fluorescence study. Each set was repeated five times. The results of the recovery experiments and the accuracy of the tests are shown in Table S1-S2 (ESI) and were also compared with other reported methods for these ions (Table 2).

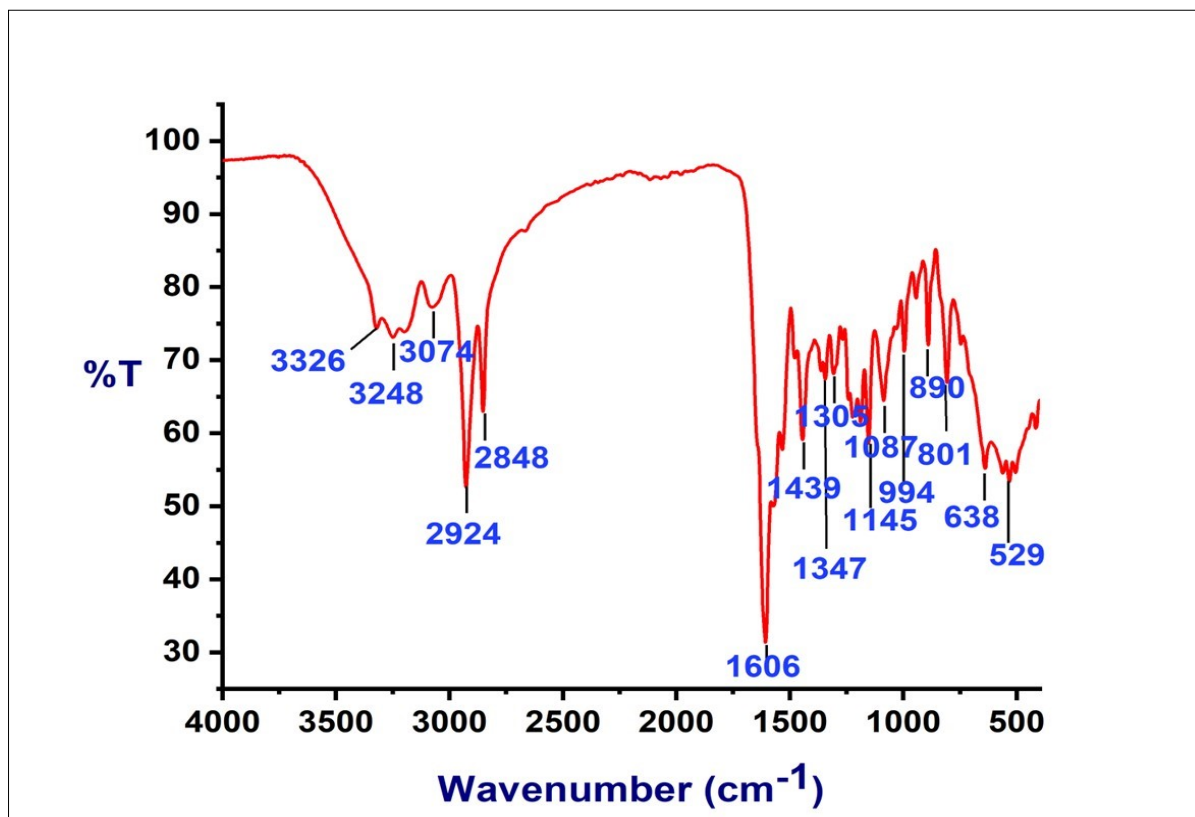
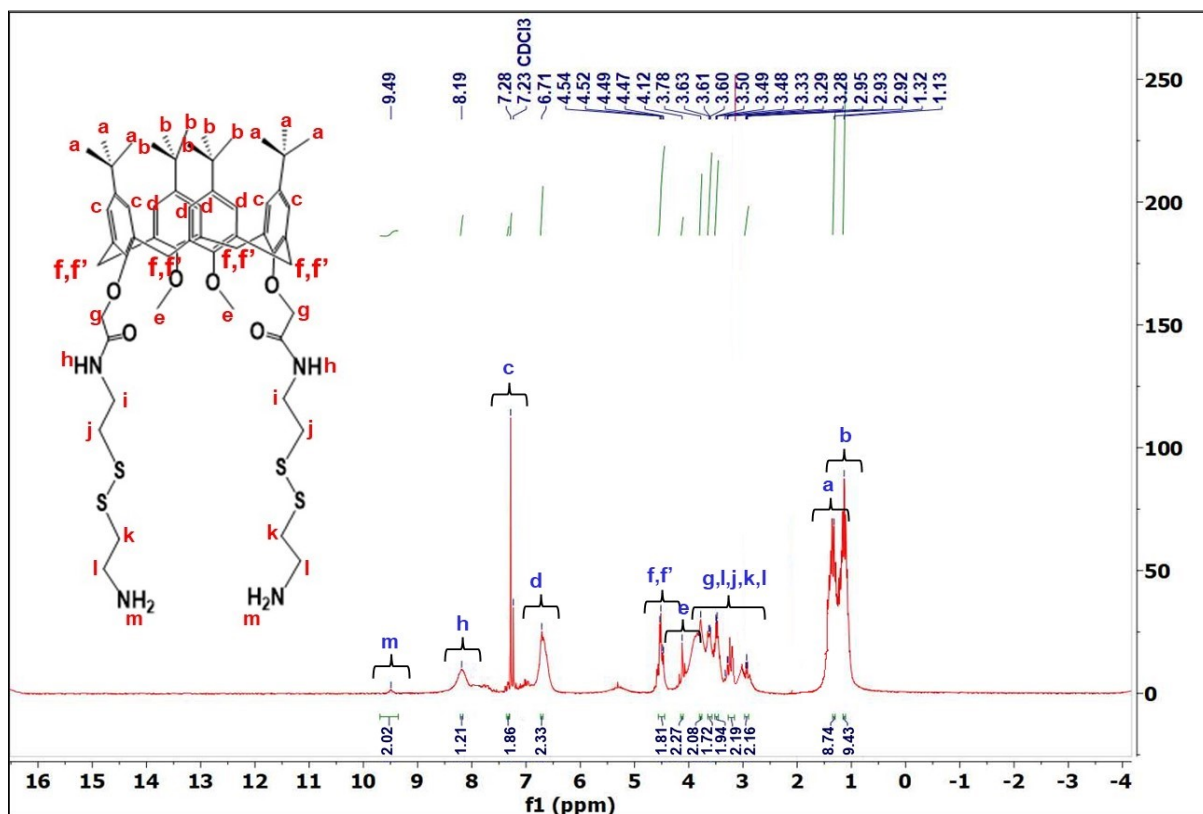


