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Efficient Herbicide Delivery through a Conjugate Gel Formulation for the Mortality of Broad Leaf Weeds

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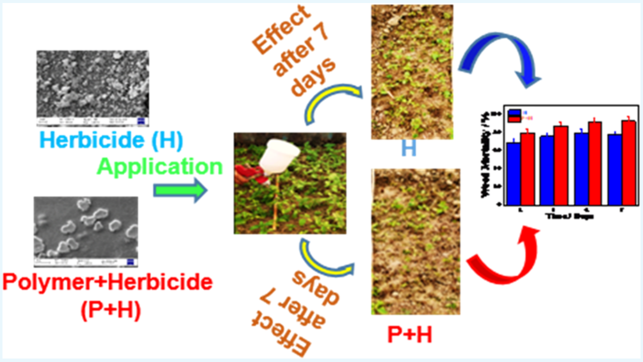
ABSTRACT: Carfentrazone-ethyl is embedded in guar gum to prepare a polymer−herbicide conjugate gel formulation for a sustained release of the active ingredient (a.i.). The sprayable gel formulation was optimized at 0.5% (w/v) concentration. Strong interactions of the prepared composition of the polymer−herbicide conjugate system are shown through spectroscopic techniques, depicting the peak broadening of hydrophilic −OH bonds in the herbicide at 1743 cm^{-1}, shifting to 1730 cm^{-1} in the polymer−herbicide sample. There is a broadening and shifting of the peak at 329 nm for the n → π^* transition at 335 nm in the polymer−herbicide conjugate system in UV spectra. Differential scanning calorimetric measurements show a lowering of endothermic melting peaks to 242 and 303 °C in the polymer−herbicide conjugate. X-ray diffraction studies showed a sharp diffraction peak of the pure polymer at a 2θ of ~20.3°, while broadening and shifting of the peak position to a 2θ of ~20.8° were observed after adding the herbicide. Diffusion of the active ingredient in the polymer−herbicide conjugate resulted in much greater coverage (most of the weed leaf stomata (>95%)) than conventional spraying. The efficacy of both the polymer−herbicide formulation and herbicide at different doses in weed nurseries showed significantly higher weed mortality in *Anagallis arvensis* (95.4%), *Chenopodium album* (~97%), and *Ageratum conyzoides* (93.16%) treated with the polymer−herbicide formulation @ 20 g a.i. ha^{-1}. Narrow SPAD readings range of *A. arvensis* (0.1−30.6) and that of *C. album* (0−5) were observed in the polymer−herbicide formulation @ 20 g a.i. ha^{-1} was at par with the conventional formulation @ 30 g a.i. ha^{-1}. Less regeneration in a weed nursery of *A. arvensis* (27%), *C. album* (77%), and *A. conyzoides* (49%) treated with gel formulations @ 20 g a.i. ha^{-1} was observed, which was significantly lower than those in conventional herbicides.



1. INTRODUCTION

Different tools and technologies have been employed in agriculture to increase productivity so as to meet the food demand of the ever-increasing population. The production and quality of agricultural produce are governed by technological, biological, and environmental factors, among which the judicious use of agricultural practices plays a key role in fetching higher yields. To achieve the global food security of 9 billion population by 2050, a sustainable increase in food production is required on an urgent basis.[1] The global food supply was greatly increased during the green revolution period but the excess and inappropriate use of the farm inputs to achieve higher production, particularly herbicides, resulted in the addition of toxic chemicals to soils, surface, and groundwater, thereby endangering life and life-supporting systems.[2] On the other hand, crop production is adversely affected, resulting in a yield reduction of up to 66% if the weeds are not controlled at the critical stages of crops.[3−5] Application of a single herbicide does not control all types of weeds, and its continuous use may lead to weed shift and the development of herbicide resistance.[6] The continuous use of conventional herbicides to control grassy weeds, the shift from conventional tillage (CT) to zero tillage (ZT), and the negligence toward broad leaf weed management in wheat resulted in a shift in weed flora.[7] An effective control of the narrow leaf weeds (*Phalaris minor* Retz.) was observed to a large extent in ZT wheat as compared to CT wheat, but the population of broad leaf weeds has increased simultaneously.[8] However, a post-emergence herbicide, carfentrazone-ethyl, is reported to have good control of broad leaf weeds present in the agricultural field and industrial and utility areas.[9] These broad leaf weeds are being controlled by employing the conventional methods in agricultural farmlands and in the

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cropped areas, which in turn is having an ill effect on both the environment and on the economy of farmers on account of the loss of 90% of the applied herbicide through volatilization, runoff, and erosion, affecting the ecosystem with increased application costs. Weed control, however, becomes the major input cost of crop production mainly in the cereal growing belt of the subcontinent. When these herbicides are applied, employing the conventional methods, there is photolytic, hydrolytic, or microbial action, which causes leaching, evaporation loss, and degradation of the herbicides. As a result, the efficiency of pesticides is greatly reduced and the pesticide active ingredient is inhibited from carrying out its purpose on the target species.[10]

To avoid these losses and to enhance the efficiency, the deposition and coverage of the herbicide droplets on the target interface should be improved in agricultural production.[11] In the case of hydrophobic or superhydrophobic weeds having low surface free energy including that of the waxy layer, mastoid process, and villi, it is quite difficult to enhance the droplet dispersion and examine their behavior on the leaf surface.[12,13] However, encapsulating the herbicides into the polymers may help in the controlled release of the formulation's active ingredient, thereby enhancing the effectiveness and reducing the adverse effects due to excess drug supply.[14,15] Remarkable thermal, mechanical, and environmental characteristics are offered by the bio-nano-composites. There are favorable interactions observed between the chemical and the polymer matrix on account of homogeneous dispersion. This exfoliation/intercalation is proven to be a fundamental factor for strengthening their characteristics.[16,17] The balance of enthalpic and entropic factors influence the nature of nanofiller dispersion in the polymer matrix and is used to characterize the thermodynamics of the mixing nanofiller and polymer.[18] The dispersion of nanofiller is achieved from the favorable thermodynamics of mixing and the melting temperature, and the heat of fusion shifts to a lower temperature due to strong interactions in polymer nanocomposites. The surface area of the nanofiller is thought to be sufficient for causing the entropic rise.[16]

Controlled release formulations (CRFs) combine biologically active agents and excipients, mainly a polymer that controls the release of agents over a predetermined span.[19,20] This delivery system permits the availability of an active agent to a particular product targeted to achieve a significant effect within the time frame and therefore may act as an alternative to the conventional method of herbicide delivery.[14] The controlled release system not only aims to alleviate the adverse side effects of the application rate on the environment but also sustains the potential herbicidal efficiency.[21] In addition, a satisfactory efficacy of herbicide at a constant active ingredient is observed for a longer period on the weeds, and the dose is reduced owing to the need for a lower amount of active ingredient for effective biological activity, thereby curtailing the weed control costs. When the controlled delivery technology is utilized in agriculture, it not only diminishes the excessive effect of the conventional techniques but also promotes judicious utilization of agrochemicals or biocides by exposing an effective concentration over a given period, which lessens the residual effect of herbicides, resulting in a decrease of costs for farmers and for companies.[22]

In the present investigation, a conjugate gel formulation of broad leaf weed herbicide carfentrazone-ethyl is prepared by embedding it in the biodegradable polymer, i.e., guar gum, to enhance the efficiency of herbicides on broad leaf weeds and control weeds in the wheat field and other manifested areas. Further, the characterization of the polymer−herbicide gel formulation, pristine polymer, and conventional herbicide is studied through spectroscopic, thermal, and structural analysis. The bioefficacy of the newly prepared formulation at different doses has been studied in three different broad leaf weeds separately for 2 years against the conventional herbicide delivery with different doses and a control.

2. MATERIALS AND METHODS

2.1. Establishment of Weed Nurseries and Experimental Design. The present experiment was conducted in the agricultural research farm of Banaras Hindu University, Varanasi (25°18′ N latitude and 83°30′ E longitude), U.P., India, during rabi seasons of 2019 and 2020. Broad leaf weeds like *Anagallis arvensis* L., *Chenopodium album* L., and *Ageratum conyzoides* L., which generally infest the wheat field, were selected as representative plants. The nursery of *Anagallis arvensis* L. was established on a total area of 420 m^2, where the area of each nursery plot was 4 m by 5 m, which was replicated three times using a randomized block design (RBD) and repeated in time with seven treatments comprising different concentrations of conventional broad leaf herbicide and polymer−herbicide (broad leaf herbicide) conjugate formulation and a control, where any weed management measures were prohibited. Similarly, each of the nurseries of *Chenopodium album* L. and *Ageratum conyzoides* L. was grown separately in a 420 m^2 area using a randomized block design (RBD) with seven different treatments consisting of herbicidal doses and a control and were replicated three times and repeated for different times. The seven treatments applied in the nursery of all three broad leaf weeds comprised three different concentrations of carfentrazone-ethyl @ 10 g a.i. ha^{-1} (gram active ingredient per hectare), 20, and 30 g a.i. ha^{-1}; three different concentrations of carfentrazone-ethyl + guar gum gel formulation @ 10, 20, and 30 g a.i. ha^{-1} and a control, where no weed control measures were taken. While establishing the nursery, the seeds of *C. album* were broadcast, whereas the rest of the two weed nurseries were prepared by transplanting the seedlings of *A. arvensis* and *A. conyzoides* in the respective plots. The weeds were allowed to grow to three to five leaf stages before different concentrations of conventional herbicides and the polymer−herbicide conjugate gel formulation was dispersed in the respective weeds nurseries, and the observations were recorded for two seasons.

