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Photoresponsive polymers based on coumarin moiety for the controlled release of pesticide 2,4-D

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We report an excellent photoresponsive controlled release formulation based on coumarin copolymer for pesticide 2,4-D. In the present work, acrylate and polyethylene glycol (PEG) based coumarin photoresponsive polymers were synthesised. The newly synthesised coumarin based polymers exhibited dual functionalities, namely "fluorophore" and "phototrigger" for controlled release of pesticide 2,4-D. Fluorescence property of coumarin based polymers helped us to monitor the release of 2,4-D from polymeric formulation. Release of pesticide by coumarin based polymers was acheived on exposure to UV light. TGA results indicated that coumarin polymeric encapsulated pesticide have good thermal stability than free pesticide 2,4-D. Further leaching experiment also showed that polymeric encapsulated pesticide leaches slowly than free pesticide 2,4-D. Bioassay studies in plant suggest that coumarin polymeric encapsulated pesticide efficiently delivered 2,4-D inside the plant tissues (pumpkin plant *Cucurbita maxima.*) improving its herbicidal activity. Our results indicated that use of fluorescent coumarin polymer based delivery device for controlled release of pesticide by light holds great interest for field application.

1. Introduction

The development of polymeric controlled-release pesticide formulations for regulated release and systemic application of pesticides has a strong potential to greatly increase the sustainable use of pesticides for plant protection. The advantages of polymeric controlled-release pesticide formulations includes i) stabilization against environmental degradation by heat, air, humidity or microorganisms, ii) decreases dosage, evaporation, leaching which in turn reduces environmental pollution, iii) ease of handling of harmful crop protecting agents, reduce irritation of the human mucous-membrane and lower phyto-toxicity, iv) lowering the mobility of the biocides in the soil and reducing their residues in the food chain and v) longer application intervals.[1-8]

There are mainly two broad categories, physical and chemical combinations in polymer based controlled release formulations. In physical combination the active agent is either encapsulated or heterogeneously dispersed or dissolved in polymeric material. Further the active agent is released through diffusion and/or erosion of capsule wall. In chemical combination the active agent is chemical attached to a natural or synthetic polymer by a specific chemical bond, either *via* an ionic or covalent linkage. The active material, which is attached to the polymeric substrate by a specific chemical bond, is released by slow degradation of the polymer itself or through cleavage of the active agent-polymer linkage by reagents or bio-reagents in the environment. It is apparent that the majority of literature published to date for controlled release of pesticides from polymer by chemical cleavage is mainly based on two classes of external stimuli pH and temperature.[9,10]

Currently, several classes of responsive stimuli are in use like ultrasonic field, light, magnetic field, electric field and enzyme action.[11-18] Among them light stimulus attracted much attention since they allow precise control over the release including location, timing and dosage.[19] Hence light activated polymers could be, in principle, useful carriers for agrochemicals. The well-known polymers responsive to light include azobenzene groups incorporated in methylcellulose and spiropyran-containing photo-responsive polymers, such as poly-(acrylic acid) (PAA), poly(2-hydroxypropyl methacrylate) (PHPMA), and Poly(N-isopropylacrylamide) (PNIPAM).[20-29] Zhang *et al.* synthesized photoresponsive template using azobenzene-containing molecularly imprinted polymer microspheres and further demonstrated the applicability of the template for photoregulated release of pesticide 2,4-D.[30]

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Coumarin-based photoresponsive releasing systems are well known for photoregulated release of active molecules.[31-34] Recently, coumarin-based polymeric systems have been extensively utilized as photoresponsive carrier for several active ingredients because of their strong fluorescence and efficient photorelease ability. In 2011 Jin *et al*. reported a coumarin based polymeric photo-triggered as a delivery device for anti-cancer drug 5-Fluorouracil.[35] Further Chung et al synthesised photoresponsive coumarin stabilised polymeric nanoparticles as a detectable drug carrier.[36] Recently, we also synthesised Coumarin containing star shaped 4-arm-polyethylene glycol for dual treatment of photodynamic therapy and chemotherapy.[37] To date the use of coumarin-based photoresponsive polymeric systems for controlled release of pesticide is unexplored. Hence, we thought to develop coumarin based photoresponsive polymeric formulation for controlled release of pesticide.

In the current study, we have synthesised photoresponsive coumarin polymers based on acrylate and polyethylene glycol (PEG) for controlled release of 2,4-D. Photophysical and photocontrolled release ability of coumarin based polymers was investigated. Further the stability and leaching ability of coumarin polymeric encapsulated pesticide 2,4-D was studied in comparison to free pesticide 2,4-D. Finally, we also investigated the herbicidal effect of coumarin–2,4-D polymers at different concentration against Pumpkin plant (*Cucurbita maxima*.).