Figure S1: ¹H NMR spectrum of compound E.

Figure S2: FT – IR spectra of compound E.

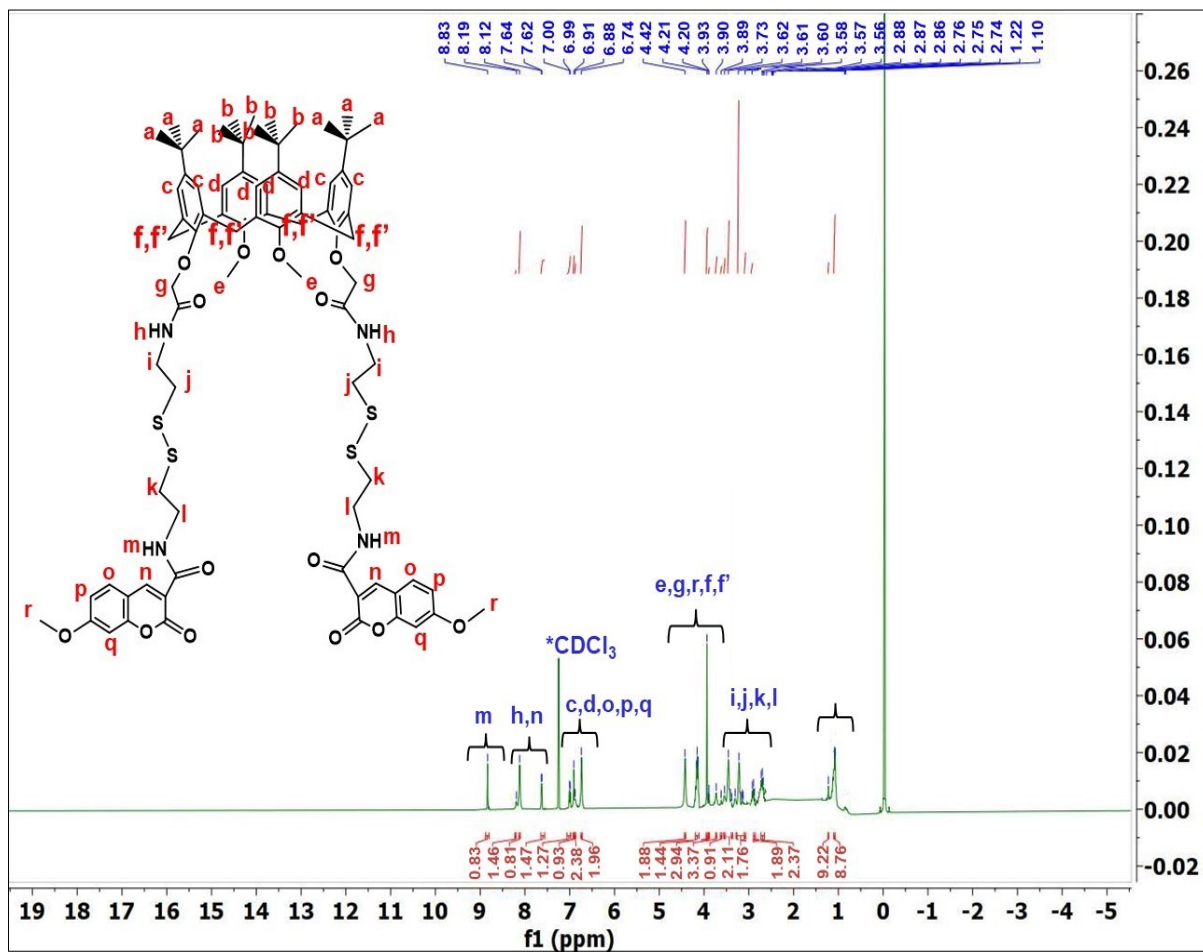


Figure S3: ¹H NMR spectrum of CouC4S.