2.2. Broad Leaf Herbicide. Carfentrazone-ethyl(ethyl-(RS)-2-chloro-3-{2-chloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl]-4-fluorophenyl}-propionate), a contact herbicide of the aryl triazolinone group, was used to control broad leaf weeds in the present investigation through a foliar application, and after being absorbed through leaves, the translocation was restricted. It controls the broad leaf weeds by inhibiting protoporphyrinogen oxidase (PPO) enzyme, resulting in cell death in these target weeds. It is used as a post-emergence herbicide in cereals like wheat, barley, oats, triticale, etc. Carfentrazone-ethyl is also known to cause foliar injury to crops; however, the crops recover from this injury within a short span of time. It is available in the form of 40 and 50% DF (dry flowable). Carfentrazone-ethyl will now be abbreviated as "herbicide" and, henceforth, will be expressed as "H". The conventional herbicide (carfentrazone-ethyl) was prepared by mixing

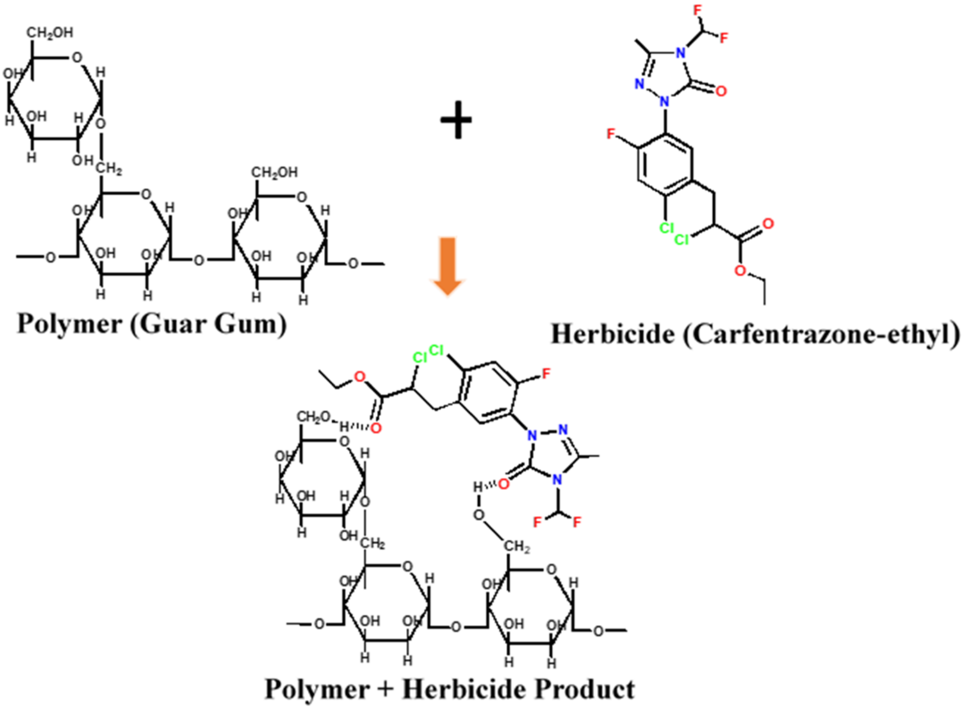

Figure 1. Schematic of the chemical structure of the polymer and herbicide with possible interaction sites to form a polymer−herbicide conjugate.

different recommended doses of broad leaf herbicide for the required area in water, and then the solution was sprayed in all of the plots of weed nurseries of all of the three different weeds.

2.3. Guar Gum as a Biopolymer. Gum (Guar gum powder, Hindustan Gum & Chemicals Ltd., Bhiwani, Birla Colony, Haryana 127021, India) derived from guar beans is a galactomannan polysaccharide (density ranges between 0.8 and 1.0 g mL^{-1}; acidity pK_a of 5−7; thixotropic above 1% concentration) has thickening and stabilizing properties. It is water-soluble and is a better emulsifier than other gums on account of more galactose branch points. Guar gum is not affected by ionic strength or pH as it is nonionic; however, it degrades at low pH with a moderate temperature (pH ∼ 3 at 50 °C). Guar gum will be named as "polymer" in this manuscript and will be abbreviated as "P".

2.4. Preparation of a Polymer−Herbicide Conjugate Formulation. The herbicide gel formulation was prepared by embedding the synthetic herbicide (carfentrazone-ethyl 40% DF @ 10, 20, and 30 g a.i. ha^{-1}) into the biodegradable polymer (guar gum) through a solution route and will be abbreviated as "P + H". To prepare the formulation, initially, a known amount of biodegradable guar gum powder was dissolved in aqueous medium in a 100 mL beaker, resulting in the concentration range of 0.25, 0.50, and 0.75% (weight/volume (w/v)). To prepare a control solution, recommended doses of herbicide @ 10, 20, and 30 g a.i. ha^{-1}, respectively, were dispersed in an aqueous solution through probe sonication for approximately 30 min at room temperature. Thereafter, both the solutions of polymer in aqueous medium and solutions of different doses of herbicide were mixed with the help of magnetic stirring on the digital hot plate at room temperature for 1 h. The concentration of the prepared formulation was optimized at various ratios to develop a sprayable solution for efficient applications through different types of conventional sprayers used by the farmers. The film was formed through the solution casting method in which the polymer−herbicide conjugate formulation of 5% (weight/weight (w/w)) was prepared through magnetic stirring on a hot plate. This solution was then poured into a petri dish and was kept in a vacuum oven for overnight drying at 60 °C for 24 h. After the solution in the petri dish was completely dried, a film was formed, which was removed later from the petri dish with the help of a tweezer. This vacuum-dried film was then used for further analysis and examinations. The schematic of the polymer−herbicide interaction is shown in Figure 1.

2.5. Morphological Studies. A scanning electron microscope (SEM) (SUPRA 40, Zeiss SEM) was used to investigate the surface morphology of pure broad leaf weed, herbicide solutions sprayed over weed leaf (@ 20 g a.i. ha^{-1}) and polymer−herbicide conjugate (@ 20 g a.i. ha^{-1}) sprayed over weed leaf, followed by Pd−Au alloy coating. Under this investigation, a variety of signals were produced by the accelerated electrons after coming in contact with the samples. Further, these different signals produce different topologies and morphologies of the sample surfaces. The morphology of the pure polymer and herbicide particles was taken as control. The effect of spraying the herbicide conjugate over the leaf was also observed macroscopically through digital photography.

2.6. Spectroscopic Investigation. The qualitative estimation of pristine polymer, broad leaf herbicide, and the polymer−herbicide conjugate gel formulation was determined through light absorption techniques where these samples were exposed to the electromagnetic radiation of UV−visible, which measures the electronic transition in the range of 200−800 nm wavelengths with a scan rate of 200 nm min^{-1} using a Jasco V-650 spectrometer. The FTIR spectrum of these specimens was recorded with the help of a Thermo Scientific Nicolet Fourier transform infrared (FTIR) spectrometer in ATR mode fitted with a diamond crystal in the range of 600−4000 cm^{-1} by taking 100 scans with a resolution of 4 cm^{-1}.

2.7. Thermal Measurements. The thermal behavior of the specimen was examined through differential scanning calorimetry (DSC) with the help of a Mettler-Toledo 832 instrument using automated STARe evaluation software, which measured the melting temperature (T_m) as well as the heat of fusion (ΔH) of the specimen in the temperature range of −50 to 350 °C with a constant heating rate of 10° min^{-1} under an inert atmosphere. Calibration of the instrument was carried out using the standard In/Zn before recording the specimen thermograms.

2.8. XRD for Structural Analysis. The crystalline structures and quantification of the conversion of the phase fraction of the polymer, herbicide, and polymer−herbicide gel formulation were determined using the X-ray powder diffraction technique, where Cu Kα acts as the source of monochromatic X-ray radiation of wavelength, λ = 0.154 nm under fixed current and voltage supply from the generator. The specimens were placed on the sample holder made of quartz at room temperature. This analyzer scanned the specimen at a diffraction angle (2θ) from 2 to 40° at a scanning rate of 3° min^{-1}.

2.9. Optimization of the Polymer−Herbicide Formulation. The polymer−herbicide conjugate gel formulations of different doses @ 10, 20, and 30 g a.i. ha^{-1} were prepared at different concentrations of 0.25, 0.5, and 0.75% (w/v) through a solution route. These prepared formulations were optimized by spraying on different leaves of French bean (*Phaseolus vulgaris* L.) to obtain the optimized sprayable concentration using a conventional sprayer. The different doses of polymer−herbicide formulations and those of conventional herbicides were sprayed at these different concentrations initially with the help of a compressed air sprayer (paint gun sprayer) calibrated at 3−4 bar (45−60 PSI) with a 1.4 mm standard nozzle and a cup capacity of 600 mL and thereafter with a conventional sprayer so as to perform the comparative study of the retention period of each concentration on the leaf surface.

2.10. Mortality of Weeds. The weed mortality percentage was determined from three different randomly selected areas in each of the seven experimental plots of all three broad leaf weed nursery plots at regular time intervals for a period of 20 days in both seasons. To compare the killing effect of different doses of polymer−herbicide gel formulation @ 10, 20, and 30 g a.i. ha^{-1} and herbicide aqueous solution @ 10, 20, and 30 g a.i. ha^{-1} on weed population, the total number of weeds before the application of the treatments and the number of weeds that survived after the application of different treatments were recorded in all three weed nursery experimental plots. The weed mortality percentage was calculated for each randomly selected area in each treatment using the following formula[23]

$$\text{weed mortality (\%)} = \frac{W_t - W_s}{W_t} \times 100 \tag{1}$$

where W_t is the total number of weeds before the application of treatment and W_s is the number of weeds that survived after treatment application; the mean value was then observed. The data recorded were then subjected to ANOVA as per statistical methods.[24] The pictures of the effect of broad leaf herbicide and polymer−herbicide gel formulations on the weed mortality of experimental plots of each *Anagallis arvensis* L., *Chenopodium album* L., and *Ageratum conyzoides* L. were captured using a smartphone camera.