2. Experimental section

2.1. Materials

Ethyl acetoacetate, 2,4-Dichlorophenoxyacetic acid, resorcinol, molecular bromine, triethylamine, acrolyl chloride, diethyl azodicarboxilate (DEAD), dimethyl sulphate, ethyl chloroformate, acetonitrile, and methanol were purchased from Merck. DMSO-d_6 was purchased from Sigma-Aldrich. Double distilled water was used in this experiment. All the stock solutions were kept in the refrigerator prior to use.

2.2 Instruments

^{1}H NMR (400 MHz) spectra were recorded on a BRUKER-AC 400 MHz spectrometer. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (deuterodimethyl sulfoxide: 2.54 ppm). Data are reported as follows: chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (Hz). ^{13}C NMR (100 MHz) spectra were recorded on a BRUKER-AC 400 MHz spectrometer with complete proton decoupling. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 77.0 ppm). Chromatographic purification was done with 60-120 mesh silica gel (Merck). For reaction monitoring, precoated silica gel 60 F254 TLC sheets (Merck) were used. UV/vis absorption spectra were recorded on a Shimadzu UV-2450 UV/vis spectrophotometer and fluorescence emission spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer. FT-IR spectra were recorded on a Perkin Elmer RXI spectrometer. High-resolution mass spectra were recorded using Qtof MicroYA263 mass spectrometer.

2.3. Experimental methods

2.3.1. Synthesis of coumarin–2,4-D conjugate (2)

We have synthesized photoremovable protecting group 4-bromomethyl-7-hydroxy-chromen-2-one (**1**) following our earlier reported procedure.[29] Next, treatment of PRPG (**1**) with 2,4-D in dry DMF in presence of potassium iodide and potassium carbonate provided coumarin–2,4-D conjugate (**2**) in good yield (80%).

2.3.2. Synthesis of acrylate based coumarin–2,4-D conjugate monomer (2a)

In a two neck round bottom flask coumarin–2,4-D conjugate (**2**) (1 mmol) and triethylamine (1.1 mmol) were dissolved in dry THF and the reaction mixture was cooled at 0° C for 15 min. To the reaction mixture acryloyl chloride (1.2 mmol) dissolved in 3 mL dry THF was slowly added for 30 min. The reaction mixture was stirred for 1 h at 0° C and then at room temperature for 12 h. Consumptions of starting material was monitored by TLC. After completion of the reaction THF was removed and ethyl acetate was added to it. The organic layer was then washed with 20 mL 1(N) HCl solution and dried over $MgSO_4$. Removal of organic solvent under reduced pressure resulted acrylate based coumarin–2,4-D conjugate monomer (yield 72 %) (**2a**).

Cream-colored solid, mp: 130-135 °C; UV–vis (MeOH 50: Water 50): λ_{max}(ε $M^{-1}cm^{-1}$) 280 (0.8×10^4), 320 (1.4×10^4); FTIR (KBr) υ_{max} (cm^{-1}): 1735, 1724, 1616, 1223, 1160, 1100, 1066, 804; ^{1}H NMR (DMSO–d_6, 400 MHz): δ 7.79 (d, 1H, *J* = 8.8 Hz), 7.56 (d, 1H, *J* = 2 Hz), 7.38 (s, 1H), 7.32 (dd, 1H, *J* = 8.8, 2 Hz), 7.22-7.17 (m, 2H), 6.57 (d, 2H, *J* = 12 Hz), 6.45-6.39 (m, 1H). 10.4), 6.18 (d, 1H, J = 12 Hz), 5.49 (s, 2H), 5.16 (s, 2H). ^{13}C NMR (DMSO–d_6, 100 MHz): δ 168.1, 164.0, 159.7, 154.0, 153.1, 152.4, 149.6, 134.7, 129.6, 128.3, 127.6, 126.3, 125.6, 122.7, 118.9, 115.2, 115.1, 112.5, 109.3, 65.7, 62.1.

2.3.3. Synthesis of acrylate based coumarin–2,4-D polymer (3a)

Monomer **2a** (0.15 gm, 0.334 mmol) was taken in a 25 mL round bottom flask equipped with magnetic stirring bar and dissolved in 2 mL 1,4–dioxane. The reaction mixture was degasified with N_2 for 30 min. AIBN (5.5 mg, 0.03 mmol) in 20 μL of 1,4–dioxane was added to the reaction mixture. The reaction mixture was stirred for 6 h at 70 °C temperature under inert atmosphere. After completion, the reaction vessel was immerged in liquid nitrogen to quench the reaction and diluted with THF. Product was precipitated from methanol/hexane mixture for two times to remove the unreacted monomer, filtered and dried in vacuum oven for 24 h (yield 68 %).