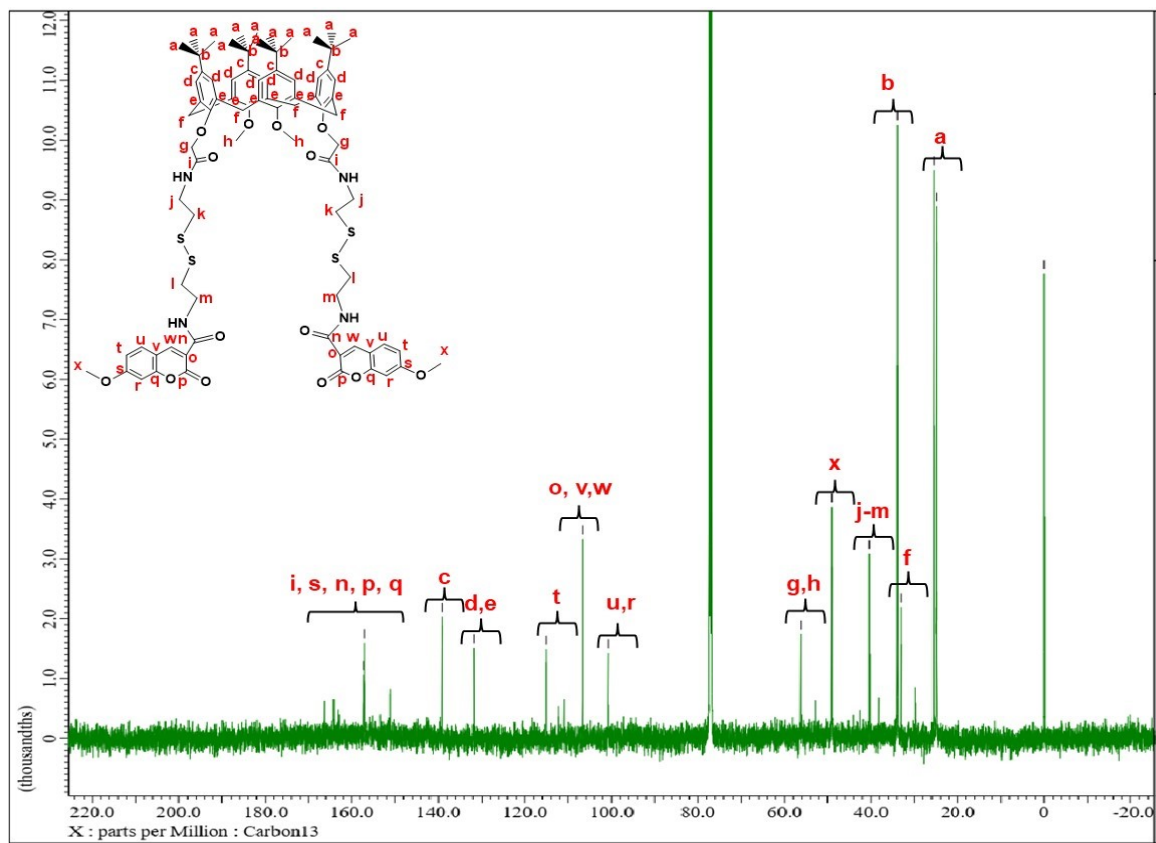


Figure S4: ^{13}C NMR spectrum of CouC4S.

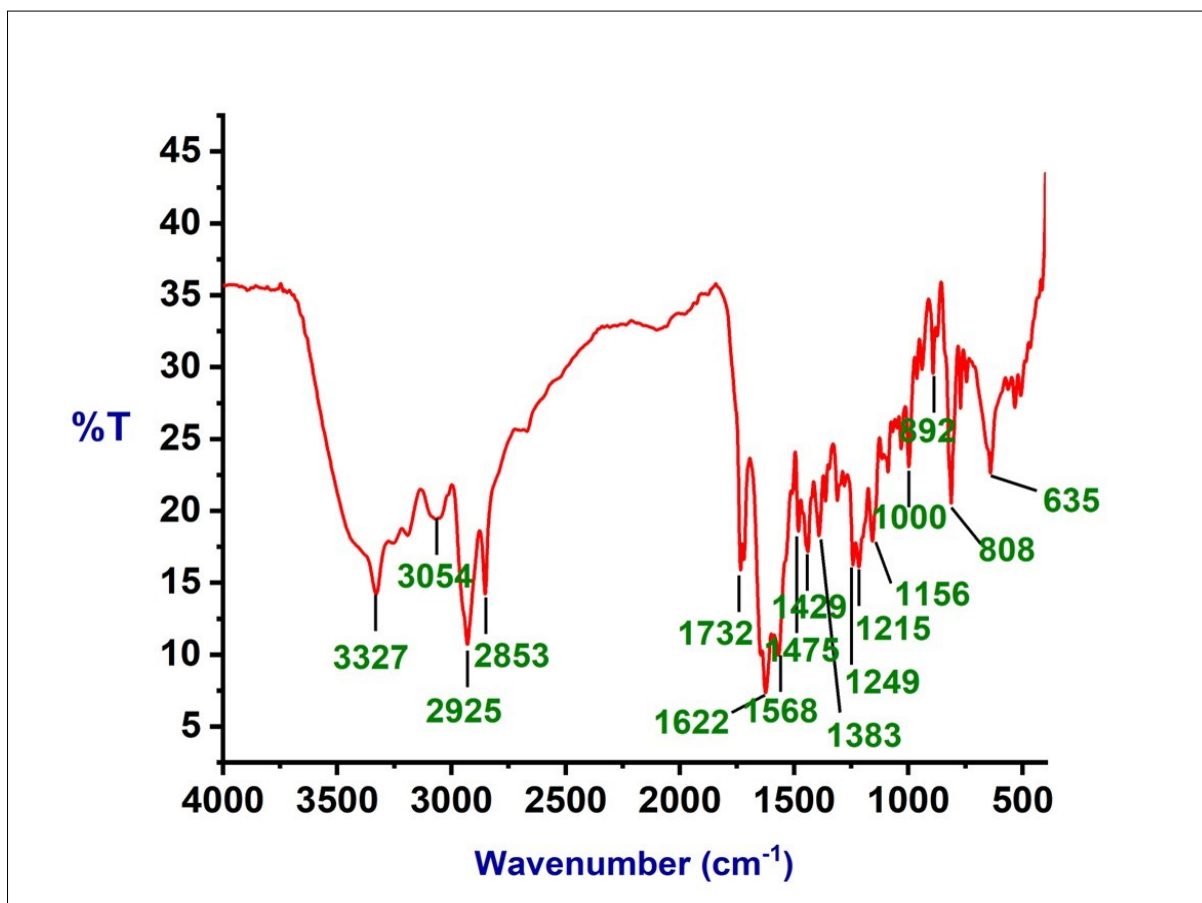


Figure S5: FT – IR spectra of CouC4S.