2.11. SPAD Reading. The Soil Plant Analysis Development (SPAD) chlorophyll meter of Minolta Camera Company (Japan) was used to estimate the SPAD readings range of all of the broad leaf weeds from their respective nursery, treated with different doses of carfentrazone-ethyl @ 10, 20, and 30 g a.i. ha^{-1} and herbicide−polymer gel formulation sprayed @ 10, 20, and 30 g a.i. ha^{-1} over weed leaves by measuring the transmission of red and infrared light through weed leaves with the help of two light-emitting diodes (650 and 940 nm) and a photodiode detector. SPAD units, which are proportional to leaf chlorophyll content were measured by placing the SPAD (502) portable chlorophyll meter on the weed leaf lamina, and subsequently, the average of 10 readings per experimental plot was used to determine the SPAD units of all of the treated plots. The SPAD readings were then subjected to ANOVA.[24] SPAD readings were recorded in the treated weed plots and in the control plot from the 5th day onward when the herbicide effect was prominently visible after the herbicide aqueous solution and polymer−herbicide gel formulation were sprayed over the weed leaves.

2.12. Anatomical Studies. The efficacies of the herbicide (carfentrazone-ethyl) aqueous solution and that of the polymer−herbicide gel formulation were also determined through the anatomical study of leaf, root, and stem of *A. conyzoides* grown in the nursery under a polarizing optical microscope (POM), Leica. The weed samples were collected from the experimental plots of the nursery showing significant results, which were treated with carfentrazone-ethyl @ 20 g a.i. ha^{-1}, herbicide−polymer gel formulation sprayed @ 20 g a.i. ha^{-1} and that of control. Three weeds were selected randomly and then uprooted from these experimental plots of the nursery of *A. conyzoides* on the third day after the treatment effect was significantly visible. The cross sections of the root, stem, and leaf were prepared using a razor blade and were then treated with a sodium hypochlorite solution (50%), resulting in the decolorization of the cross sections.[25] These cross sections were stained with methylene blue[26] after being washed with distilled water and were then placed onto the histological slides prepared from common plant anatomy procedures.[27,28]

3. RESULTS AND DISCUSSION

3.1. Dose Optimization of the Herbicide−Polymer Conjugate Formulation for Application on Broad Leaf Weeds.

Various polymer concentrations have been employed to optimize the appropriate polymer−herbicide conjugate for its application in fields. The polymer−herbicide gel formulation at 0.25% (w/v) concentration formed comparatively smaller droplets when sprayed through a conventional sprayer; however, it was less viscous and the film formation after a 4 h observation period was not so prominent, as shown in Figure S1. In the case of 0.75% (w/v) gel formulation, the formation of smaller droplets through a conventional sprayer was very difficult due to the higher viscosity of the solution formed. A similar observation has been recorded, where the viscoelastic properties of the solution changed with the addition of a guar gum-based polysaccharide,[29] thereby efficiently reducing the spray droplet to ≤150 μm.[30] Smaller droplets were formed easily with the help of a paint gun sprayer attached to a compressor (pressure supplied 2.5 bar), and the spraying was smoothly carried out. The polymer−herbicide gel formulation was optimized at 0.5% (w/v) concentration as the formulation, which was easily sprayable using a conventional sprayer at this concentration. It was found that the gel formulation, after being sprayed over the leaves of *P. vulgaris*, formed smaller droplets, which further spread out and covered a larger area on the leaf surface. The gel formulation @ 10, 20, and 30 g a.i. ha^{-1} creates a film and is retained for a longer time on the leaf surface, whereas the herbicide aqueous solution (conventional herbicide) @ 10, 20, and 30 g a.i. ha^{-1} sprayed on the leaf evaporated just after a period of half an hour. The film formed on the leaf surface using a conjugate was visible in the form of patches when the observation was made 4 h later (Figure 2).

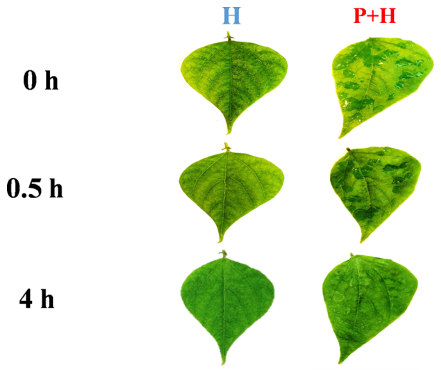

Figure 2. Optimization of dose at 0.5% w/v concentration and analysis of retention of herbicide (H) and polymer−herbicide conjugate gel formulation (P + H) immediately after spray (0 h), after half an hour, and after 4 h from the time of application of herbicide aqueous solution (D) and polymer−herbicide gel formulation (P + H). Patches of polymer films over the leaf are evident in the P + H system.

The polymer−herbicide gel formulation produced sticky droplets, which spread uniformly covering a larger weed leaf area, thereby enhancing the wetting of the leaf surface due to the presence of the polymer. The leaf surface was covered with nearly cuticular polymer films, thus improving the foliar retention and herbicide penetration. The polymer−herbicide gel formulation does not get evaporated easily as compared to the herbicide aqueous solutions at different doses, which in turn promotes herbicide uptake. The retention period of the herbicide was increased by embedding it within the biopolymer, which generates a sticky gel as compared to the aqueous solution of conventional herbicide. The retentivity of spray solution of pesticide is said to be based on spray properties as well as on the intrinsic wetting property of the leaf surface. The retention of water droplets and that of pesticides on crops and nontarget plants to a large extent depends on the wetting ability of the leaf surface.[31] This smart delivery system when combined with the active ingredient of the herbicide through the polymer−herbicide gel formulation helps in the reduction of the herbicide dose in controlling the broad leaf weeds. The reduction in size allows easy absorption of these active ingredients on the soil particles, thereby preventing the growth of weeds, which developed resistance in the case of conventional herbicides.[32] The off-target movement of the herbicide in the adjacent plots was controlled by the use of guar gum, a polysaccharide commonly used as a drift control agent (DCA).[33]

3.2. Morphological Study. The surface morphological investigation was carried out by scanning electron microscopy of the polymer (P), herbicide (H), and polymer−herbicide conjugate (P + H) in the film form. Figure 3b shows the polymer−herbicide, herbicide, and polymer aqueous solution sprayed over the leaves of *A. conyzoides*. Now, it is evident that most of the stomata (>95%) are covered with the biodegradable polymer−herbicide conjugate as opposed to most stomata open in the case of a conventional spray of herbicide solution. The stomata are mainly responsible for the gaseous exchange in the process of photosynthesis in plants.

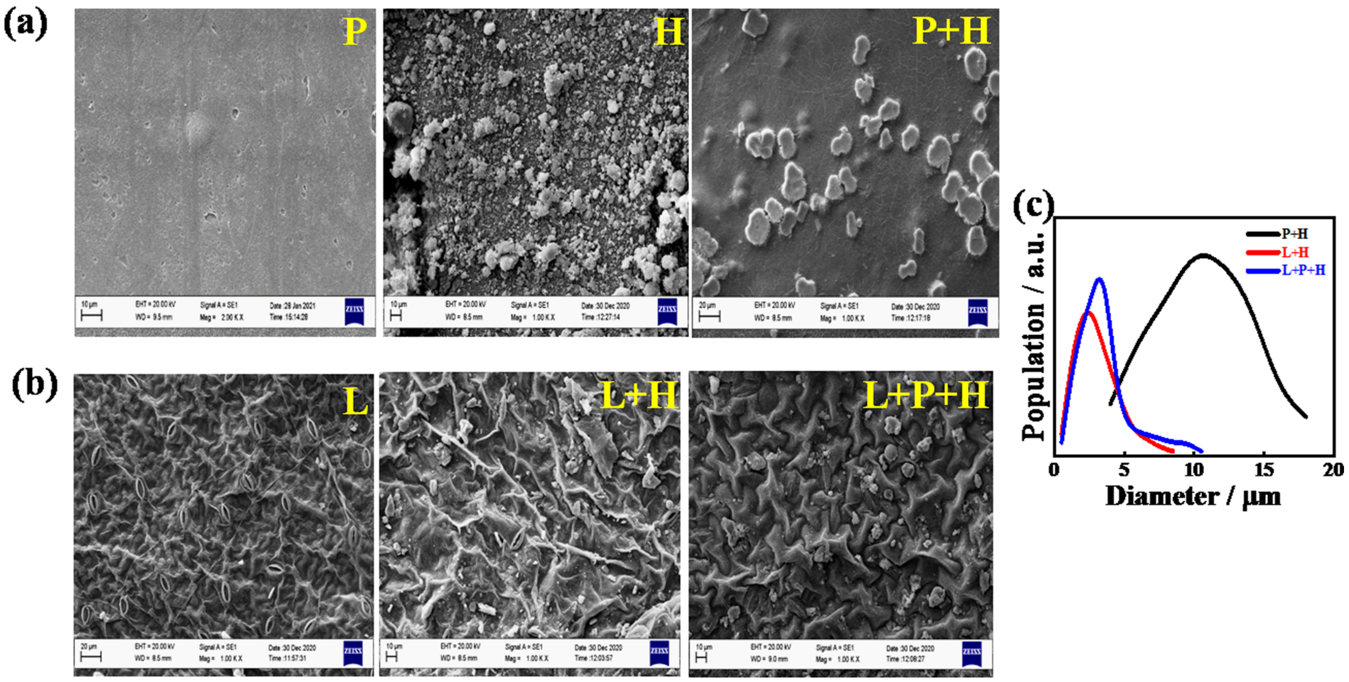

Figure 3. Morphological investigation: (a) SEM images of the pure polymer (P), pure herbicide (H), and the polymer−herbicide conjugate (P + H) film; (b) SEM images of pure weed leaf (L), herbicide dispersed weed leaf (L + H), and polymer−herbicide dispersed on weed leaf (L + P + H); and (c) distribution of the herbicide particles with and without weed leaf against pristine herbicide.

