Spectral, structural and energetic study of acephate, glyphosate, monocrotophos and phorate: an experimental and computational approach

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ABSTRACT
In the current study, experimental (UV-visible, Fourier transform infrared [FTIR], 1H-NMR and scanning electron microscope) and computational (UV-visible, FTIR, 1H-NMR, HOMO-LUMO, steric and geometric parameters) analyses of acephate, glyphosate, monocrotophos and phorate were performed for the first time. Computational studies were performed at the HF/6-311G(d,p) level of theory. It was found that experimental values of UV-visible, FTIR, 1H-NMR and geometric data were in very good agreement with the computational ones. The current study may assist future studies, like spectral analysis, pesticide(s) detection, surface behaviour and decomposition analysis of top selling titled pesticides of world market.

1. Introduction
Plant protection has become necessary to increase the food production due to increase of the worldwide growth of the human population [1–3]. It is expected that the chemical pressure on the environment will further increase. Consequently, the uses of pesticides become a necessary part of agriculture, and their analysis is a prime most necessity of current time [1–3]. To analyse these toxic compounds, either on their development stages or in the environmental compartments, it is obligatory to develop theoretical studies to assist the experimental studies. In literature, few studies have been reported to check the spectral, geometrical, toxicological and biodegradation aspects of contaminants/pesticides [2–14].

Organophosphate pesticides (OPs) are the major class of pesticides, used as replacement of the toxic organochlorine pesticides in agricultural applications [2,3]. These are easily degradable, but more toxic than organochlorines [2,3,15]. OPs inhibit the phosphorylation of acetylcholinesterase and exert primarily a cholinergic toxicity [3]. From a health perspective, it is always risky to analyse the spectral, environmental and physicochemical properties of the toxic compounds including OPs [2–4,15,16]. As per the review reports of Terry [1] and Kumar et al. [2], OPs are toxic to humans and non-target organisms, exposure of OPs has been associated with prolonged impairments in attention, memory and other domains of cognition, as well as chronic illnesses, where these symptoms are manifested (e.g. Gulf War Illness, Alzheimer’s disease) [1,2]. Maximum exposure of OPs and other chemicals is experienced during analysis, detection, formulation and applications in the agriculture. Computational studies may provide the advantage over traditional analysis and detection processes, and the same may allow to chemists/researchers/users to overcome the excess exposure of OPs and other chemicals.

In literature, few computational studies on OPs have been reported [1–7]. Among these studies, maximum studies were reported on the herbicide, glyphosate. Ali et al. [8] have reported an extensive ab initio Molecular Orbital calculations at the HF/3–21G(d) level to study the conformational energy surfaces of N-hydroxy-glyphosate and N-amino-glyphosate [8]. Kaliannan et al. [17] have reported fully optimized structure and rotational potential around the active site nitrogen (about the C–N and N–C bond) at the HF/3–21G(d) level [17]. Zhang et al. [18] have studied THz spectra of molecular and solid forms of acephate using first principles calculations based on the density functional method [18]. A tandem mass spectrometry of methamidophos was performed by Rifai et al. [19], which was supported by B3LYP/6–31G(d) calculations [19].

As per our best information, the current study is the first detailed report on the experimental and computational analysis of titled molecules. Wide applications of titled molecules in the world market may make
this study more significant [20]. Future studies including the spectral analysis, pesticide(s) detection, surface behaviour and decomposition analysis of these pesticides may be acerated by this study.

In the current topic, we have performed the experimental and computational studies of acephate (1), glyphosate (2), monocrotophos (3) and phorate (4) (detailed information under supplementary Table S1). The specific objective of the current study was to perform experimental and computational study with the aim to evaluate and understand the structural, energetic and stability properties of titled pesticides 1–4.

2. Experimental

2.1. Material and instruments

All the pesticides were gifted by Gautmi Ltd, Andhra Pradesh, India, and having purity more than 95%. Ultraviolet–visible (UV–visible) experiments were performed on Shimadzu-1800s UV–visible spectrometer (cubed 1 cm length) in aqueous medium at different pH (4, 7 and 10) maintained by using HCl and NaOH dilute solutions. The wavelength-dependent molar absorptivity coefficient $\epsilon$ (M$^{-1}$ cm$^{-1}$) was obtained using Beer–Lambert’s law: $A = \log \frac{I_0/I}{L \times C}$, where $A$ is the absorbance (dimensionless), $I_0$ is the reference intensity taken with the cell filled with distilled water, $I$ is the intensity with the cell filled with an aqueous solution of the studied pesticide, $L$ is the path length of the two cells (cm) and $C$ is the concentration of pesticide (in mol L$^{-1}$).