UV–vis (MeOH 50: Water 50): λ_{max}(ε $M^{-1}cm^{-1}$) 283 (0.7×10^4), 322 (1.3×10^4); FTIR (KBr) υ_{max} (cm^{-1}): 1748, 1734, 1615; ^{1}H

NMR (DMSO–d_6, 400 MHz): δ 7.87-7.7.62 (br, 1H), 7.58-73 (m, 1H), 7.38-7.25 (br, 1H), 7.22-7.08 (br, 3H), 6.55-6.37 (bs, 1H), 5.41 (bs, 2H), 5.15 (bs, 2H), 1.73 (bs, 1H), 1.37 (bs, 2H).

2.3.4. Synthesis of PEG based coumarin–2,4-D polymer (3b)

In a 50 mL two neck round bottom flask PEG–monomethyl ether (M_n – 2000) was dissolved in dry DCM. To the PEG solution triphosgene (1 mmol) and coumarin–2,4-D conjugate (**2**) (1 mmol) dissolved in dry DCM was added. The reaction mixture was then degasified by bubbling with nitrogen gas for 30 min. After degasification the reaction mixture was sonicated for 30 min. A solution of diethyl azodicarboxylate (DEAD) in dry DCM was added slowly to the reaction mixture and reaction mixture was further sonicated for 40 min. The product was purified by precipitation in cold diethyl ether for three times for complete removal of unreacted monomer. The purified product (**3b**) was then dried in vacuum oven overnight (yield 70 %).

UV–vis (MeOH 50: Water 50): $\lambda_{max}(\varepsilon\ M^{-1}cm^{-1})$ 282 (0.6×10^4), 319 (1.2×10^4); FTIR (KBr) υ_{max} (cm^{-1}): 2889, 1756, 1705, 1638, 1466, 1343, 1280,1241, 1114; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.34 (d, 2H, *J* = 1.2 Hz), 7.14 (d, 2H, *J* = 7.4 Hz), 6.79 (d, 2H, *J* = 9 Hz), 6.23 (s, 1H), 5.34 (s, 2H), 4.80 (s, 2H), 3.95-3.86 (m, 2H), 3.78-3.55 (br, 170H), 3.35-3.28 (m, 14H), 2.99-2.47 (br, 9H).

2.3.5. Characterization of acrylate based coumarin–2,4-D polymer (3a)

Molecular weights and polydispersity of polymer **3a** was recorded by gel permeation chromartography (GPC) and the corresponding results are presented in **Figure S1**. GPC was carried out at ambient temperature by using a Viscotek-GPC system equipped with two GMH HR-H non polar organic columns in series. THF was used as eluent at a flow rate of 1mL/min. For calibration polystyrene standards in the M_n range of 2000-39, 500 were used. The polymers were also characterized by UV, IR and ^{1}H NMR spectroscopy.

2.3.6. Photophysical properties of coumarin–2,4-D polymers (3a–b)

The UV–vis absorption and emission spectra of degassed 5×10^{-5} M solution of polymers (**3a–b**) in MeOH:Water (50:50) were recorded. The Stokes' shift has been calculated from the difference in the absorption and the emission maxima of the coumarin–2,4-D polymers. Fluorescence quantum yield of the polymers (**3a–b**) was calculated using the equation (1).

$$(\Phi_f)_P = (\Phi_f)_{ST} \frac{(\mathrm{Grad}_P)}{(\mathrm{Grad}_{ST})} \frac{(\eta_P^2)}{(\eta_{ST}^2)} \qquad (1)$$

Where, the subscript 'P' and 'ST' denotes polymer and standard respectively. Quinine sulphate in 0.1 (N) H_2SO_4 solutions was taken as standard.[38] Φ_f is fluorescence quantum yield; *Grad* is the gradient from the plot of integrated fluorescence intensity *vs* absorbance, and η the refractive index of the solvent.

2.3.7. Thermogravimetric analysis of 2,4-D and coumarin–2,4-D polymers 3a-b

Thermal decomposition characteristics of coumarin–2,4-D polymers (**3a–b**) were investigate through thermogravimetric analysis. They are thermally decomposed on a PerkinElmer Redcroft 870 thermal analyzer under inert nitrogen atmosphere at linear temperature heating rate 10 °C min^{-1} over a thermal range of 25–600 °C. Samples (8–10 mg) were loaded in alumina pans and used for analysis.

2.3.8. Leaching experiment of 2,4-D and photoresponsive polymers based on coumarin–2,4-D conjugate (3a–b)

The leaching of pesticide (2,4-D) and photoresponsive polymers (**3a–b**) through a thin layer soil was carried out in a Buchner funnel with a diameter of 8 cm.[39] A thin layer of soil (loam soil) weighing 50 g was deposited on a Whatman filter paper in a funnel. The tested compound weighing 1 mg *(M_0)* was applied top of the soil in the funnel and then the soil was covered with another piece of Whatman filter paper. The funnels were irrigated by 40 mL of water at 2 h intervals. Each sample was irrigated for a total of eight times. After each irrigation, the leachate was collected and analyzed by fluorescence spectrometry. The amount of compound leached each time *(M_t)* was calculated with respect to the initial concentration of the compound *(M_0)*. For each sample leaching test was performed in triplicates.