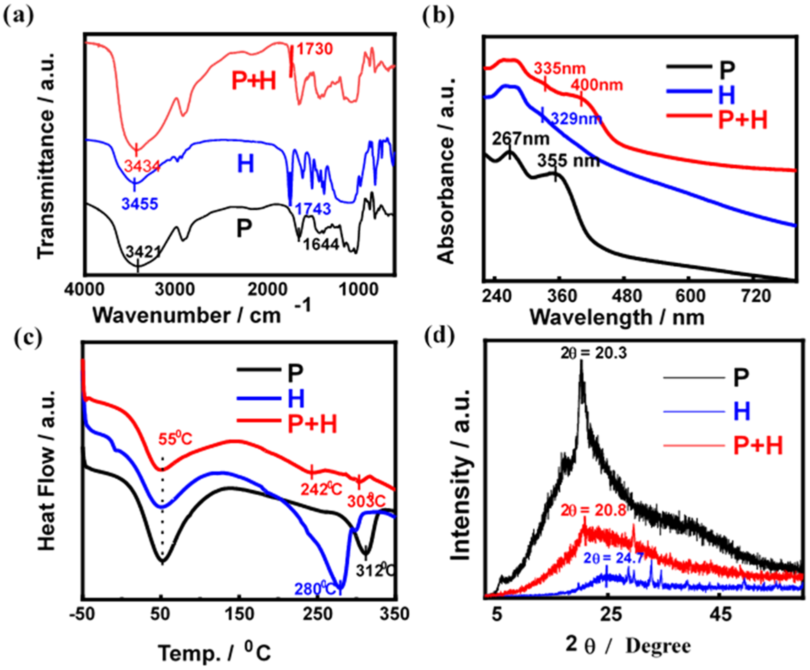

Figure 4. (a) FTIR spectra of the indicated specimens; the vertical lines indicate the position of the peak position; (b) UV−vis spectra; (c) DSC thermograms of samples showing the peak position; and (d) X-ray diffraction pattern (XRD) of the polymer (P), herbicide (H), and polymer−herbicide (P + H) conjugate.

Hence, the active ingredient diffusion on the weed leaf by herbicides blocked the photosynthesis process, thereby killing the weed plants. It is clear that the diffusion of the active ingredient from the polymer−herbicide conjugate on the weed leaf is much greater than the active ingredient diffusion from conventional spraying. So, the biodegradable polymer−herbicide conjugate solution is more effective than the pure herbicide aqueous solution. A similar result was found in the optical microscope images (Figure 3a−c). This is to mention that clear stomata are observed in pure weed leaf as usual. The distribution and dimension of the herbicide particles with and without weed leaf were analyzed through ImageJ software (Figure 3c) and found that the herbicide particle size in the P + H film was of a wider range of 5−20 μm size. The broad range of distribution became narrow (0−10 μm) when herbicide particles and polymer−herbicide particles were sprayed over the weed leaf. It is noticed that the polymer−herbicide conjugate is better dispersed and uniform on the weed leaf and covered most of the stomata as compared to only herbicide dispersion on the leaf presumably because of the formation of the polymer−herbicide gel formulation on the leaf surface.

3.3. Polymer−Herbicide Interactions and Structural Alteration.

Polymer−herbicide interactions were confirmed through spectroscopic measurements like UV−vis and FTIR, and structural modifications were examined through DSC and XRD studies. In IR spectra, the change (shift) in the vibration frequencies indicates the polymer−herbicide interactions (Figure 4a). The hydrophilic −OH bond vibrational stretching frequencies of the polymer, herbicide, and polymer−herbicide conjugate were observed at 3421, 3455, and 3434 cm^{-1}, respectively, and the peak broadening occurred in the polymer−herbicide conjugate specimen. This shift and broadening were primarily due to the hydrogen bonding between the herbicide and the polymer, as shown in Figure 4a.[34,35] The carbonyl stretching in the herbicide sample was observed at 1743 cm^{-1}, which shifted to 1730 cm^{-1} in the polymer−herbicide conjugate sample, and the shifting of ∼13 cm^{-1} indicates the strong interactions of the prepared composition of the polymer−herbicide conjugate. Further, Figure 4b shows the UV−visible spectra of pristine biodegradable polymer, herbicide, and polymer−herbicide conjugate. Pure polymer film shows two sharp transitions at 267 and 355 nm due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively. On the other hand, pure herbicide exhibited the electronic transitions at 269 and 329 nm, respectively.[36] Considerable broadening and shifting of the peak at 329 nm for the $n \rightarrow \pi^*$ transition occurred at 335 nm in the polymer−herbicide conjugate. The red shift and broadening of the peak indicate a strong interaction between the polymer and herbicide molecules. Moreover, the polymer peak at 355 nm also shifted to 400 nm, indicating stronger interactions of the herbicide with the polymer chain.

Differential scanning calorimetric (DSC) measurements of the specimens were performed to understand the melting (T_m) behavior and heat of fusion (ΔH). The endothermic peak at a lower temperature (∼55 °C) indicates the loss of water molecules (moisture loss) in all specimens. A pure biodegradable polymer exhibits an endothermic peak (T_m) at 312 °C, assigned to melting, having a heat of fusion (ΔH) of 53.1 J g^{-1}, while a pure herbicide shows the peak at 280 °C with a heat of fusion (ΔH) of 37.1 J g^{-1} (Figure 4c). The endothermic melting peaks decreased to 242 and 303 °C in the polymer−herbicide conjugate having a corresponding heat of fusion values of 4.9 and 53.1 J g^{-1}, respectively.[34,35,37] The lowering of the endothermic peak (melting temperature) along with a lower heat of fusion indicates the strong polymer−herbicide interactions, which influence the overall properties of the conjugate as compared to pure components. Further, structural changes were analyzed using the X-ray diffraction studies, and the patterns are shown in Figure 4d. In a pure polymer, a sharp diffraction peak was obtained at a 2θ value of ∼20.3°, while with the addition of a herbicide to the polymer

Table 1. Effect of Different Doses of the Herbicide and the Polymer−Herbicide Conjugate System on the Mortality of *Anagallis arvensis* L

treatments[a]	weed mortality (%)					
	day 1	day 2	day 3	day 4	day 6	day 7
H @ 10 g a.i. ha^{-1}	25.7	48.3	52.7	59.6	64.7	72.3
H @ 20 g a.i. ha^{-1}	40.9	71.4	75.2	77.1	80.5	87.6
H @ 30 g a.i. ha^{-1}	54.0	80.0	85.7	92.0	92.3	94.9
P + H @ 10 g a.i. ha^{-1}	33.3	50.7	57.0	64.0	65.7	77.7
P + H @ 20 g a.i. ha^{-1}	52.2	69.3	77.2	81.8	88.6	95.4
P + H @ 30 g a.i. ha^{-1}	60.3	75.3	89.6	89.7	96.0	96.6
control	0.0	0.0	0.0	0.0	0.0	0.0
SEM ±	2.3	1.9	1.9	2.4	2.2	2.3
C.D. ($P = 0.05$)	7.3	6.1	5.9	7.4	6.7	7.2

[a] H: Herbicide (carfentrazone-ethyl); P + H: polymer + herbicide (carfentrazone-ethyl).