Transmission Fourier transform infrared (FTIR) experiments were performed on a Shimadzu-8400. Molecules 1 and 2 are solid and these were analysed by taking 1.5 mg of the compound one by one in 200 mg KBr, mixed and crushed well. Then the KBr powder was pressed at a pressure of 10 tons into 200 mg KBr, mixed and crushed well. Then the KBr–sample mixture was pressed into 16 mm diameter discs, using a Specac, UK die-press. A pure disc of potassium bromide (KBr) of 200 mg 1 cm length) in aqueous medium at different pH (4, 7 and 10) maintained by using HCl and NaOH dilute solutions. The wavelength-dependent molar absorptivity coefficient $\epsilon$ (M$^{-1}$ cm$^{-1}$) was obtained using Beer–Lambert’s law: $A = \log \frac{I_0/I}{L \times C}$, where $A$ is the absorbance (dimensionless), $I_0$ is the reference intensity taken with the cell filled with distilled water, $I$ is the intensity with the cell filled with an aqueous solution of the studied pesticide, $L$ is the path length of the two cells (cm) and $C$ is the concentration of pesticide (in mol L$^{-1}$).

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Nuclear magnetic resonance (NMR) analyses were performed on an NMR spectrophotometer (Bruker Avance III, 400 MHz) by taking trimethylsilane as an internal reference. Compounds 1–4 were prepared in deuterated dimethyl sulfoxide (d$_6$–DMSO) in different sample tubes having 2 mL capacity. Approximately 2 mg of the solid compounds of molecules 1 and 2 was used and 500 μL of molecules 3 and 4 was used. The samples in tubes were allowed to settle down and soluble liqueurs poured into NMR sample analysis tubes. These NMR sample analysis tubes were caped well and put into the NMR instrument automated ballet. Finally, the NMR spectra were recorded in ppm values.

Scanning electron microscopy (SEM) was performed to check the surface topography and distribution of elemental composition on the surface. Different elements and surface topographies emit different quantities of electrons. SEM analysis was performed at SAIF Coachi Kerala, India, on the instrument, JEOL Model JSM-6390LV in the following conditions; resolution: 3 nm (Acc V 30 KV, WD 8 mm, SEI); 8 nm (Acc V 3.0 KV, WD 6 mm, SEI); 15 nm (Acc V 1.0 KV, WD 6 mm, SEI); Magnification: 10x, 100x and 1000x with Probe Current: 1 pA–1 mA.

2.2. Computational study

The computational studies of molecules 1–4 were performed using the Personal Computer-General Atomic and Molecular Electronic Structure System (PCGAMESS) version 13.0 [4–14]. The theoretical, steric parameters (stretch, bend, stretch-bend, torsion, non-1,4 VDW, 1,4 VDW, dipole/dipole and steric energy for frame), polarizability, infrared intensities (FTIR), UV–visible and $^1$H-NMR study of the titled pesticides in the ground state (a minimum energy state of molecule) were calculated. For the first time, computational analysis was performed using the Hartree Fork (HF) method with the 6–311++G(d,p) basis set through the GAMESS programme [4–13], using Cambridge Software ChemBio3D Ultra 13.0.

To calculate the above-mentioned parameters, the executable programme file of GAMESS (using Cambridge Software ChemBio3D Ultra 13.0) was run on PC. In first step, structures of molecules 1–4 were prepared by using Chemdraw [7–13], and then converted into 3D structures. One by one, all the 3D structures were optimized into minimum energy structure by using the appropriate commands (calculations – GAMESS interface – set basis (HF-6–311++G(d,p)) – temperature (298°C) – energy minimization – run). In the next step, the desired parameters were calculated by using the appropriate commands (calculations – GAMESS interface – desired parameter, e.g. prediction of IR spectrum or UV spectrum or NMR spectrum or polarization or dipole moment – set basis (HF-6–311++G(d,p)) – temperature (298°C) – run). The steric parameters were optimized through MM2 computational optimization. Finally, output files of all the parameters...
were obtained (calculations – GAMESS interface – view output files). All the qualitative and quantitative studies were done on the basis of output files obtained after complete optimizations.

3. Results and discussion

Structurally, OPs are pentavalent phosphorus-containing organic compounds that possess three different substituents including P9O/S moiety to fill the valences on the phosphorus atom [1–3]. Considering per configurational and conformational arrangement aspects, all the molecules have different arrangements around the P atom with different energy values [1–3,13,14,17–19]. In this study, the analysis of single conformer of each molecule was performed which has a minimum energy value because only the stable or minimum energy value molecule can provide accurate data [9–14,17–19]. The structural view (top, side and front view) of minimum energy structures as well HOMO–LUMO and dipole views of 1–4 are shown in the supplementary figure (S2). To make the study more effective and interesting, a relationship between molecules 1–4 was established on the basis of structures of titled molecules (Supplementary S2), i.e. “for glyphosate, two moieties are OH, for monocrotophos these two moieties are –OCH₃, for phorate these moieties are –OEt, and for acephate these moieties are –OCH₃ and –SCH₃”.