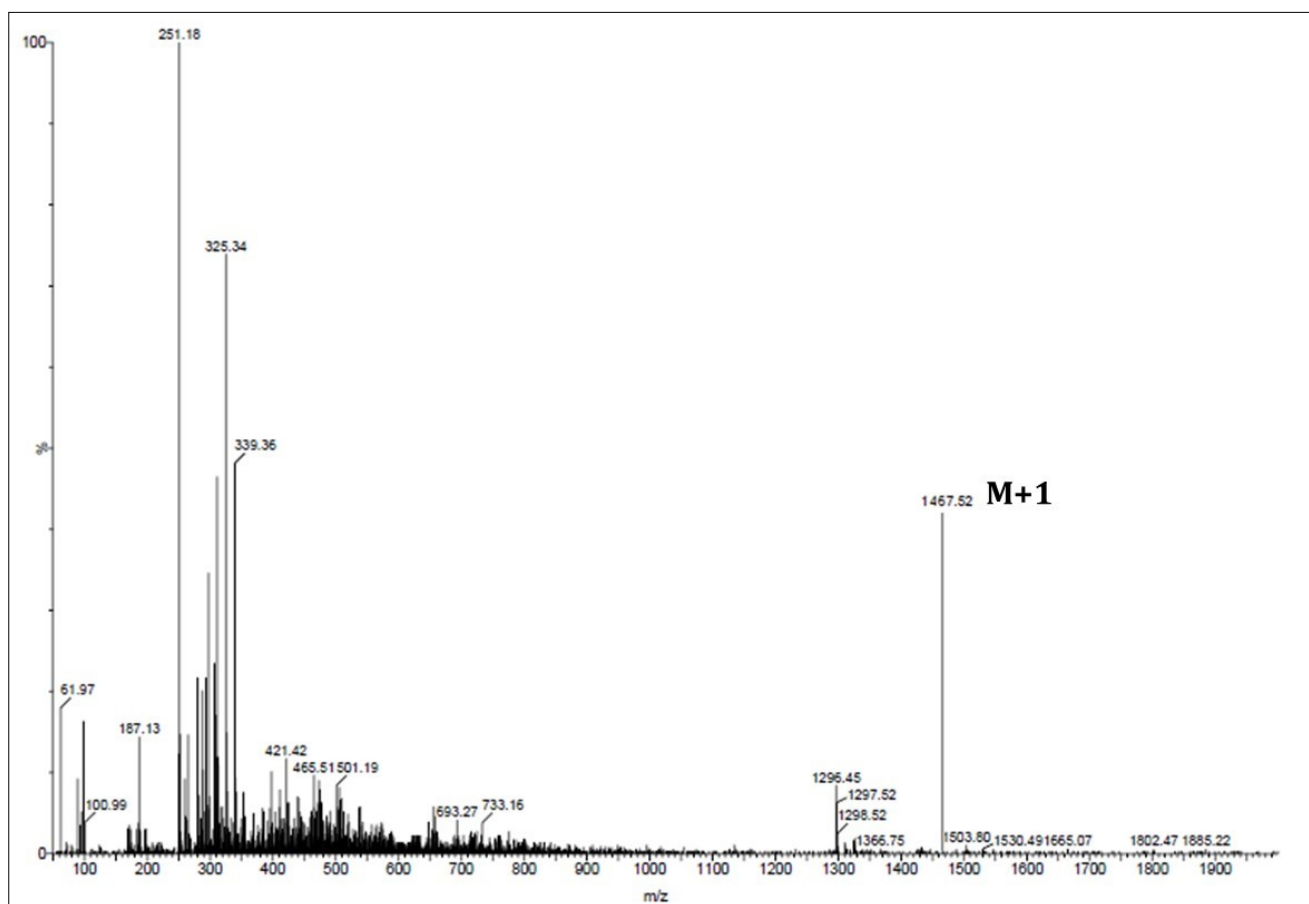


Figure S6: ESI – MS spectra of CouC4S.