2.3.9. Stability of coumarin–2,4–D polymers (3a–b) under dark

To check the stability of the coumarin–2,4–D polymers (**3a–b**), the suspensions of the polymers individually in EtOH 20: Water 80 (1.5×10^{-4} M) were incubated at 35 °C in the dark for 2 weeks, individually. At regular interval of time (2 days) we carried out UV–vis and fluorescence spectroscopy of the incubated solution and analyzed with respect to 0 day.

2.3.10. Photolysis of coumarin–2,4-D polymers (3a–b) in aqueous ethanolic solution

To check the photorelease of 2,4-D from coumarin–2,4-D polymers (**3a–b**), 5×10^{-5} M solution of individual polymer (**3a–b**) were prepared in aqueous ethanolic [EtOH:Water (20:80)] solution. The polymer solution was exposed under UV–vis light (310 and 350 nm) individually, using a 125 W medium pressure Hg lamp filtered by suitable filter with continuous stirring. At regular intervals of irradiation, 3 mL of the reaction mixture was taken and analyzed using UV–vis and fluorescence spectroscopy. Based on UV–vis spectroscopy data for each caged compounds, we plotted natural logarithm of the concentration of caged compound (ln*C*) versus irradiation time. We observed an exponential correlation for the disappearance of polymers which suggested a first order reaction. Further, the quantum yields for the photolysis of polymers were calculated using equation (2).

$$(\Phi_p)_P = (\Phi_p)_{act} \frac{(k_p)_P}{(k_p)_{act}} \frac{(\eta_{act})}{(\eta_P)} \quad (2)$$

Where, the subscript 'P' and 'act' denotes polymer and actinometer respectively. Potassium ferrioxalate was used as an actinometer.[40] Φ_p is the photolysis quantum yield, k_p is the photolysis rate constant and η is the fraction of light absorbed.

2.3.11. Bioassay of 2,4-D and coumarin–2,4-D polymers (3a–b)

The pumpkin plant (*Cucurbita maxima.*) seeds were obtained from local market in Midnapore, West Bengal, India. The seeds were surface sterilized with 95% ethanol and 2.5% sodium hypochlorite and were washed several times with distilled water, dried and stored in sterile condition in closed container before being used in the treatment. Pumpkin plant (*Cucurbita maxima.*) is an important vegetable plant of the world. Pumpkin fruit is a powerhouse of nutrients such as a highly valued omega 3 fatty acids, unsaturated fatty acids, high protein, beta-carotene, vitamin E and minerals. Pumpkin has powerful antioxidants, antidiabetic and anticancer properties. Pumpkin plant tended to be expressed preferentially in the root and shoot system of ten-day-old plant and it is also ease of handling.

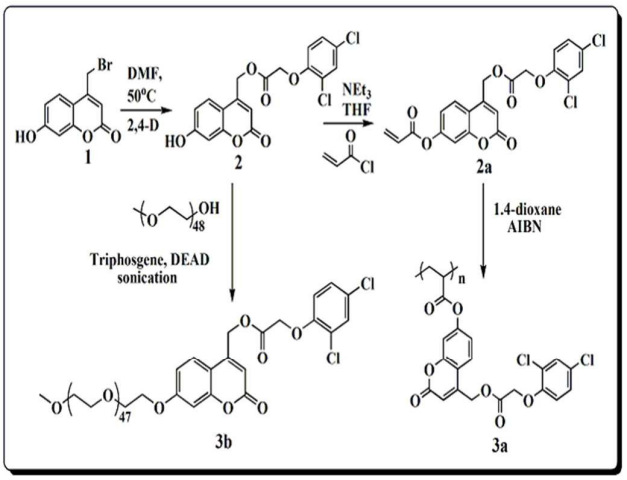
For bioassay study two sets of experiments were designed. In experimental set-1, effect of different concentrations (10^{-4} to 10^{-7} M) of 2,4-D in pumpkin plant was studied. The root and shoot length was measured by using centimeter-scale at regular time interval such as every 2 days. Morphological study (lateral root and root hair formation) of pumpkin plant was also observed. After completion of experimental set-1, experimental set-2 was conducted by using 10^{-4} to 10^{-7} M polymers (**3a–b**) to understand the effect of released 2,4-D on growth of the root and shoot length of pumpkin plant at a stipulated time interval.