3.1. Effect of pH variation on titled molecules

The effect of pH on molecules 1–4 was observed experimentally. The changes in absorbance were measured at 235 nm for 1, 215 nm for 2, 330 nm for 3 and 260 nm for 4 using a concentration of 0.01 M (where M means molar) (Supplementary figure S3). Concentration was taken as a dependent factor over variable wavelength. The absolute values of molar absorptivity were decided to find out the significant results at different pH values. The calculated values of molar absorptivity (ε) were as shown in supplementary figure S3; for molecule 1 at 235 nm, 96.5 ± 0.22 M⁻¹ cm⁻¹ at pH 4, 122.6 ± 0.31 M⁻¹ cm⁻¹ at pH 7 and 233.0 ± 0.42 M⁻¹ cm⁻¹ at pH 10. For molecule 2, the calculated values of molar absorptivity (ε) were as at 215 nm, 24.1 ± 0.08 M⁻¹ cm⁻¹ at pH 4, 29.2 ± 0.12 M⁻¹ cm⁻¹ at pH 7 and 148.0 ± 0.34 M⁻¹ cm⁻¹ at pH 10. Similarly, for molecule 3, the calculated values of molar absorptivity (ε) were as at 330 nm, 181.7 ± 0.23 M⁻¹ cm⁻¹ at pH 4, 188.0 ± 0.29 M⁻¹ cm⁻¹ at pH 7 and 199.9 ± 0.37 M⁻¹ cm⁻¹ at pH 10. Finally, for molecule 4, the calculated values of molar absorptivity (ε) were as at 260 nm, 147.8 ± 0.21 M⁻¹ cm⁻¹ at pH 4, 160.1 ± 0.26 M⁻¹ cm⁻¹ at pH 7 and 162.9 ± 0.29 M⁻¹ cm⁻¹ at pH 10. Overall, it was found that the 0.01 M solution of molecule 3 has the highest values of molar absorptivity (ε) from pH 4 to 10, followed by 4, 1 and 2 (Supplementary figure S3).

Compared to molar absorptivity (ε) values at pH 4 with pH values at 7 and 10, there were 27% and 141% increase observed for molecule 1, 21% and 514% increase observed for molecule 2, 3% and 10% increase observed for molecule 3, and 8% and 11% increase observed for molecule 4. In terms of percentage increase with an increase in pH values, molecule 2 has shown excellent increase, followed by 1, 4 and 3. Among the titled molecules 1–4, at pH 4, the order of percentage change among the molar absorptivity (ε) was 3 > 4 > 1 > 2, at pH 7, the order of molar absorptivity (ε) was 1 > 2 > 4 > 3 at and pH 10, the order of molar absorptivity (ε) was 2 > 1 > 4 > 3. At pH 10, all molecules show greater molar absorptivity (ε) followed by pH 7 and 4. Interestingly, there were drastic changes in absorbances or molar absorptivity (ε) observed in case of molecules 1 and 2 at higher pH 10 (Supplementary figure S3).

To find out the reason behind drastic changes with pH, we have compared literature studies with the current study. It was found that at pH 4 molecule 1 is in cationic form (at N atom) due to the acidic environment of the solution (Figure 1). As the pH increases to 7, the molecule starts to attain its free or original state (lone pairs with every hetero atom like N and O, and especially C9O group which acts at the chromophoric group allowing π − π* and n − π* transitions). At pH 10, it was observed that molecule 1 was involved in H-bonding leading to the formation of six-membered ring(s) and increases the conjugation. As a result of conjugation, increase in the π − π* transition was observed, which leads to the higher molar absorptivity (ε) (Figure 1) [21–27]. A similar fact was applicable for molecule 2 and molecule 3 due to the formation of five- and four-membered chelation rings through H-
bonding. In case of molecule 4, there was almost stationary change in molar absorbivity (ε) with an increase in pH due to non-conjugation and non-chelation ring nature.

Recent studies have revealed that hydrolysis of molecules 1–3 is very slow in water, and half life increases up to tenfold [28–31]. The slow decomposition was attributed to the formation of the six-membered ring through H association (Figure 1). From the above discussion, the observed order of stability was as: 2 > 1 >> 3. The fact behind this stability order was the formation of kinetically and thermally stable five-member ring by 2, followed by 1 (six-member less stable, i.e. kinetic stability only) and 3 (four member not stable, i.e. kinetically and thermally non-stable). As per Baeyer Strain theory, four-membered ring is non-stable due to angle strain.