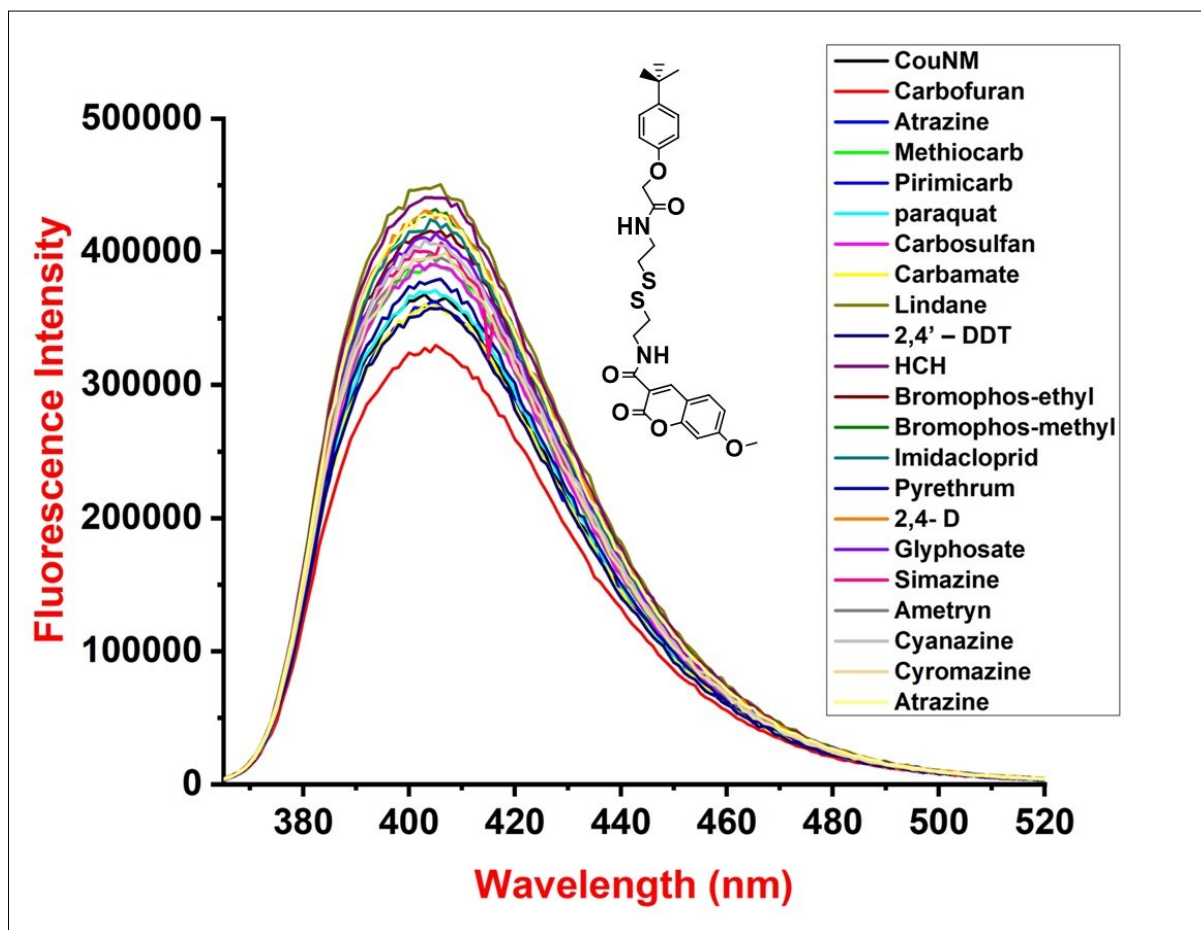


Figure S7: Emission spectra obtained of CouNM (1×10^{-6} M) with solutions of different types of