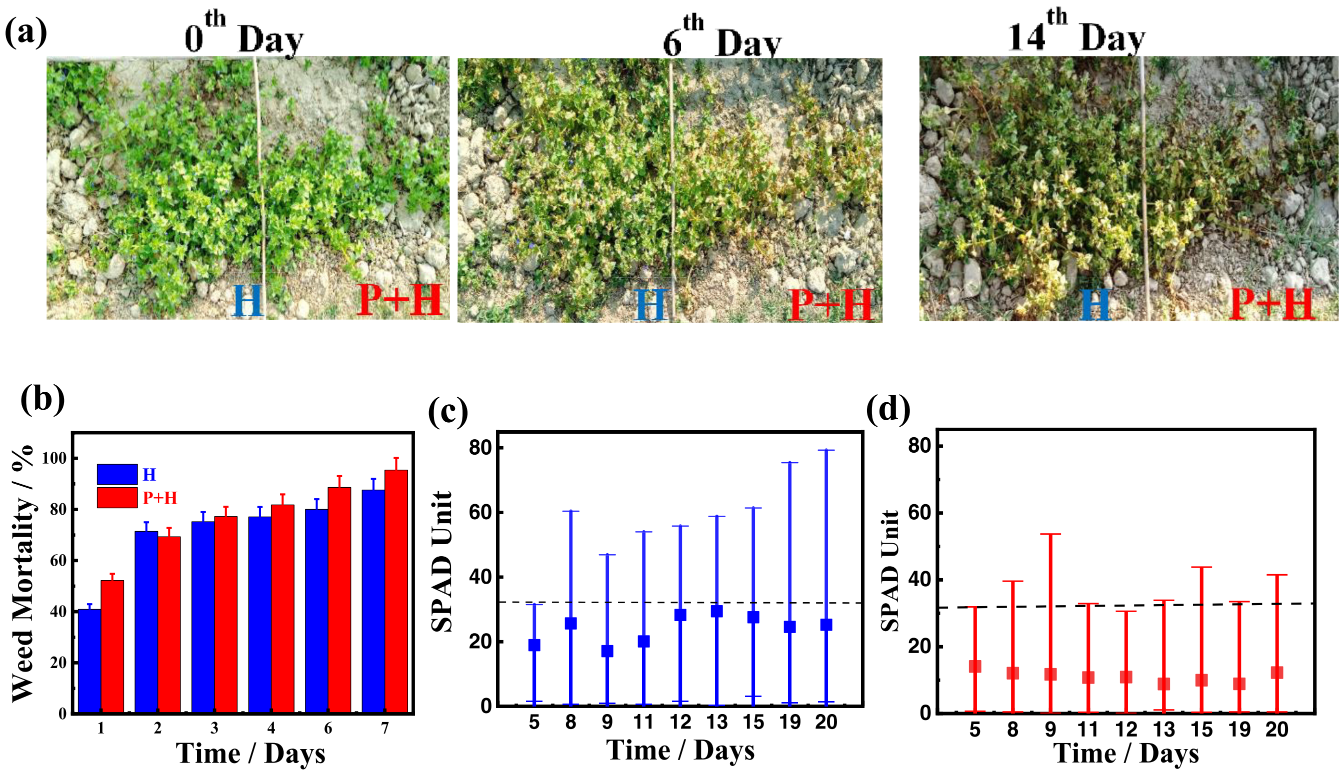

Figure 5. Mortality of *Anagallis arvensis* L. (a) Photographic images showing the relative mortality rate of treatments with a significant result as a function of time using the conventional herbicide @ 20 g a.i. ha^{-1} and polymer−herbicide conjugate spray @ 20 g a.i. ha^{-1} on weed leaves grown in the nursery; (b) weed mortality percentage for both treatments as a function of time; (c) SPAD unit reading of weeds treated with herbicide aqueous solution @ 20 g a.i. ha^{-1}; and (d) SPAD unit reading of weeds treated with polymer−herbicide conjugate gel formulation @ 20 g a.i. ha^{-1}. The horizontal lines indicate the average value of the SPAD unit of healthy weeds of control plots (without any treatment).

matrix, the peak broadened and slightly shifted to a 2θ value of ∼20.8°. In the pure polymer, there is order in the polymer chain due to the extensive hydrogen bonding between various layer structures. However, after mixing the herbicide in the polymer, the sharpness of the XRD peak becomes broad due to the insertion of the herbicide particles in the polymer layer structure due to greater interactions. A few new XRD diffraction peaks also appeared in the conjugate, which correspond to herbicide moieties as observed in the XRD pattern of the pure herbicide. Hence, the polymer−herbicide conjugate formulation is confirmed through spectroscopic techniques, thermal, and XRD studies, which make it versatile for its application.[34,35]

3.4. Effect of the Conjugate System on the Mortality of *Anagallis arvensis* L.

Anagallis arvensis L., a broad leaf weed predominant in wheat fields, was effectively controlled by spraying the polymer−herbicide conjugate gel formulation as compared to a herbicide aqueous solution (conventional herbicide). In the weed nursery, among all of the treatments, the polymer−herbicide conjugate gel formulation @ 30 g a.i. ha^{-1} recorded a significant effect on weed mortality and was at par with that of the conventional herbicide @ 30 g a.i. ha^{-1} up to 7 days after treatments were applied (Table 1). The toxicity symptoms of the herbicide could be observed on the very next day after application in the form of a water-soaked appearance of leaves before wilting, along with a drooping effect. After 6 days from the day of application, a prominent change in the

Table 2. Effect of Different Doses of the Herbicide and the Polymer−Herbicide Conjugate System on SPAD (502) Units of *Anagallis arvensis* L

treatments[a]	SPAD (502) readings								
	day 5	day 8	day 9	day 11	day 12	day 13	day 15	day 19	day 20
H @ 10 g a.i. ha^{-1}	30.8	31.7	27.4	30.6	37.5	37.5	34.6	30.7	34.5
H @ 20 g a.i. ha^{-1}	19.0	25.7	17.1	20.1	28.3	29.4	27.6	24.6	25.3
H @ 30 g a.i. ha^{-1}	13.9	11.5	11.3	11.5	14.0	20.6	24.4	20.6	24.6
P + H @ 10 g a.i. ha^{-1}	22.0	22.5	22.7	21.8	22.5	19.5	20.3	18.5	20.3
P + H @ 20 g a.i. ha^{-1}	14.1	12.1	11.7	10.8	10.9	8.8	10.0	8.9	12.2
P + H @ 30 g a.i. ha^{-1}	11.6	11.6	11.2	9.9	10.7	9.4	9.3	8.5	10.5
control	32.0	30.7	32.1	31.4	30.6	32.9	31.3	31.9	31.6
SEM ±	1.1	1.4	1.0	1.2	1.0	1.3	1.1	1.3	1.4
C.D. (P = 0.05)	3.5	4.4	3.0	3.8	3.1	3.9	3.4	4.0	4.3

[a] H: Herbicide (carfentrazone-ethyl); P + H: polymer + herbicide (carfentrazone-ethyl).

leaf morphology in the form of weed leaf browning and quick death of weeds were observed as the chlorophyll content in the leaves gradually reduced (Figure 5a). Carfentrazone-ethyl is a quick-acting herbicide, which successfully controls the broad leaf weeds in wheat by inhibiting protoporphyrinogen oxidase enzyme[38,39] and disruption of the membrane causing cell death.[40] Further, on the 14th day from the day of application of herbicide and polymer−herbicide gel formulation, weed leaves turned dark brown, thereby reducing weed density and resulting in weed mortality. When the killing effect of both treatments was compared, it was found that the weed mortality using the polymer−herbicide conjugate gel formulation-treated plot was considerably higher as compared to the only herbicide-treated plot on the 4th, 6th, and 14th days after application (Figure 5a). The mortality percentage of *A. arvensis* treated with the polymer−herbicide gel formulation @ 20 g a.i. ha^{-1} was 52.2% on the very next day (1st day) and 95.4% 7 days after application of treatments, which was significantly higher than that with the herbicide aqueous solution @ 20 g a.i. ha^{-1} and was at par with that with the herbicide aqueous solution @ 30 g a.i. ha^{-1} on the very next day (1st day) and 7 days after treatments were applied (Figure 5b, Table 1). The herbicide efficiency against the weeds increased on adding an adjuvant[41] (guar gum); therefore, the polymer−herbicide conjugate gel formulation had a higher edge on weed mortality as compared to the herbicide aqueous solution (conventional herbicide). A similar study reported that if the post-emergence herbicides are applied to the weeds without any adjuvant added to them, then there is either problem of rolling off of the herbicide or retention of the herbicide on the weed leaves without any penetration into the system.[40]

After 5 days in the weed nursery, the herbicide toxicity in the weeds was reported in the form of leaf chlorosis and browning, and SPAD readings of the weeds treated with the polymer−herbicide conjugate gel formulation and herbicide aqueous solution were observed using a SPAD-502 chlorophyll meter. On day 5, SPAD-502 readings in *A. arvensis* treated with the polymer−herbicide conjugate gel formulation @ 20 g a.i. ha^{-1} were in the range of 0.7−31.9, and the average reading was 14.08, which was at par with that of weeds treated with the polymer−herbicide conjugate gel formulation as well as herbicide aqueous @ 30 g a.i. ha^{-1} and was significantly less than that of weeds treated with the herbicide @ 20 g a.i. ha^{-1} with a SPAD reading range of 1.6−31.5 and an average reading of 18.96 (Figure 5c,d, Table 2). The SPAD readings recorded in healthy *Amaranthus vlitus*, a common widespread weed, lie in the range of 7.9−54.2,[42] which is in accordance with the SPAD reading range of 9.8−70.4 of healthy *A. arvensis* grown in the control plot with an average SPAD reading of 32.1, shown by the horizontal line in Figure 5c,d. On the 12th day in the weeds treated with the polymer−herbicide gel formulation @ 20 g a.i. ha^{-1}, there was a narrowing in the range of SPAD readings, and a significantly lowest 0.1−30.6 SPAD reading range was recorded with an average reading of 10.91 against the SPAD reading in the range of 1.5−55.8 with an average reading of 28.28 in the group treated with a herbicide solution @ 20 g a.i. ha^{-1} and was at par with SPAD readings of the polymer−herbicide gel formulation as well as herbicide aqueous solution both sprayed @ 30 g a.i. ha^{-1}. The decline in the average SPAD-502 readings up to the 13th day in the polymer−herbicide conjugate-treated weeds can be attributed to the lowering of the photosynthetic rate, which has a linear correlation with the SPAD readings[42] as well as the leaf chlorophyll content[43] (Figure 5d). Further, on the 20th day, the range of SPAD readings gradually increased in the weeds treated with herbicide @ 20 g a.i. ha^{-1} and recorded a significantly higher range of 1.4−79.3 SPAD readings having an average reading of 25.2 than the SPAD reading range of 0.5−41.5 with an average reading of 12.2 in the polymer−herbicide gel formulation sprayed over the broad leaf *A. arvensis* @ 20 g a.i. ha^{-1}, which in turn was at par with polymer−herbicide gel treatment @ 30 g a.i. ha^{-1}. This increase in the SPAD reading range of *A. arvensis* treated with herbicide aqueous solution @ 20 g a.i. ha^{-1} was mainly due to the regeneration of broad leaf weeds in this herbicide-treated plot (43%), which was significantly higher as compared to regeneration (27%) reported in polymer−herbicide gel formulation dispersed weeds @ 20 g a.i. ha^{-1}, which in turn was at par with polymer−herbicide gel formulation sprayed as well as herbicide-treated weeds both @ 30 g a.i. ha^{-1}, observed 30 days after the application of treatments in the weed nursery, as shown in Figure S5 and Table 5. Similar findings have been reported, where *A. arvensis* weeds treated with carfentrazone-ethyl @ 20 g ha^{-1} showed some recovery as compared to carfentrazone-ethyl + metsulfuron at 25 g ha^{-1} + 0.2% NIS.[44] However, the SPAD reading was significantly lower in all polymer−herbicide conjugate gel-treated weeds as opposed to conventional herbicide spray at different doses, indicating the greater efficacy of the developed system.