Moreover, the acid dissociation constant (pKa values) has a direct relationship with pH values, so pKa values can play a significant role in decomposition (thermally, chemically and biochemically), persistence and reactivity of molecules [28–33]. The pKa values depend upon the number of dissociated hydrogen ions, and it is directly proportional to the stability of the molecule (ΔG = 2.303 RT pKa). The reported pKa value of 1 is 6.54 (at nitrogen), 15.73 (at nitrogen) for 3 and −0.58 (at OH near P), 2.95 (at OH of COOH), 8.37 (at OH near P) and 6.34 (at nitrogen) for 2. At acidic pH, the protonated form predominates, while at a higher pH these molecules (1–3) exist in the anionic form. Hence, the electrostatic interaction between the positively charged surface and protonated favours adsorption and increases their removal.

### 3.2. Experimental cum computational comparative spectroscopic analysis

In spectroscopic analysis, very good agreement between computational and experimental data was observed. The noticeable differences were observed because theoretical calculations were performed in the gaseous phase, while the experimental results were obtained in the solid/liquid phase [32–38]. Excellent results were obtained for UV–visible analysis, where negligible difference was observed between the computational and experimental analysis. The observed difference was 3 nm for molecule 1, 4 nm for molecule 2, 2 nm for molecule 3 and 1 nm for molecule 4 (Table 1 and Supplementary figure S4).

The comparative FTIR analyses showed that the difference between the computational and experimental analysis was between 71–10 cm⁻¹ (v(N–H); 71, v(C(O)); 50, δ(N–H); 63, v(N–C); 10, v(P9O); 45, δ(C–O); 14 and δ(N–C); 50)) for molecule 1, 56–2 cm⁻¹ (v(N–H); 25, v(C9O); 56, δ(N–H); 51, v(N–C); 19, v(P9O); 4, δ(C–O); 2 and δ(N–C); 5)) for molecule 2, 22–2 cm⁻¹ (v(N–H); 19, v(C–O); 2, δ(N–H); 22, v(N–C); 3, v(P9O); 7, δ(C–O); 12 and δ(N–C); 6)); for molecule 3 and 17–3 cm⁻¹ (v(P9S); 8, v(P–O); 17, δ(C–S); 3, v(C–O); 11 and δ(C–S); 5)) for molecule 4 (Table 1 and Supplementary figure S5).

### Table 1. Details of observed and optimized vibrational frequencies, UV plots and 1H-NMR analysis of titled molecules 1–4.

| Molecule | FTIR analysis (cm⁻¹) | 1H-NMR analysis (ppm) | UV–visible analysis (nm) |
|----------|----------------------|-----------------------|-------------------------|
|          | O   | E   | MA | Shift | O | E | MA | Shift | O | E | Shift |
| Acephate or 1 | 3283 | 3212 | v(N–H) | 71 | 8.00 | – | – | NH | – | 215.0 | 212.0 | 3 |
|          | 1648 | 1698 | v(C9O) | –50 | 3.78 | 3.74 | – | O–CH3 | – | 0.04 |
|          | 1583 | 1520 | δ(N–H) | 63 | 2.04 | 2.25 | – | CO–CH3 | – | 0.21 |
|          | 1360 | 1370 | v(N–C) | –10 | 2.00 | 2.03 | – | S–CH3 | – | 0.03 |
|          | 1269 | 1224 | v(P9O) | 45 | | | | | |
|          | 544  | 530  | δ(C–O) | 14 | | | | | |
|          | 470  | 420  | δ(N–C) | 50 | | | | | |
| Glyphosate or 2 | 3268 | 3243 | v(N–H) | 25 | 13.03 | – | – | COOH | – | 208.0 | 204.0 | 4 |
|          | 1676 | 1732 | v(C9O) | –56 | 4.80 | 4.70 | – | OH | – | 0.10 |
|          | 1519 | 1570 | δ(N–H) | –51 | 3.49 | 3.88 | – | CH2–CO | – | 0.39 |
|          | 1370 | 1389 | v(N–C) | –19 | 2.80 | 3.10 | – | F–CH3 | – | 0.30 |
|          | 1226 | 1222 | v(P9O) | 4 | 2.00 | – | – | – | – |
|          | 552  | 550  | δ(C–O) | 2 | | | | | |
|          | 475  | 470  | δ(N–C) | 5 | | | | | |
| Monocrotophos or 3 | 3296 | 3315 | v(N–H) | –19 | 7.80 | 7.90 | – | NH | – | 0.10 |
|          | 1681 | 1683 | v(C9O) | –2 | 5.89 | 5.83 | – | 9CH3 | – | 0.06 |
|          | 1578 | 1600 | δ(N–H) | –22 | 3.78 | 3.75 | – | O–CH3 | – | 0.03 |
|          | 1362 | 1365 | v(N–C) | –3 | 2.24 | 2.77 | 9C–CH3 | – | 0.53 |
|          | 1269 | 1276 | v(P–O) | –7 | 2.13 | 2.13 | NH–CH3 | – | 0.00 |
|          | 588  | 600  | δ(C–O) | –12 | | | | | |
| Phorate or 4 | 1008 | 1016 | v(P9S) | –5 | 4.01 | 4.33 | – | O–CH2 | – | 0.32 |
|          | 945  | 962  | v(P–O) | –17 | 3.71 | 3.98 | S–CH2–S | – | 0.27 |
|          | 652  | 655  | v(C–S) | –3 | 2.48 | 1.85 | S–CH2 | – | 0.63 |
|          | 479  | 490  | v(C–O) | –11 | 1.29 | 1.31 | O–CH2–CH3 | – | 0.02 |
|          | 405  | 410  | δ(C–S) | –5 | 1.25 | 1.31 | S–CH2–CH3 | – | 0.06 |