The stock solution (20 mL) was prepared as follows: required amount of test compounds were measured and dissolved in ethanol and then mixed with distilled water, individually. Similar-sized seeds (10 nos.) of germinated pumpkin seeds were selected and were put on to petri dish containing Whatman No. 1 filter paper moisten with distilled water, supplemented with 10 mL of stock solution of test compound. Control plants were grown in similarly prepared petri dishes during each experiment. For control experiment 10 mL distilled water was used instead of 10 mL of stock solution of test compound. The Petri dishes were incubated in the constant temperature-light room. The plants were grown for 10 days at 32 °C in a 14 h photoperiod with a light intensity of 70 µmol photon m^{-2} s^{-1} provided by fluorescent lamps. All the petri dishes were exposed to direct sunlight for 2 h (9.30 to 11.30 am) every day throughout the experiment period. Each sample was moistened with an equal volume of distilled water for daily watering.

Each treatment was replicated thrice. The data were analyzed by analysis of variance of shoot and root length using ANOVA followed by Duncan's multiple range tests to delineate the treatment means using SPSS computer software. The percentage stimulation or inhibition activity was assessed in comparison with the control experiment.

3. Results and Discussion

3.1. Synthesis of acrylate and PEG based coumarin–2,4-D polymers



Scheme 1 Synthesis of monomer (2a) and acrylate based coumarin–2,4-D polymer (3a) and PEG based coumarin-2,4-D polymer (3b).

Acrylate based coumarin–2,4-D conjugate monomer (**2a**) was synthesized as outlined in **Scheme 1**. First, photoremovable protecting group (PRPG) 4-bromomethyl-7-hydroxy-chromen-2-one (**1**) was synthesized following our earlier reported procedure.[29] Next, we synthesized coumarin–2,4-D conjugate (**2**) by treating PRPG (**1**) with 2,4-D in dry DMF in presence of potassium iodide and potassium carbonate.[30] The monomer **2a** was synthesized by slow addition of THF solution of acroloyl chloride to the ice cooled reaction mixture of coumarin–2,4-D conjugate (**2**) and triethylamine in dry THF (**Figure S1)**. Polymer **3a** was synthesized from monomer **2a** using 1,4-dioxane as a solvent and AIBN as an initiator under inert atmosphere. After synthesis the polymer was characterized by ^{1}H NMR and GPC spectroscopy (**Figure S2-S3)**.

PEG based coumarin–2,4-D polymer (**3b**) was synthesized from **2** as shown in **Scheme 1**. To the DCM solution of PEG–monomethyl ether (M_n–2000), triphosgene and coumarin–2,4-D conjugate (**2**) were added. The reaction mixture was then degasified by bubbling with nitrogen gas for 30 min. After degasification the reaction mixture was sonicated for 30 min. A solution of diethyl azodicarboxylate (DEAD) in dry DCM was added slowly to the reaction mixture and the reaction mixture was further sonicated for 40 min. The purified product was then dried in vacuum oven for overnight. The polymer was characterized by UV and ^{1}H NMR spectroscopy (**Figure S4)**.

3.2. Photophysical properties of coumarin–2,4-D polymers (3a–b)

The UV–vis absorption and emission spectra of degassed 5 × 10^{-5} M MeOH : Water (50:50) solution of coumarin-2,4-D polymers (**3a** and **3b**) were represented in **Fig. 1** and the results are summarized in **Table 1**. UV–vis spectra showed two intense absorption bands centered in the region of λ = 322–326 nm and λ = 280 nm (**Fig. 1.A**). Both the polymers exhibited similar type of UV–vis absorption spectrum. Further the emission spectra (**Fig. 1.B**) showed that the polymers (**3a–b**) are fluorescent in nature with maximum emission wavelength at around 470 nm and the magnitude of the Stokes' shift in the range of 150–155 nm with moderate fluorescence quantum yields (0.13 < Φ_f < 0.15).

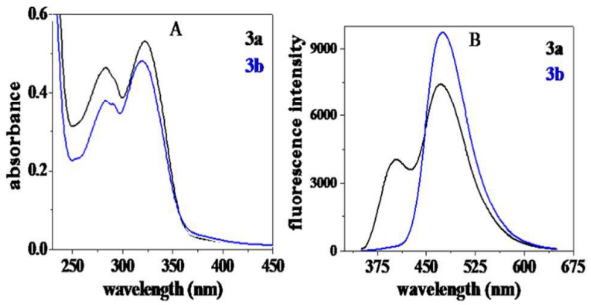


Fig. 1 (A) UV–vis absorption and (B) emission spectra of the 5 × 10^{-5} M solution of polymers (**3a** and **3b**) in MeOH : Water (50:50).