3.5. Effect of the Conjugate System on the Mortality of *Chenopodium album* L.

The effect of polymer−herbicide (carfentrazone-ethyl) gel formulation in the weed nursery on the mortality of *Chenopodium album* L., a broad leaf weed forming the complex weed flora of the wheat field, was more

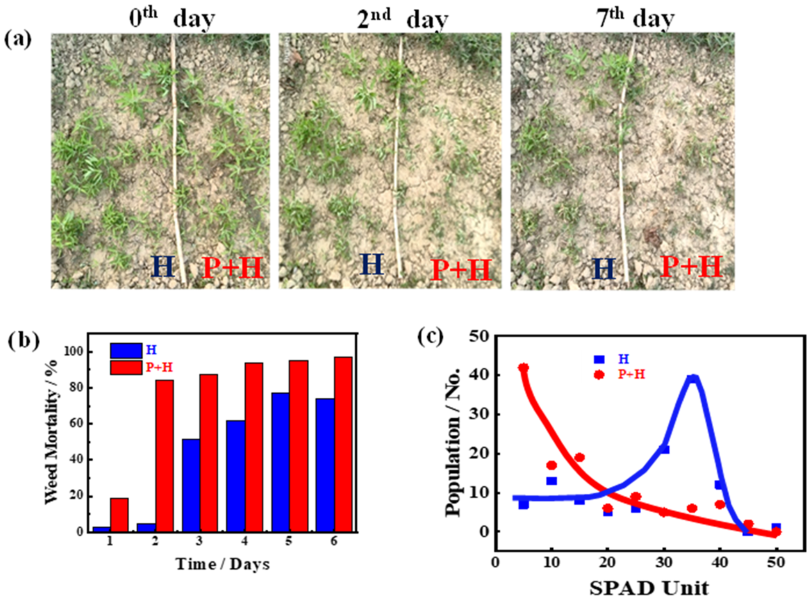

Figure 6. Mortality of *Chenopodium album* L. (a) Photographic images showing the relative mortality of weeds grown in nursery treated with herbicide (H) and the polymer−herbicide conjugate system (P + H) @ 20 g a.i. ha^{-1} having significant differences at indicated treatment times; (b) weed mortality percentage in the indicated system of treatments of herbicide (H) and polymer−herbicide conjugate system (P + H) @ 20 g a.i. ha^{-1} over a period of time; and (c) SPAD unit reading of weeds treated with the conventional herbicide (H) @ 20 g a.i. ha^{-1} and polymer−herbicide (P + H) conjugate gel formulation @ 20 g a.i. ha^{-1}.

prominent as compared to the herbicide aqueous solution (Figure 6a). On the 2nd day after application of the treatments, the number of weeds showing wilting symptoms was higher in all of the polymer−herbicide gel formulation-treated plots as compared to the herbicide aqueous solution-treated weed plots. Browning of leaves, dehiscence of leaves, and death of more number of polymer−herbicide conjugate gel formulation-treated *C. album* were recorded as compared to herbicide aqueous solution-treated weeds in which a slow killing effect was observed on the 7th day after the treatment was applied. Similar findings were reported when the premix of herbicide (carfentrazone-ethyl) + herbicide (metsulfuron) @ 25 g ha^{-1} + 0.2% surfactant provided significant control of *C. album* and other weeds as compared to the sole application of these herbicides.[38]

Table 3. Effect of Different Doses of the Herbicide and the Polymer−Herbicide Conjugate System on the Mortality of *Chenopodium album* L

	weed mortality (%)				
treatments[a]	day 1	day 2	day 3	day 4	day 6
H @ 10 g a.i. ha^{-1}	2.5	3.2	35.33	40	54.66
H @ 20 g a.i. ha^{-1}	3.09	4.5	51.55	61.85	77.32
H @ 30 g a.i. ha^{-1}	21.3	48.7	72.7	79.7	79.66
P + H @ 10 g a.i. ha^{-1}	5.6	9.3	37	47	47
P + H @ 20 g a.i. ha^{-1}	19.05	84.13	87.33	93.65	95.2
P + H @ 30 g a.i. ha^{-1}	29.667	88.7	89.7	94	95.37
control	0	0	0	0	0
SEM ±	0.9	1.7	2.1	2.4	1.9
C.D. (P = 0.05)	2.8	5.3	6.4	7.4	6.0

[a] H: Herbicide (carfentrazone-ethyl); P + H: polymer + herbicide (carfentrazone-ethyl).

On day one, after all of the treatments were applied in the weed nursery of *C. album*, the weed mortality percentage was reported to be significantly lowest (2.5%) in the weeds treated with herbicide aqueous solution @ 10 g a.i. ha^{-1}, which was at par with that of weeds treated with herbicide aqueous solution @ 20 g a.i. ha^{-1} (Table 3). However, weed mortality in plots treated with the polymer−herbicide conjugate gel formulation @ 20 g a.i. ha^{-1} was significantly higher (19.05%) than that in weeds treated with herbicide aqueous solution @ 20 g a.i. ha^{-1} and was at par with that of the conventional spray of herbicide @ 30 g a.i. ha^{-1} (Table 3). A sharp increase in the weed mortality percentage of 84% was observed in weeds treated with the polymer−herbicide gel formulation @ 20 g a.i. ha^{-1}, which was not only at par with that of weeds treated with polymer−herbicide gel formulation @ 30 g a.i. ha^{-1} but was significantly higher than all of the treatments including control. On day 6, a significantly higher weed mortality percentage of ∼97% was recorded in the polymer−herbicide conjugate gel formulation sprayed @ 20 g a.i. ha^{-1} as compared to 74% weed mortality in weeds treated with the conventional herbicide at the same concentration (Figure 6b, Table 3). The weed control efficiency of carfentrazone-ethyl @ 20 g ha^{-1} ranged from 90 to 100% at 60 days' crop stage of wheat.[38]

The SPAD readings of herbicide and polymer−herbicide conjugate gel formulation sprayed on weeds were recorded over 20 days following the application of the different treatments and were plotted against the weed population from the respective experimental plots (Figure 6c). The SPAD readings in the 0−5 range were recorded by the large number (42) of weeds treated with the polymer−herbicide conjugate gel formulation @ 20 g a.i. ha^{-1}, which was at par with that of weeds (45) treated with the polymer−herbicide conjugate gel formulation @ 30 g a.i. ha^{-1} and significantly higher as compared to only ∼7 weeds from the plot treated with the herbicide @ 20 g a.i. ha^{-1} (Table 4). Reduced regeneration of *C. album* and higher weed mortality percentage (42.8%) observed on the 20th day after the application of treatment (Figure S3) showed less greenness in the polymer−herbicide conjugate gel formulation sprayed @ 20 g a.i. ha^{-1} in the plot/

Table 4. Effect of Different Doses of the Herbicide and the Polymer−Herbicide Conjugate System on the SPAD (502) Reading Range of *Chenopodium album* L

	population of weeds (no.)									
	SPAD (502) reading range									
treatments[a]	0−5	5.1−10	10.1−15	15.1−20	20.1−25	25.1−30	30.1−35	35.1−40	40.1−45	45.1−50
H @ 10 g a.i. ha^{-1}	3.0	6.9	5.1	3.5	3.7	28.6	45.5	19.6	7.8	5.3
H @ 20 g a.i. ha^{-1}	7.3	12.9	8.0	5.0	6.0	20.8	38.8	12.0	0.0	1.5
H @ 30 g a.i. ha^{-1}	19.5	17.6	12.8	10.8	9.9	12.9	32.5	10.5	0.0	0.0
P + H @ 10 g a.i. ha^{-1}	32.6	10.5	11.5	4.2	4.6	10.6	26.5	14.7	5.6	2.2
P + H @ 20 g a.i. ha^{-1}	42.4	17.0	19.3	6.0	8.9	4.7	6.0	7.2	1.8	0.0
P + H @ 30 g a.i. ha^{-1}	45.0	20.3	20.8	15.5	11.9	4.5	5.2	5.6	0.0	0.0
control	0.0	4.6	3.9	3.7	3.1	33.9	57.6	44.3	19.8	14.3
SEM ±	1.2	0.9	0.9	0.6	0.5	0.7	1.1	0.8	0.7	0.4
C.D. (P = 0.05)	3.8	2.9	2.7	1.9	1.7	2.3	3.4	2.5	2.2	1.4

[a] H: Herbicide (carfentrazone-ethyl); P + H: polymer + herbicide (carfentrazone-ethyl).

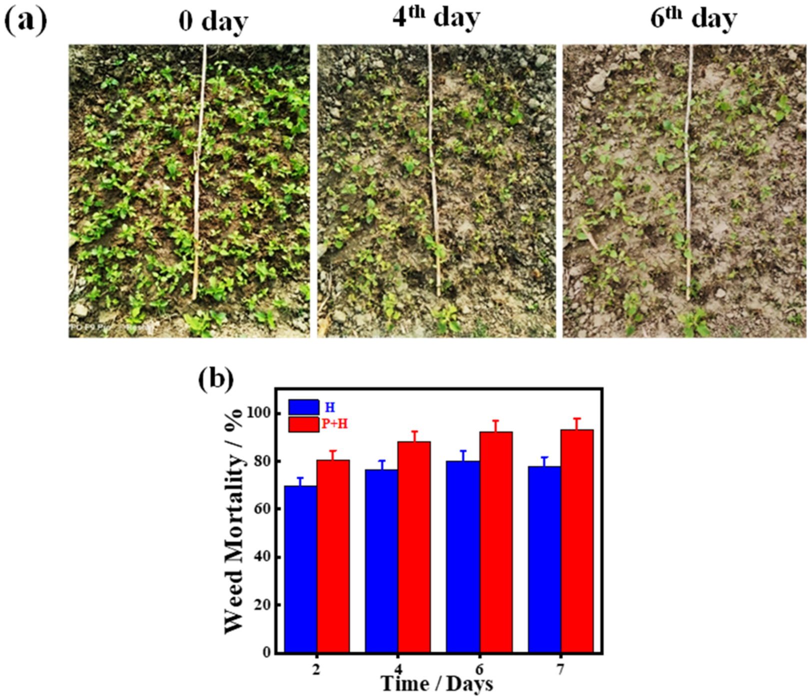

Figure 7. Mortality of weeds (*Ageratum conyzoides* L.). (a) Photographic images of weeds showing the relative mortality of weeds grown in the nursery using the indicated systems, (H)-conventional system @ 20 g a.i. ha^{-1} and polymer−herbicide conjugate gel (P + H) @ 20 g a.i. ha^{-1}. (b) Bar diagram showing quantitative mortality of weeds in the indicated systems showing significant differences.