Note: O = Optimized frequencies, E = Experimental frequencies, MA = Mode assigned.
The major differences in the C90 and N–H frequencies of molecules 1 and 2 were observed, which was greater than 40 cm\(^{-1}\). In addition to the above-mentioned fact (computational data in the gaseous phase and experimental data in the solid/liquid phase), the other most effective reason behind it was the H-bonding [21–24]. H-bonding was not counted or optimized in computational analysis. Due to the presence of H-bonding, the experimental frequencies were observed at lower wave numbers as compared to computational ones. The higher difference (>20 cm\(^{-1}\)) was observed at the N–H frequencies of molecules 3. It was due to the involvement of N–H in resonance with C90 [34–37]. There was less than 20 cm\(^{-1}\) difference observed in the P–O frequency molecule 4. It may be due to the average frequency of two free rotating P–O bonds having different environment.

In addition to FTIR analysis, “Liquid drop on KBr disc”, a shortcut and time-saving method was performed. To check the accuracy, the FTIR data of molecule 3 and 4 were compared with the NIST data (Supplementary figures S6 and S7). It observed that both the spectrums were very similar to each other at observable FTIR bands. Hence, we can apply this method to the FTIR analysis of toxic liquid chemicals including pesticides. This method can avoid consumption of the time to change the liquid sample holder and exposure of toxic pesticide(s). Same can avoid consumption of the time to change the liquid chemical(s) including pesticides. This method hence, we can apply this method to the FTIR analysis of toxic liquid chemicals including pesticides. This method can avoid consumption of the time to change the liquid sample holder and exposure of toxic pesticide(s). Hence, the P–O frequencies of molecules 3 and 4 (Table 1) and (Supplementary figures S8 and S9). The reasons behind it were very much similar as discussed in the UV and FTIR section. The additional fact was the influence of solvent with molecule(s) [33–38].

### 3.3. Chemical reactivity

Reactivity of any molecule, especially of the agrochemicals is always an interesting point of chemical study. Based on the HOMO–LUMO energy difference, the order of reactivity was as 4 (5.84 eV) > 2 (10.31 eV) > 3 (10.46 eV) > 1 (12.59 eV). HOMO–LUMO analysis has shown that these molecules are chemically less reactive at RT because all these molecules have an energy gap of more than 1.30 eV [13,14,39]. Often, in organic chemistry, we like to know the “partial” charge on a carbon atom to see if it might be a good nucleophile or the site for electrophilic attack. The charge density is important as these are the electrons most likely to be involved in the chemical reactivity [11–14,18]. The exact order of charge densities at phosphorus (P) atom of titles molecules was 3 (2.094) > 2 (1.970) > 1 (1.857) > 4 (1.797) (Table 2). Similarly, the order of charge density at oxygen or sulphur of P90/P9S was 3 (−1.213) > 1 (−1.187) > 2 (−1.17) > 4 (−0.961) (Table 2).

Huckel charge densities have been considered as useful quantities to illustrate the charge distributions of molecules [2]. Charge distributions can give the information about how the molecules interact with another molecule. The current study has shown that P atoms of all titled pesticides suspected for nucleophilic attack due to positive charge densities and oxygen or sulphur directly attached to the P atom acted as a nucleophile [1–3,40]. This information may help the decomposition mechanism designing processes.