pesticides (1×10^{-6} M) in DCM: methanol (70:30) at 25 °C.

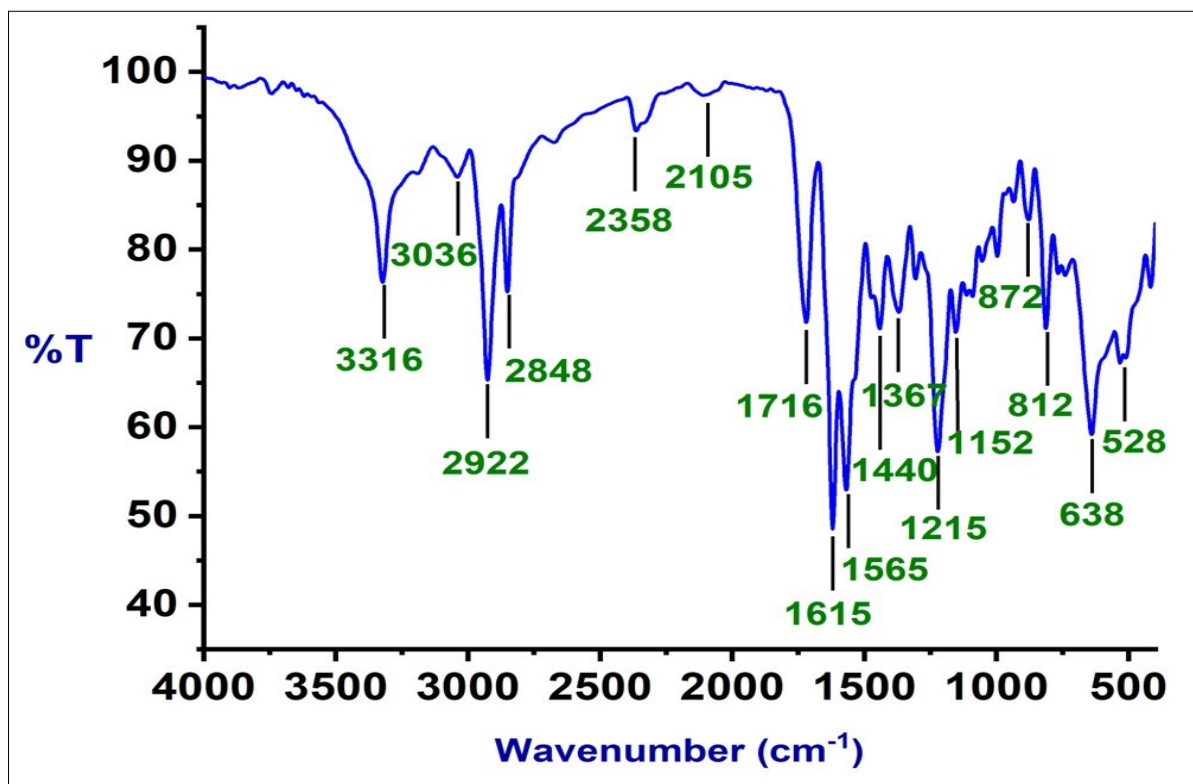
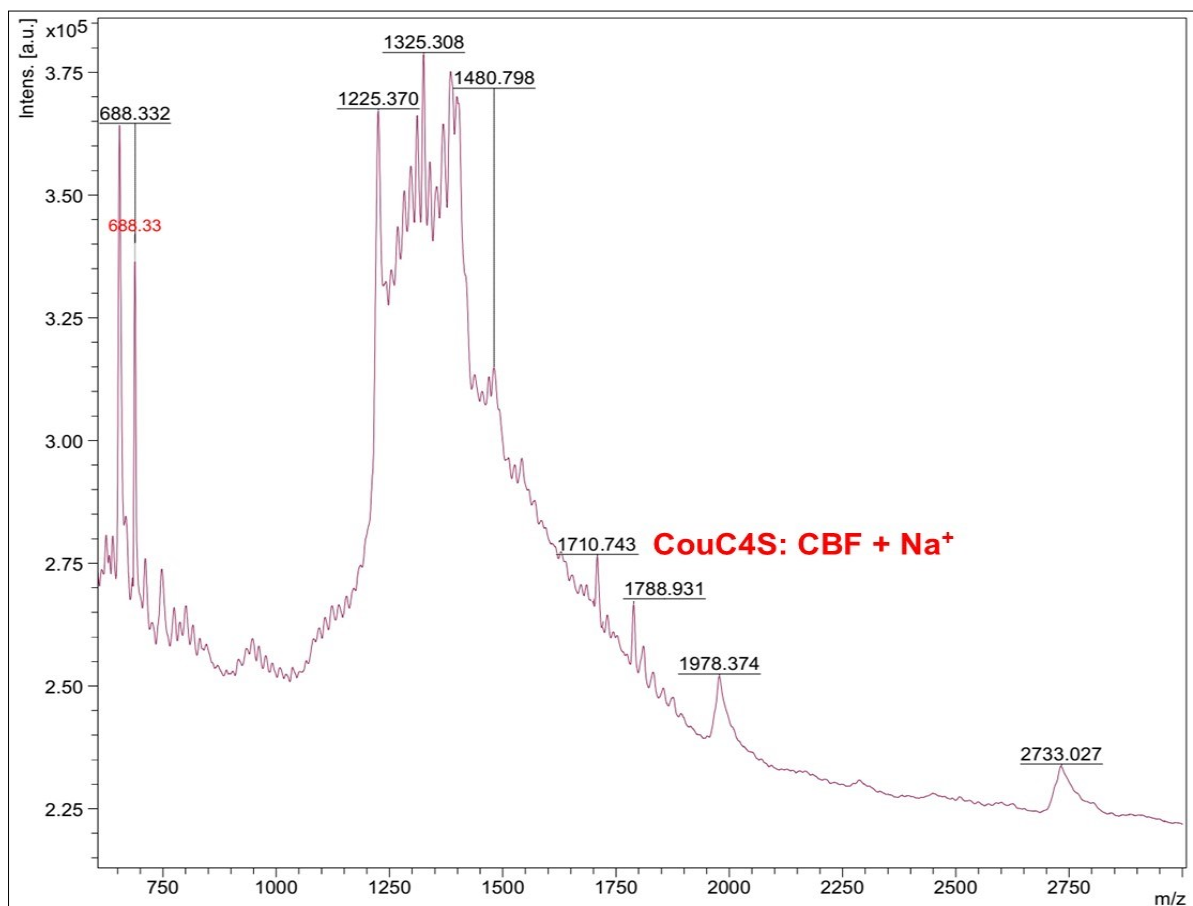