on the weeds (Table 6). Hence, the ≤5 SPAD reading range was recorded by the large gel formulation-treated weed density as compared to herbicide aqueous dispersed weeds. On the other hand, a significantly higher range of SPAD reading of 30.1−35 was reported by a larger population (39) of weeds treated with herbicide @ 20 g a.i. ha^{-1} against only ~6 in polymer−herbicide gel formulation dispersed plot @ 20 g a.i. ha^{-1}, which was at par with that of *C. album* (~5) treated with gel formulation @ 30 g a.i. ha^{-1} (Table 4) because the mortality was not so effective with a mere 15.5%, which was due to the increased regrowth of the weeds over 20 days following the application of the treatments, as depicted in Figure S3 and Table 6. Similar results were observed over 4 days of glyphosate-treated *C. album* grown under high temperatures and elevated carbon dioxide (HT/ECO_2), where rapid chlorophyll reduction (estimated through SPAD units) was observed as compared to the same plants grown under low temperature and ambient CO_2 (LT/ACO_2), and 5 days after application of herbicide, severe chlorosis as well as turgor loss was observed in the treated weed leaves grown under HT/ECO_2.[45] However, the evidence correlating SPAD values with the leaf total chlorophyll concentration in any weeds is majorly lacking but the SPAD values in weeds are correlated with the physiological parameters of the leaves and has inadequately been reported.[46] Regeneration in *C. album* was about 90% in weeds treated with the conventional herbicide @ 20 g a.i. ha^{-1}, which was significantly higher compared to only 77% regrowth in weeds treated with the polymer−herbicide gel formulation @ 20 g a.i. ha^{-1}, which was at par with that of the regeneration reported for weeds treated with the formulation @ 30 g a.i. ha^{-1} 30 days after application (Figure S6, Table 6). Similar results were reported in the case of carfentrazone-ethyl-treated variable leaf milfoil (*Myriophyllum heterophyllum* Michx.) in which shoot regrowth was seen from the root crown after 42 days after treatment at the time of harvest.[47]

3.6. Effect of the Conjugate System on the Mortality of *Ageratum conyzoides* L.

Ageratum conyzoides L., a troublesome weed of the cultivated and agricultural field growing along the road verges, showed a significant response toward the herbicide toxicity in the case of polymer−herbicide conjugate gel formulation sprayed in the experimental plots of the weed nursery than in the conventional herbicide sprayed weed nursery plots (Figure 7a). Spraying of the polymer−herbicide conjugate gel formulation at different doses resulted in the quick wilting of weeds, followed by the browning of leaves and death of *A. conyzoides* over 4 days after application as compared to the different doses of herbicide dispersed weeds in which the toxic effect of the herbicide aqueous solution was significantly less. On the 6th day, the greenness of the weeds was adversely affected and high mortality was reported in the polymer−herbicide conjugate gel formulation sprayed over weeds in comparison with the herbicide-treated weeds. The photosynthetic rate of herbicide-treated *A. conyzoides* decreased due to the inhibition of protoporphyrinogen oxidase enzyme[38,39] as compared to the healthy weed having a photosynthetic rate of 13.2 μmol carbon dioxide (CO_2) m^{-2} s and leaf nitrogen content (2.3%) of 1.28 mg g^{-1} fresh weight chlorophyll.[48] Weed mortality percentage (80.33%) recorded in polymer−herbicide conjugate gel formulation dispersed @ 20 g a.i. ha^{-1} on *A. conyzoides* was significantly higher than that of weeds treated with herbicide-treated weeds (69.46%) @ 20 g a.i. ha^{-1} and was at par with weeds sprayed with polymer−herbicide conjugate gel formulation dispersed @ 20 g a.i. ha^{-1} 2 days after application of the said treatments (Figure 7b, Table 5). There was an increase in the mortality percentage (93.16%) in weeds treated with polymer−herbicide conjugate gel formulation @ 20 g a.i. ha^{-1}, which was at par in weeds treated with polymer−herbicide conjugate gel formulation as well as herbicide @ 30 g a.i. ha^{-1} up to the 7th day after the treatments and was significantly higher than the mortality percentage in the case of weeds treated with only herbicide aqueous solution @ 20 g a.i. ha^{-1}, which declined to 77.8%. This decline in weed mortality can be attributed to the regeneration of the weeds reported on the 7th day after spraying of herbicide aqueous solution. *A. conyzoides* in polymer−herbicide conjugate gel formulation-treated plot @ 20 g a.i. ha^{-1} showed significantly lower regeneration of weeds (49%) than weeds treated with the conventional herbicide aqueous solution (70%) @ 20 g a.i. ha^{-1} and was at par with the regeneration reported in the weeds (46%) treated with a higher dose of polymer−herbicide conjugate gel formulation @ 30 g a.i. ha^{-1}. This low regeneration can be attributed to the controlled delivery and longer retention of herbicide on the weed leaf surface through gel formulation (Figure S7, Table 6). Further, this regrowth commonly occurs in the weed tissue that is not initially killed when contact herbicides, sprayed conventionally, are applied because the translocation is limited throughout the plant tissues.[49] The herbicide carfentrazone-ethyl @ 20 g ha^{-1} is reported in other studies to control ∼83.7% of broad leaf weeds, thereby increasing the seed yield significantly using the polymer−herbicide conjugate gel system in this study.[50]

Table 5. Effect of Different Doses of the Herbicide and the Polymer−Herbicide Conjugate System on the Mortality of *Ageratum conyzoides* L

	weed mortality (%)			
treatments[a]	day 1	day 2	day 3	day 4
H @ 10 g a.i. ha^{-1}	33.83	58	65	65
H @ 20 g a.i. ha^{-1}	69.46	76.33	80.15	77.86
H @ 30 g a.i. ha^{-1}	74	82.67	91.53	91.53
P + H @ 10 g a.i. ha^{-1}	36.667	63	74	82.73
P + H @ 20 g a.i. ha^{-1}	80.33	88.03	92.31	93.16
P + H @ 30 g a.i. ha^{-1}	84.33	90	94.27	94.6
control	0	0	0	0
SEM ±	2.1	3.0	2.5	2.5
C.D. (P = 0.05)	6.5	9.4	7.8	7.7

[a] H: Herbicide (carfentrazone-ethyl); P + H: polymer + herbicide (carfentrazone-ethyl).

Table 6. Effect of Different Doses of the Herbicide and the Polymer−Herbicide Conjugate System on the Regeneration of Broad Leaf Weeds

	regeneration (%)		
treatments[a]	*Anagallis arvensis* L.	*Chenopodium album* L.	*Ageratum conyzoides* L.
H @ 10 g a.i. ha^{-1}	56	91	78
H @ 20 g a.i. ha^{-1}	43	90	70
H @ 30 g a.i. ha^{-1}	26	81	55
P + H @ 10 g a.i. ha^{-1}	46	82	69
P + H @ 20 g a.i. ha^{-1}	27	77	49
P + H @ 30 g a.i. ha^{-1}	25	72	46
control	0	0	0
SEM ±	2	2	1
C.D. (P = 0.05)	5	5	4

[a] H: Herbicide (carfentrazone-ethyl); P + H: polymer + herbicide (carfentrazone-ethyl).

The off-target movement of the herbicide was controlled through the use of biopolymer (guar gum) in which the herbicide was embedded to generate a polymer−herbicide conjugate gel formulation. The guar gum acted as the thickening agent[51] and altered the viscoelastic properties of the water-based spray on weeds.[29] The liquid stretching of dispersed droplets was limited by the extensional viscosity and a decrease in shear viscosity allowed the formation of coarser droplets[33] having a larger volume median diameter (VMD), resulting in lowered drift potential. Further, polymer−herbicide conjugate gel @ 10, 20, and 30 g a.i. ha^{-1} has been applied on three different broad leaf weeds demonstrating greater mortality as compared to conventional spray @ 10, 20, and 30 g a.i. ha^{-1} in addition to the greater suppression of regeneration of weeds after a considerable time of treatment because of the sustained delivery of active ingredients of herbicide from the conjugate gel on the leaf surface. There is diverse weed flora in the crop field, and this broad leaf herbicide is not found effective against some of the weeds. To overcome the problems of weed infestation in the case of diverse weed flora, which is not controlled by the existing herbicide at their different doses, either a new herbicide or a herbicide mixture with different modes of action is recommended, e.g., carfentrazone-ethyl is recommended to be applied in combination with other herbicides, viz. metsulfuron, over sole application of carfentrazone-ethyl, which may help in improving the killing effect on broad leaf weeds. This is how the diverse spectrum of weeds is controlled

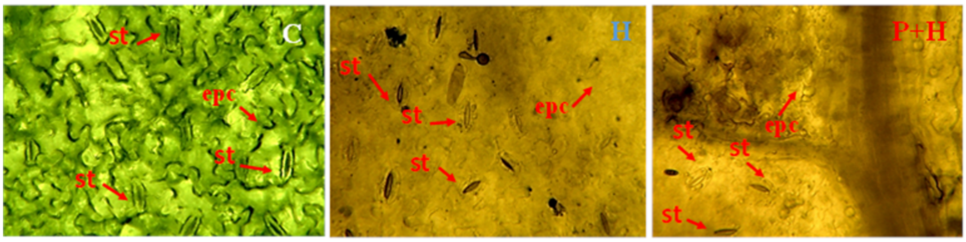

Figure 8. Abaxial view: leaf blade of *A. conyzoides* L. showing epidermal cells (epc) and stomata (st) in C (control), H (herbicide @ 20 g a.i. ha^{-1}), and P + H (polymer−herbicide gel formulation) @ 20 g a.i. ha^{-1}; magnification of C, H, and P + H = 200×.