| Acepate | Glycophosate | Monocrotophos | Phorate |
|---------|--------------|---------------|---------|
| O –1.187 [O(11)] | O –0.500 [O(11)] | O –1.213 [O(11)] | C 0.166 [C(11)] |
| P 1.857 [P(2)] | P 1.970 [P(2)] | P 2.094 [P(2)] | O –0.514 [O(2)] |
| S –0.070 [S(1)] | O –0.496 [O(3)] | O –0.455 [O(3)] | P 1.797 [P(3)] |
| C –0.153 [C(4)] | O –1.171 [O(4)] | O –0.455 [O(4)] | O –0.518 [O(4)] |
| O –0.477 [O(5)] | C –0.211 [C(5)] | C 0.083 [C(5)] | C 0.155 [C(5)] |
| C 0.045 [C(6)] | N –0.187 [N(6)] | C 0.083 [C(6)] | C –0.145 [C(6)] |
| N –0.009 [N(7)] | C –0.020 [C(7)] | O –0.279 [O(7)] | C –0.145 [C(7)] |
| C 0.467 [C(8)] | C 0.580 [C(8)] | C 0.325 [C(8)] | S –0.961 [S(8)] |
| O –0.791 [O(9)] | O –0.663 [O(9)] | C –0.149 [C(9)] | S –0.117 [S(9)] |
| C –0.266 [C(10)] | O –0.121 [O(10)] | C –0.332 [C(10)] | C –0.090 [C(10)] |
| H 0.039 [H(11)] | H 0.200 [H(11)] | H 0.377 [C(11)] | S 0.071 [S(11)] |
| H 0.035 [H(12)] | H 0.200 [H(12)] | O –0.853 [O(12)] | C –0.075 [C(12)] |
| H 0.047 [H(13)] | H 0.035 [H(13)] | N 0.231 [N(13)] | C –0.122 [C(13)] |
| H 0.020 [H(14)] | H 0.016 [H(14)] | C –0.045 [C(14)] | H 0.012 [H(14)] |
| H 0.061 [H(15)] | H 0.103 [H(15)] | H 0.026 [H(15)] | H 0.013 [H(15)] |
| H 0.035 [H(16)] | H 0.045 [H(16)] | H 0.025 [H(16)] | H 0.018 [H(16)] |
| H 0.108 [H(17)] | H 0.026 [H(17)] | H 0.024 [H(17)] | H 0.013 [H(17)] |
| H 0.113 [H(18)] | H 0.194 [H(18)] | H 0.026 [H(18)] | H 0.043 [H(18)] |

Table 2. Calculated Huckel charged densities of titled molecules.
under acidic (electrophilic or cationic attack (metal ions)) and basic (nucleophilic attack) conditions [40]. The hydrolysis or decomposition of any molecule by biological microorganisms (bacteria, enzyme, fungi, etc.) depends upon the electrophilic and nucleophilic attacks too. The most suspected bonds for the chemical decomposition of titled molecules are P–N bond in acephate, C–N bond glyphosate, P–O bond in monacrotophos and P–S bond in phorate [28–31]. During the bio-decomposition, the most suspected bonds are C–N bond in acephate and glyphosate, P–O bond in monacrotophos and P–S bond in phorate [41–46]. Final products of all processes are phosphate oxides, ammonia and carbon dioxide [28–33].

3.4. Molecular stability of molecules on the basis of computational study

The steric energy parameters (stretch, bend, stretch-bend, torsion, non-1,4 VDW, 1,4 VDW, dipole/dipole and steric energy for frame) of molecules 1–4 were analysed computationally. The exact order of steric energy (kcal/mol) for molecules was as 4 >> 3 > 1 > 2 (Figure 2 and Table 3). A reciprocal order was analysed with melting points of molecules (Figure 2). Figure 2 has revealed that molar mass and size of molecules can increase the steric parameters.

3.5. Polarizabilities and the prediction of the biodegradation

Bio-decomposition of the agrochemical has been considered as a huge task. For the last 50 years, it has become a mammoth problem for scientific communities. Computational optimizations of any molecule may assist chemists to design the biodegradation pathway(s) of the agrochemicals. In recent studies, few authors have reported the biodegradation of chemicals on the basis of polarizabilities optimization of molecules [4–12]. In the current study, out of four molecules 1–4, three molecules 1–3 are highly water soluble, so the risk of leaching into the aquatic environment is high if these are not quickly decomposed. The persistence of these toxic molecules in soils is a threat for soil fertility as they may inhibit the growth of beneficial microorganisms of soils [24–31,41–50]. So, parameters including the dipole moment and polarizability of any molecule can play the decisive role for its biodegradation including toxic agrochemicals [47–49]. The average half life period of 1 on soil was 0.5–6 days, 5–197 days for 2, 2–10 days for 3 and 3–20 days for 4. These half lives periods of titled molecules were varying reciprocally with computational parameters namely dipole moment and polarizability (Figure 3). The computed averaged static dipole polarizability and dipole moment of molecules 1–4 were calculated. The component of the polarizability tensor optimized along the z-axis, i.e. long molecular axis. The values of averaged polarizabilities depend upon the positions of substituents bound to the moiety [5–11]. The average polarizability \( \langle \alpha \rangle \) was calculated as per the formula reported in the literature [5–11], i.e. 

\[
\langle \alpha \rangle = \frac{\alpha_{xx} + \alpha_{yy} + \alpha_{zz}}{3}
\]

On the basis of average polarizability and dipole moment, the order of bio-decomposition was as 1 > 3 >> 4 > 2. This order was in excellent agreement with the experimental decomposition as mentioned in the literature [41–50] (Figure 3).