Table 1. UV–vis and fluorescence data for coumarin-2,4-D polymers (**3a–b**)

Coumarin–2,4-D polymer	UV–vis		Fluorescence		
	λmax (nm)a	ε^b	λmax (nm)^c	Stokes' shift(nm)^d	$\Phi_f{}^e$
3a	322	1.3	472	150	0.15
3b	319	1.2	474	155	0.13

[a]Maximum absorption wavelength. [b]Molar absorption coefficient (10^4 $M^{-1}cm^{-1}$) at the maximum absorption wavelength. [c]Maximum emission wavelength. [d]Difference between maximum emission wavelength and maximum absorption wavelength. [e]Fluorescence quantum yield (error limit within ± 5%) were calculated using quinine sulphate as standard (Φ_f = 0.54 in 0.1 N H_2SO_4).

3.3. Thermogravimetric analysis (TGA) of 2,4-D and coumarin–2,4-D polymers (3a–b)

The weight loss TGA curves of coumarin–2,4-D polymers (**3a–b**) and free 2,4-D were represented in **Fig. 2**. By comparing the thermograms, it is evident that coumarin–2,4-D polymers volatilizes at a higher temperature compared to 2,4-D. 50% of the sample of coumarin–2,4-D polymers **3a** and **3b** were lost at higher temperature 326 °C and 306 °C, respectively in comparison to pure pesticide 2,4-D at 247 °C. The above experiment indicates polymerization of 2,4-D decreased the degree of vaporization.

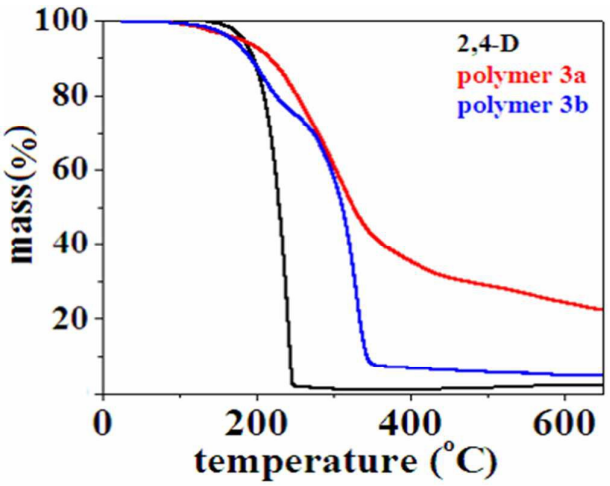


Fig. 2 Thermograms of 2,4-D and coumarin–2,4-D polymers (3a–b).

3.4. Leaching experiment of 2,4-D and coumarin-2,4-D polymers (3a–b)

Leaching through a thin soil layer was used to evaluate the pattern of leaching of free 2,4-D and coumarin–2,4-D polymers. **Fig. 3** indicates that caging of 2,4-D by polymer decrease the leaching ability of free 2,4-D. After eight irrigations of the soil, we observed 2,4-D to be completely leached, while its coumarin–2,4-D polymers (**3a–b**) were leached only in the range of 35–70%. Among the coumarin–2,4-D polymers, **3a** showed less leaching potential since polymer **3a** are less soluble in water and highly adsorbed in the soil.

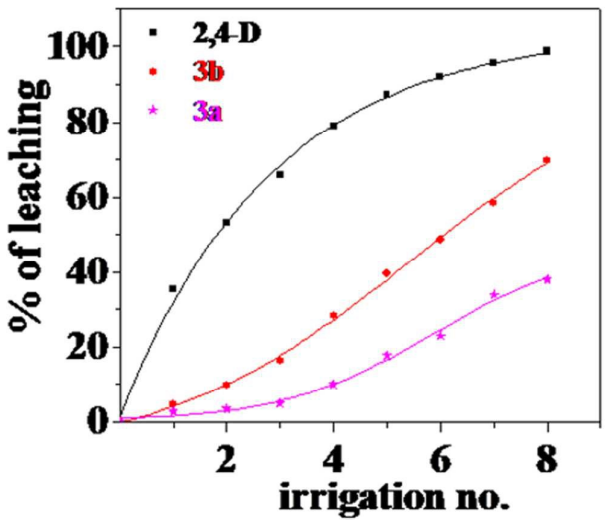


Fig. 3 Plot of % of leaching of 2,4-D and coumarin–2,4-D polymers (3a–b).

3.5. Stability of coumarin–2,4-D polymers (3a–b) under dark

The stability of the coumarin–2,4-D polymers was tested by keeping them in EtOH: Water (20:80) at 35 ˚C in dark for a

period of 2 weeks. From the UV–vis and fluorescence spectroscopy, we observed insignificant photodecomposition of polymers **3a** (~5%) and **3b** (~8%), which proves that the coumarin–2,4-D polymers are stable under the dark condition.