Figure S8: MALDI-TOF analysis plot of CouC4S: CBF complex.

Figure S9: FT-IR spectra of CouC4S: CBF complex.

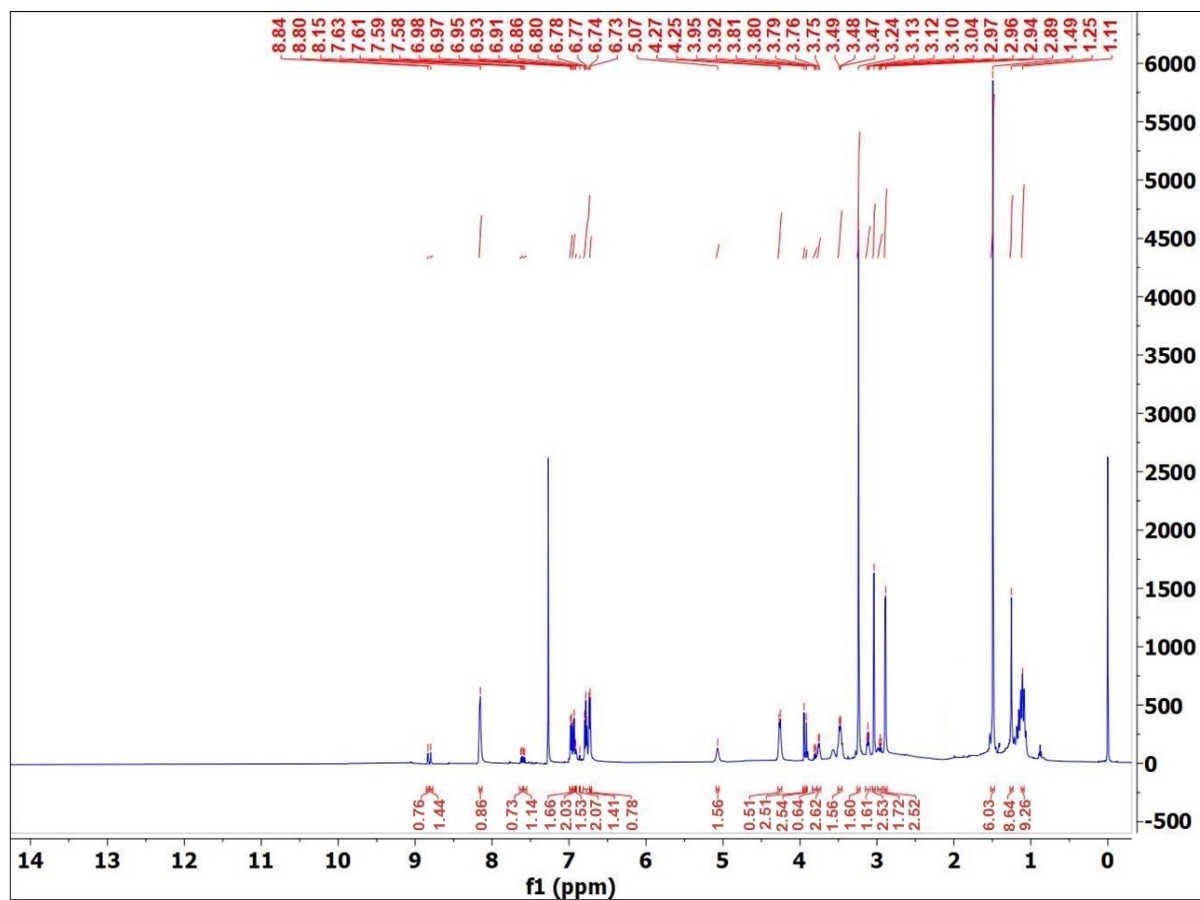


Figure S10: ^1H NMR spectrum of CouC4S: CBF complex.

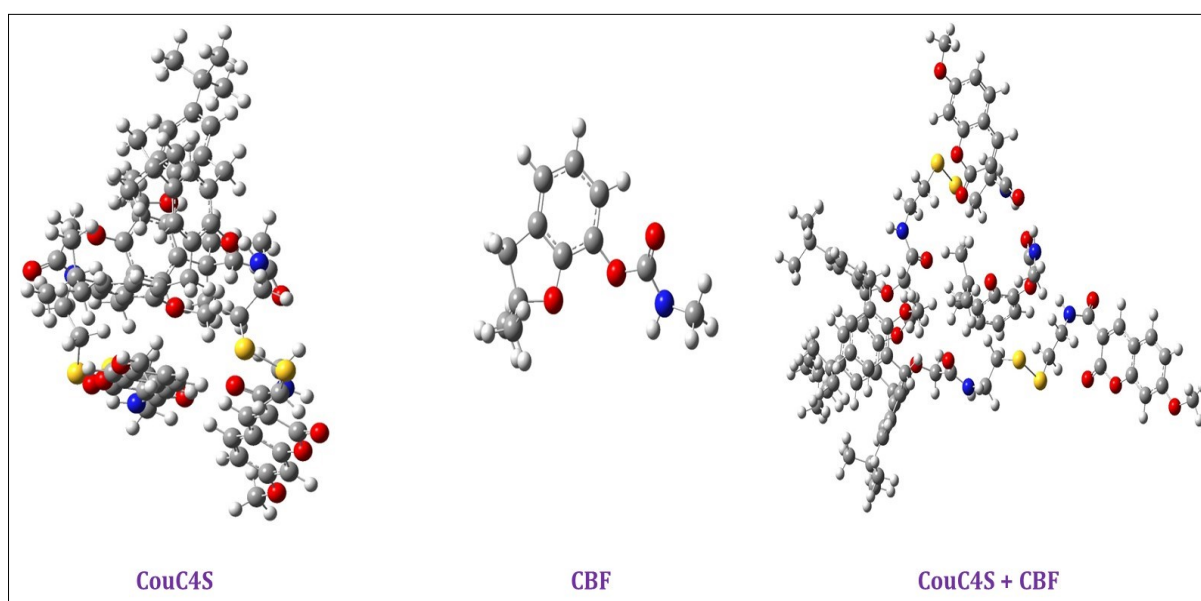


Figure S11: The optimized structures of CouC4S, CBF, and CouC4S: CBF complex.

| No. | 2 θ | Rel. Int. [%] | Plane | Interplanar Spacing (\AA) | Crystalline Size (\AA) | Lattice strain(%) |
|-----|------------|---------------|---------|--------------------------------------|-----------------------------------|-------------------|
| 1. | 11.0769 | 37.7 | (1 1 0) | 7.98782 | 98 | 3.685 |
| 2. | 17.3629 | 31.92 | (4 1 0) | 5.10754 | 159 | 1.463 |
| 3. | 19.0822 | 52.97 | (0 0 1) | 4.65106 | 114 | 1.859 |
| 4. | 20.3794 | 52.62 | (2 0 1) | 4.35784 | 123 | 1.614 |
| 5. | 22.1644 | 100 | (1 1 1) | 4.01076 | 134 | 1.368 |
| 6. | 26.0511 | 23.05 | (4 1 1) | 3.42053 | 101 | 1.545 |
| 7. | 28.6719 | 49.79 | (6 0 1) | 3.11355 | 125 | 1.135 |
| 8. | 32.0916 | 30.79 | (9 0 0) | 2.78915 | 109 | 1.164 |
| 9. | 36.3885 | 10.65 | (5 3 0) | 2.46906 | 118 | 0.95 |
| 10. | 38.346 | 15.76 | (6 3 0) | 2.3474 | 167 | 0.642 |
| 11. | 40.6416 | 21.39 | (1 1 2) | 2.21812 | 91 | 1.103 |

| a [\AA] | b [\AA] | c [\AA] | Alpha [$^\circ$] | Beta [$^\circ$] | Gamma [$^\circ$] | Cell Volume |
|--------------------|--------------------|--------------------|--------------------|-------------------|--------------------|-------------|
| 25.1 | 8.46 | 4.62 | 90 | 90 | 90 | 986.36 |

| Crystal type | Bravais type | Space group |
|--------------|---------------|-------------|
| orthorhombic | Body-centered | l m a m |