![Figure 2](image2.png)

**Figure 2.** Relation among the physicochemical and steric parameters.

![Figure 3](image3.png)

**Figure 3.** Relation among the half life periods of molecules 1–4, dipole moment and polarizability.

| Code | Stretch (kcal/mol) | Bend (kcal/mol) | Torsion (kcal/mol) | VDW (kcal/mol)* |
|------|-------------------|----------------|-------------------|-----------------|
| 1    | 0.34              | 6.02           | 24.60             | −3.42           |
| 2    | 0.26              | 5.48           | 20.28             | −5.21           |
| 3    | 0.95              | 8.86           | 33.67             | −4.93           |
| 4    | 5.08              | 52.3           | 00.30             | −4.05           |

*Both 1,4 and non 1,4 VDW.
In recent studies, it has been analysed that under the recommended dose-level nitrogen containing molecules like glyphosate, monocrotophos and acephate stimulate the soil microbial growth of nitrification bacteria as well as soil fungi [41–46]. Glyphosate breakdown provided N to microbes for microbial activity. But high doses, long-term or repeated use inhibits the count of total fungi, Acremonium strictum, Penicillium glabrum and Aspergillus fumigatus significantly [46]. On the other hand, the presence of phorate at a recommended level significantly decreased dinitrogen fixation [45,46], numbers of Staphylococcus, Micrococcus, Fusarium, Humicola and Rhizopus [46]. Phorate and glyphosate persisted in the rhizospheric soils, the difference is that glyphosate is non-toxic to microorganisms while phorate cause toxicity in short life time [41–50]. The large-scale use of pesticides in crop management systems emphasizes the need to understand their effects on soil microbial communities. The pesticides may change the soil environment due to toxicity to soil microorganisms. The relationships of different structures of pesticides on the growth of various groups of soil microorganisms are not easily predicted. Some pesticides stimulate the growth of microorganisms, but other pesticides have depressive effects or no effects on microorganisms. So, the prediction of biodegradation through the theoretical studies may assist the experimental studies which are also a demand of time.

### 3.6. Geometric parameters optimization and comparison with literature

The geometric parameters (bond length and bond angles) were performed on the crystalline molecules only. To check the crystallinity, SEM analysis was performed of molecules 1–4. SEM analysis showed that molecules 1 and 2 have a rectangular cube and ice cube like morphology and both were crystalline in nature [51–53]. The morphology (noncrystalline) of 3 and 4 was similar to each other, but quite different form 1 and 2 (Figure S10). Here, bond length and bond angles of the molecules 1 and 2 were optimized and compared with the literature [51]. The optimized theoretical geometric parameters of glyphosate were found to be in good agreement with the

| Bond | Bond length (angstrom) | Bond angle (degrees) |
|------|------------------------|----------------------|
| N(7)–C(8)–C(10)–H(20) | 109.43 | (H20)–C(10)–C(8) | 106.746 |
| O(9)–C(8)–C(10)–H(18) | 172.42 | H(18)–C(10)–C(8) | 110.616 |
| O(9)–C(8)–C(10)–H(19) | 47.0617 | H(19)–C(10)–C(8) | 108.386 |
| O(9)–C(8)–C(10)–H(20) | –66.8808 | O(9)–C(8)–C(10) | 116.349 |
| P(2)–N(7)–C(8)–O(9) | –167.654 | P(2)–N(7)–C(8)–O(9) | 108.386 |
| P(2)–N(7)–C(8)–C(10) | 15.9728 | C(10)–N(7)–C(8) | 119.838 |
| H(17)–N(7)–C(8)–O(9) | 3.9295 | H(18)–N(7)–C(8)–O(9) | 118.188 |
| P(2)–O(5)–C(6)–H(14) | –32.7556 | O(5)–C(6)–H(14) | 110.681 |
| P(2)–S(3)–C(4)–H(13) | 86.4444 | P(2)–S(3)–C(4)–H(13) | 117.573 |
| O(1)–P(2)–N(7)–H(17) | 19.1661 | O(1)–P(2)–N(7)–H(17) | 133.5 |
| S(3)–P(2)–N(7)–C(8) | 62.2191 | S(3)–P(2)–N(7)–C(8) | 124.387 |
| O(5)–P(2)–N(7)–C(8) | –43.7024 | O(5)–P(2)–N(7)–C(8) | 108.267 |
| O(1)–P(2)–O(5)–C(6) | 46.781 | O(1)–P(2)–O(5)–C(6) | 105.283 |
| S(3)–P(2)–O(5)–C(6) | 172.185 | O(5)–S(3)–P(2) | 108.036 |
| N(7)–P(2)–O(5)–C(6) | –75.2163 | N(7)–P(2)–O(5)–C(6) | 89.6666 |
| O(3)–P(2)–S(3)–C(4) | 142.14 | C(4)–P(2)–S(3) | 102.045 |
| O(5)–P(2)–S(3)–C(4) | 15.396 | O(5)–P(2)–S(3) | 109.328 |