3.6. Photolysis of coumarin–2,4-D polymers (3a–b)

Irradiation of coumarin–2,4-D polymers (**3a–b**) in EtOH:Water (20:80) at two different wavelengths (≥ 310 and 350 nm) resulted in controlled release of 2,4-D (**Table 2**). We monitored the course of photorelease of coumarin–2,4-D polymers (**3a–b**) using UV–vis and fluorescence spectroscopy (**Fig. 4** and **Fig. 5**). The decrease in the intensity of the absorption and emission maxima indicates the decomposition of the coumarin-2,4-D polymers to their corresponding photoproducts (**Scheme 2**). In each case, the photolysis was stopped when conversion reached at least 90% (as indicated by UV–vis spectroscopy). For polymers mentioned in **Table 2**, the photoproduct 2,4-D was confirmed by isolating and matching with ^{1}H NMR spectra of authentic sample. Further, the results indicated that the photolytic rate constant of polymers **(3a-b)** decreases as the irradiation wavelength increases. (incident intensity (I_0) = 1.25 ×10^{17}, 9.661× 10^{16}, photons $s^{-1}cm^{-1}$ for 310 nm and 350 nm respectively).

Table 2. Photolytic data of coumarin–2,4-D polymers (**3a–b**) at different irradiation wavelengths in EtOH:Water (20:80)

Coumarin–2,4-D Polymer	≥ 310 nm			≥ 350 nm		
	ε^a	$k_p{}^b$	$\Phi_p{}^c$	ε^a	$k_p{}^b$	$\Phi_p{}^c$
3a	0.7	0.259	0.015	0.4	0.086	0.010
3b	0.6	0.540	0.020	0.3	0.197	0.012

[a]Molar absorption coefficient (10^4 M^{-1} cm^{-1}) at the irradiation wavelength. [b]Rate constant (10^{-3} min^{-1}) under photolytic conditions. [c] Photochemical quantum yield (error limit within ± 5%).

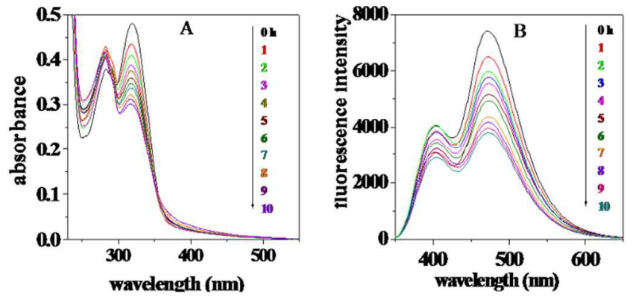


Fig. 4 (A) Absorption and (B) emission spectrum of 3a in EtOH:Water (20:80) solvent system under different irradiation time using UV light (≥ 350 nm).

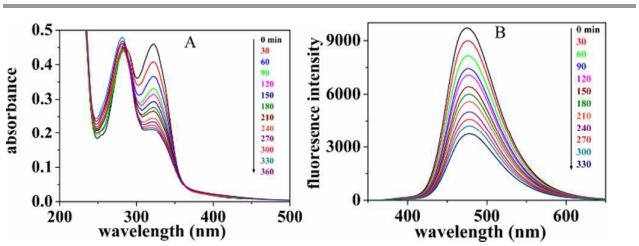
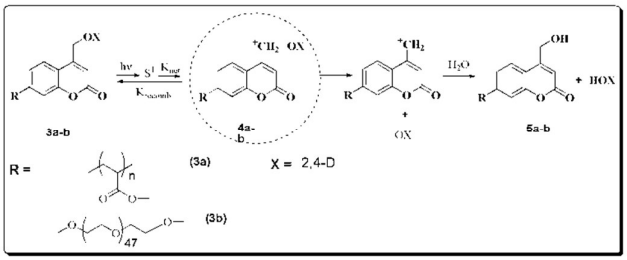


Fig. 5 (A) Absorption and (B) emission spectrum of **3b** in EtOH:Water (20:80) solvent system under different irradiation time using UV light (≥ 350 nm).



Scheme 2 Photorelease mechanism of coumarin-2,4-D polymers .

Coumarin-2,4-D polymers on excitation proceeds through singlet excited state followed by either direct heterolytic C–O bond cleavage or homolytic cleavage of C–O bond followed by electron transfer to produce the ion pair as shown in **Scheme 2**. Finally, in aqueous solvent the resulting ion pair escapes from the solvent cage and produce the corresponding photoproducts (2,4-D and hydroxylmethylcoumarin **5a-b**).