Table S1: PXRD analysis of CouC4S.

| No. | 2 θ | Rel. Int. [%] | Plane | Interplanar Spacing (\AA) | Crystalline Size (\AA) | Lattice strain(%) |
|-----|------------|---------------|---------|--------------------------------------|-----------------------------------|-------------------|
| 1. | 10.262 | 100 | | 8.62029 | 158 | 2.488 |
| 2. | 11.7909 | 12.44 | (1 1 0) | 7.50567 | 105 | 3.241 |
| 3. | 15.51 | 20.87 | | 5.71328 | 132 | 1.967 |
| 4. | 17.2924 | 59.96 | (0 1 1) | 5.12821 | 159 | 1.469 |
| 5. | 18.8382 | 56.24 | | 4.71075 | 123 | 1.749 |
| 6. | 20.3765 | 53.74 | (0 2 0) | 4.35847 | 128 | 1.554 |
| 7. | 22.1421 | 54.2 | (2 1 1) | 4.01475 | 91 | 1.996 |
| 8. | 25.9244 | 20.19 | (1 2 1) | 3.43695 | 89 | 1.746 |
| 9. | 31.8417 | 22.94 | (2 0 2) | 2.81047 | 121 | 1.057 |
| 10. | 33.0873 | 3.26 | (4 1 1) | 2.70746 | 137 | 0.902 |
| 11. | 40.4826 | 6.45 | (4 0 2) | 2.22646 | 114 | 0.886 |

| a [\AA] | b [\AA] | c [\AA] | Alpha [$^\circ$] | Beta [$^\circ$] | Gamma [$^\circ$] | Cell Volume |
|--------------------|--------------------|--------------------|--------------------|-------------------|--------------------|-------------|
| 12.76 | 8.74 | 6.24 | 90 | 90 | 90 | 695.98 |

| Crystal type | Bravais type | Space group |
|--------------|---------------|-------------|
| Tetragonal | Body-centered | l m a m |

Table S2: PXRD analysis of **CouC4S: CBF**.

| Name | Dipole moment (Debye) | HOMO (eV) | LUMO (eV) | Energy Gap (eV) | Hardness | Softness | Chemical Potential | Electrophilicity Index |
|--------------------|------------------------------|------------------|------------------|------------------------|-----------------|-----------------|---------------------------|-------------------------------|
| CouC4S | 6.339616 | -8.15852 | -1.343 | 6.814 | 3.4072 | 0.1467 | -4.7512 | 3.3126 |
| CBF | 3.408035 | -8.52478 | 0.127 | 8.652 | 4.3260 | 0.1155 | -4.1987 | 2.0375 |
| CouC4S: CBF | 15.04578 2 | -8.43879 | -1.428 | 7.010 | 3.5051 | 0.1426 | -4.9336 | 3.4722 |

Table S3: Electronic properties of CouC4S, CBF and CouC4S: CBF complex.

| No. | Functional Group | CouC4S (cm ⁻¹) | CouC4S:CBF (cm ⁻¹) |
|-----|---------------------|----------------------------|--------------------------------|
| 1 | -NH (Str) | 3327 | 3316 |
| 2 | Aromatic C-H (Str) | 3054 | 3036 |
| 3 | Aliphatic C-H(Str) | 2925 | 2922 |
| 4 | Aliphatic C-H (Str) | 2853 | 2848 |
| 5 | Amide –CONH (Str) | 1732 | 1716 |
| 6 | Aromatic C-H (ben) | 1622, 1568, 1475 | 1615, 1565,1440 |

Table S4: FT-IR analysis of CouC4S and CouC4S: CBF complex.

| Sample | Spiked pesticide | Spiked amount (μM) | Found by proposed sensor (μM) | Recovery (%) | No. of observations |
|------------------|------------------|---------------------------------|--|--------------|---------------------|
| Cabbage Sample 1 | CBF | 5 | 4.86 | 97 ± 0.2 | 5 |
| Cabbage Sample 2 | | 10 | 9.43 | 94 ± 0.3 | 5 |
| Cabbage Sample 3 | | 15 | 14.8 | 98 ± 0.8 | 5 |
| Cabbage Sample 4 | | 20 | 18.8 | 94 ± 0.1 | 5 |
| Cabbage Sample 5 | | 25 | 24.8 | 99 ± 0.2 | 5 |
| Cabbage Sample 6 | | 30 | 28.4 | 94 ± 0.6 | 5 |

Table S5: Results of the determination of CBF in different cabbage samples.

| Sr. No. | Method | No. of Tested Pesticides | Concentration range | Limit of detection | Ref. |
|---------|--|--------------------------|--|-------------------------|----------------------|
| 1 | Colourimetric Determination | 07 | - | 1.4 $\mu\text{g/L}$ | [51] |
| 2 | Amperometric Flow-injection analysis | 01 | $0.1 - 100 \times 10^{-6}$ M | 3.8 nM | [52] |
| 3 | Molecularly-imprinted polymer | 01 | $0.01 - 100 \times 10^{-6}$ M | 1.7 nM | [53] |
| 4 | Ratiometric Nanosensor | 01 | 9.8×10^{-6} M to 1.4×10^{-4} M | 12.2×10^{-6} M | [54] |
| 5 | Microcantilever-based immunosensor | 01 | $1.0 \times 10^{-7} - 1.0 \times 10^{-3}$ M | 0.1×10^{-9} M | [55] |
| 6 | Calix[4]arene-coumarin-based fluorescence sensor | 20 | $5 - 65 \times 10^{-6}$ M | 5.55×10^{-6} M | Present Paper |

Table S6: Comparative study table of detection of carbofuran by different methods.