| Bond | Bond length (angstrom) | Bond angle (degrees) |
|------|------------------------|----------------------|
| O(10)–H(18) | 0.9681 | 1.038 | H(18)–O(10)–O(8) | 112.6421 | 114.8 |
| C(8)–O(10) | 1.3571 | 1.308 | O(10)–C(8)–O(9) | 122.7405 | 125.5 |
| C(8)–O(9) | 1.1996 | 1.201 | O(10)–C(8)–C(7) | 110.2864 | 122.3 |
| C(7)–H(17) | 1.0908 | 1.057 | O(9)–C(8)–C(7) | 126.9414 | 124.2 |
| C(7)–H(16) | 1.0791 | 0.957 | H(17)–C(7)–H(16) | 108.3558 | 116.2 |
| C(7)–C(8) | 1.5058 | 1.507 | H(17)–C(7)–C(8) | 108.0841 | – |
| N(6)–H(15) | 1.0047 | 0.698 | H(17)–C(7)–N(6) | 113.3367 | – |
| N(6)–C(7) | 1.4581 | 1.479 | C(7)–N(6)–C(5) | 115.747 | 114.4 |
| C(5)–H(14) | 1.0865 | 0.936 | N(6)–C(5)–P(2) | 105.9734 | 112.2 |
| C(5)–H(13) | 1.0804 | 0.903 | C(12)–O(3)–P(2) | 118.5828 | 105.9 |
| C(5)–N(6) | 1.4562 | 1.491 | H(11)–O(1)–P(2) | 121.1267 | 105.9 |
| O(3)–H(12) | 0.9659 | 1.021 | C(5)–P(2)–O(4) | 117.3632 | 109.9 |
| P(2)–C(5) | 1.8145 | 1.816 | C(5)–P(2)–O(3) | 106.4119 | 106 |
| P(2)–O(4) | 1.5366 | 1.501 | C(5)–P(2)–O(1) | 100.006 | 104.2 |
| P(2)–O(3) | 1.6299 | 1.568 | O(4)–P(2)–O(3) | 112.3894 | 111.4 |
| O(1)–H(11) | 0.9647 | 1.018 | O(4)–P(2)–O(1) | 116.8543 | 118.2 |
| O(1)–P(2) | 1.6296 | 1.568 | O(3)–P(2)–O(1) | 101.7085 | 105.7 |

**Table 4.** Calculated geometric parameters of acephate (molecule 1) and glyphosate (molecule 2) and comparison with literature data of glyphosate.
corresponding experimental literature data (Table 4) [5–9]. In literature, not a single study on geometric parameters of molecules 1 is reported till date, so the current study may assist the future study like single crystal analysis of molecule 1 (Table 4). All the results on geometric parameters (bond length and bond angles) of crystalline molecules 1 and 2 are tabulated in Table 4.

4. Environmental applicability

These studies are very important, especially with environmental concern and real-world analysis applications. For example, the detection of glyphosate by UV–visible spectrophotometric methods which are reliable and belongs to real-time analysis, interactions of pesticides with the soils and their adsorption on clay and humates [41–50,54–63]. These studies are the foundation of detection of various contaminants and their metabolites through the chromatographic methods in water, soils, fruits, crops, vegetables and other samples [41–46,50–56,64]. The derivatization of glyphosate, acephate and monocrotophos at NH through various molecules (Sanger’s reagent) may be detectable in real time [53–55,64]. In literature, the analysis of the glyphosate has been mentioned, where derivatization done was analysed by UV–visible and HPLC methods only [53–55,64]. These studies are applicable to all the molecules containing secondary amine [60–63]. After the derivatization, UV–visible analysis can be used to detect these molecules from various compartments of the environment [54–63]. In our recent study, we have found that acephate and glyphosate were detectable by UV–visible, FTIR, mass and NMR studies [60,63]. Most importantly, these new derivatives have multiple bioactivities with different stability factors as mentioned in the current study also [60,63]. Glyphosate derivative was prepared after the computational studies which have shown easy biodegradation and low toxicity [63]. Moreover, the detection of pesticides and their metabolites through the spectroscopic methods is still an unexposed area, especially of OPs including the titled molecules, except glyphosate [51–54,64]. At the same time, computational or theoretical analysis may fill the gap, especially to analyse the properties which are experimentally tough to perform.

6. Safety

Acephate, glyphosate, monocrotophos and phorate are organophosphates that inhibit the activity of cholinesterase. Direct contact with these should be avoided. The work performed with these pesticides in the open should take place in a fume hood using gloves and eye protection.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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