3.7. Bioassay of 2, 4-D, and coumarin–2,4-D polymers (3a–b)

After successful demonstration of photorelease of 2,4-D, bioassay experiments were conducted to investigate the effect of pesticide 2,4-D and polymers (**3a–b**) on the morphology of shoot and root length of pumpkin plant (*C. maxima*). Pumpkin seedlings were grown in the presence of different concentrations, ranging from 10^{-4} to 10^{-7} M of 2,4-D and polymers (**3a–b**), individually. The results on the shoot and root growth inhibition of pumpkin plant under different concentration, during 10 days experiments are represented in **Fig. 6, Table S1 and Table S2, ESI†**. Coumarin–2,4-D polymers (**3a–b**) displayed root growth inhibition at four different concentration (10^{-4}–10^{-7} M). Both at 10^{-4} and 10^{-5} M concentrations, root growth was found to be highly inhibited by coumarin–2,4-D polymers (**3a–b**). While 10^{-6} and 10^{-7} M concentration, polymers showed delayed inhibition of main root elongation, but promotes lateral root formation.

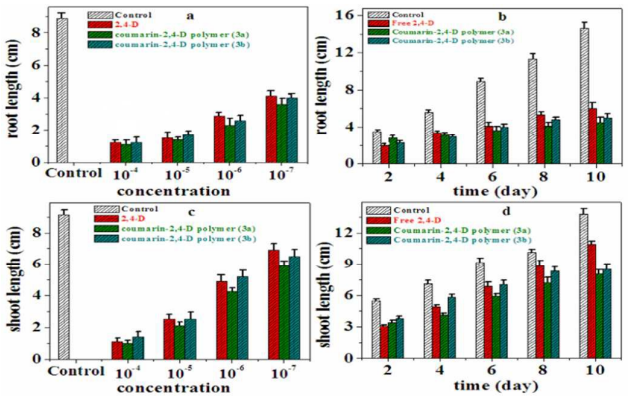


Fig. 6 (a and c) Effect of 2,4-D and polymers (3a–b) of concentration 10^{-4}–10^{-7} M) for 6 days, **(b and d)** Effect of 2,4-D and polymers (3a–b) of concentration 10^{-7} M for 10 days on the root and shoot length of C. Maxima.

Further at 10^{-6} M concentration, coumarin–2,4-D polymers (**3a–b**) showed lower root length inhibition compared to 2,4-D at initial days (up to 6 days). But after 10 days of experiment, coumarin–2,4-D polymers showed an improved root length inhibition compared to free 2,4-D indicating the controlled release of 2,4-D. Moreover similar inhibition trend was also noted at 10^{-7} M concentration (**Fig. 6.a** and **Fig. 6.b**). The similar effect was also observed in case of shoot length growth experiment (**Fig. 6c** and **Fig. 6.d**). **Fig. 7 & 8** represented the effect of polymer **3b and 3a** on the shoot and root growth of pumpkin plant.

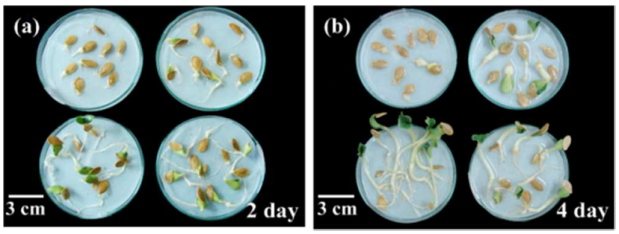


Fig. 7 Effect of different concentration (10^{-4}–10^{-7}M) of polymer 3b on *C. maxima* irradiation under sunlight (clockwise from upper to lower 10^{-4}–10^{-7}M).

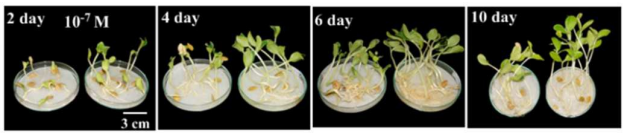


Fig. 8 Effect of 10^{-7} M of polymer 3a on *C. maxima* after regular time intervals of irradiation under sunlight (In each set left side represents treatment and right side control).

Thus, the 2,4-D-polymer serves as a effective controlled release formulation for the pesticide 2,4-D.

4. Conclusions

We have developed polymeric controlled-release formulations for pesticide 2,4-dichlorophenoxyacetic acid (2,4-D) using acrylate and polyethylene glycol (PEG) based coumarin–2,4-D polymers. Photophysical studies revealed that coumarin–2,4-D polymers are fluorescent in nature. Both acrylate and polyethylene glycol (PEG) based coumarin–2,4-D polymers were shown to release 2,4-D in aqueous ethanolic solvent by UV light (≥ 310 and 350 nm). Leaching and TGA experiment showed that coumarin–2,4-D polymers have very good thermal stability and less leaching property compared to free 2,4-D. Further, we also demonstrated the herbicidal effect of coumarin–2,4-D polymers at different concentration against *C. maxima* plant. Results of 10 days bioassay experiment revealed that at low concentration, coumarin–2,4-D polymers were active than their corresponding free 2,4-D.

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