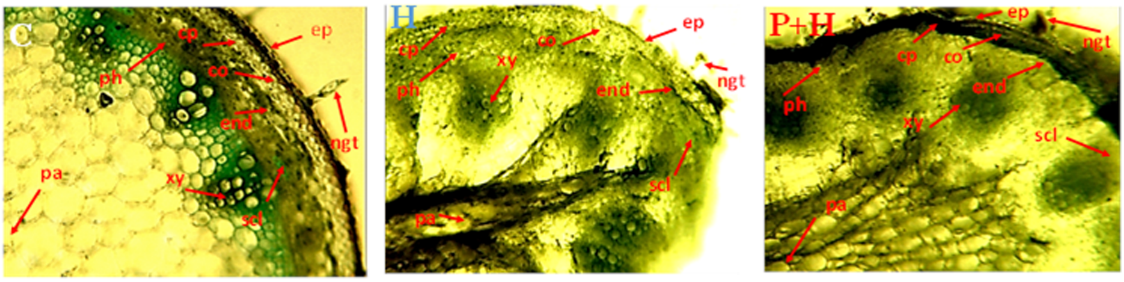

Figure 9. Cross-sectional view: stem of *A. conyzoides* L. showing epidermis (ep) with nonglandular trichome (ngt), cortical region formed by collenchyma (co) and parenchyma (cp), endodermis (end), sclerenchyma fibers (scl), phloem (ph), xylem (xy), and the medullar region composed of parenchyma (pa) in C (control), H (herbicide @ 20 g a.i. ha^{-1}), and P + H (polymer−herbicide gel formulation @ 20 g a.i. ha^{-1}); magnification of C, H, and P + H = 50×.

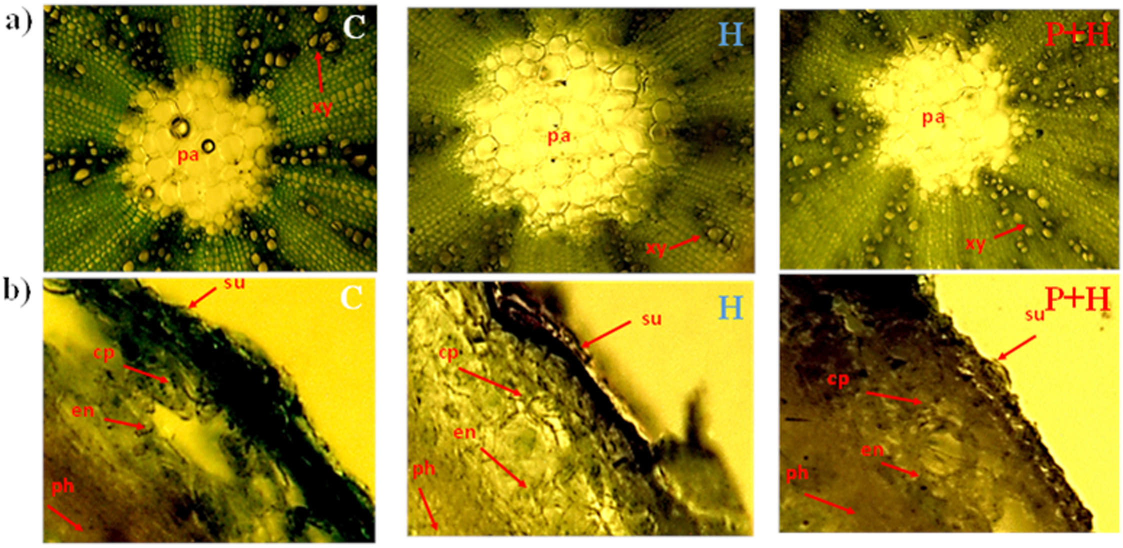

Figure 10. Cross-sectional view: root of *A. conyzoides* L. showing (a) details of the parenchymatic medullar region (pa), (b) suber (su), and cortical parenchyma (cp) with some intercellular air gaps, endodermis (en), phloem (ph), and xylem (xy) in C (control), H (herbicide @ 20 g a.i. ha^{-1}), and P + H (polymer−herbicide gel formulation @ 20 g a.i. ha^{-1}). Magnification: a and b = 50×.

chemically, which may hamper the soil health through leaching, runoff, and volatilization losses, which in turn may affect the environment adversely. The other way to manage the weed infestation is by improving the properties of herbicide toxicity through release technology utilizing altered formulations and adjuvants, which may also help in mitigating the spray drift as in the case of present experimentation. However, this concept may be applied for better weed control over a wide spectrum of crops.

3.7. Effect of the Conjugate System on the Anatomy of *Ageratum conyzoides* L.

The anatomical characterization of *A. conyzoides* treated with carfentrazone-ethyl @ 20 g a.i. ha^{-1} and herbicide−polymer gel formulation sprayed @ 20 g a.i. ha^{-1} under a polarizing optical microscope (POM) showed disruption in the cellular structures of the botanical materials (stem and leaf) from the normal observation in weeds grown in the control plot (Figures 8−10).

3.7.1. Leaf Blade.

The effect of carfentrazone-ethyl both in aqueous solution and in the polymer conjugate system was significantly visible on the leaves of *A. conyzoides*, which caused burning and drying of leaves after the treatments were applied. The microscopic observation reveals that the leaf blade of the weed was found to be amphistomatic having both anomocytic and anisocytic stomata.[52] However, the distribution of stomata on the abaxial surface of leaves was more in healthy *A. conyzoides* of the control plot, while in the case of herbicide and the herbicide−polymer gel formulation sprayed @ 20 g a.i. ha^{-1}, the density of stomata was very less (Figure 8). There is a decrease in the photosynthetic rate, and the stomata are distorted in the leaves of weeds treated with herbicide and herbicide−polymer formulation.

3.7.2. Stem. The cross sections of the stem of healthy herbicide and polymer−herbicide gel-treated *A. conyzoides* of experimental plots showed cylindrical contour, with well-defined uniseriate epidermis covered with a thin layer of cuticle,[52] multicellular, and uniseriate nonglandular trichomes commonly found in Asteraceae[53] (Figure 9C). Below the epidermis lies the corticular region with cellular inclusions, which is composed of two to four layers of angular collenchyma and parenchymatic cells arranged in five layers observed in healthy *A. conyzoides* of the control plot, whereas these parenchymatic layers were found distorted in both herbicide (Figure 9H) and herbicide−polymer gel formulation-treated weeds sprayed @ 20 g a.i. ha^{-1} (Figure 9 P + H). The vascular system comprising of continuous bundles of xylem and phloem was distributed in a single ring and capping of the phloem was observed by isolated sclerenchyma fibers located externally in the healthy sample of control.[52] However, the epidermal layers were not found distinct, and squeezing of the vascular ring was observed in herbicide and herbicide−polymer gel formulation-treated *A. conyzoides* both sprayed @ 20 g a.i. ha^{-1} on account of drying of stem after spraying of carfentrazone-ethyl.

3.7.3. Root. There was no difference observed in the cross-sectional view of roots of *A. conyzoides* of the control plot and that treated with herbicide and herbicide−polymer gel formulation both sprayed @ 20 g a.i. ha^{-1} in the experimental plots. It is reported that carfentrazone-ethyl is a contact herbicide that causes leaf dehiscence[44] and its translocation is inhibited after being absorbed by leaves; therefore, the herbicide effect is not reported in roots. The parenchymatic medullar region of the roots consists of thin-walled parenchymal cells tightly packed with no intercellular spaces, and the presence of distinct xylem bundles in both healthy as well as treated weeds was observed (Figure 10a). The cross-section of roots of healthy and weeds treated with herbicide as well as polymer−herbicide gel formulation both @ 20 g a.i. ha^{-1} was found to consist of cortical parenchyma made up of five layers of cells having straight or curved walls, cylindrical contour consisting of suber, intercellular air gaps called aerenchyma generally absent in the cortical region of the stem, and an innermost layer called endodermis surrounding the phloem[52] (Figure 10b).

The biopolymer (guar gum)-embedded broad leaf herbicide (carfentrazone-ethyl) was prepared through a solution route to create polymer−herbicide conjugate gel to enhance the efficiency of the herbicide on the broad leaf weed mortality. The morphological and structural changes due to the incorporation of herbicide into the polymer matrix were analyzed using SEM, FTIR, DSC, and XRD studies, which suggest better interactions of the herbicide with the polymer, and this interaction is effective in enhancing the bioefficacy of the conventional herbicide on weed mortality. Carfentrazone-ethyl when sprayed alone without any combination with other herbicides or any adjuvants showed low herbicidal efficiency, and the killing effect of the nongrassy weeds was reduced. Utilizing the control release system, the polymer−herbicide conjugate gel formulation was prepared and sprayed in the weed nursery of the broad leaf weeds, which increased the retention period of herbicide over the weed leaf surface, thereby allowing the penetration of active ingredient in the desired period. This improved the biological efficacy of the herbicide (carfentrazone-ethyl) as the weed mortality percentage increased by 30% on the 6th day in *C. album*, 9% in *A. arvensis*, and 20% in *A. conyzoides* on the 7th day after application of the treatments as compared to the herbicide aqueous solution-dispersed weeds. Further, the SPAD unit ranges in all three broad leaf weeds narrowed down as compared to the herbicide dispersed weeds, which showed more greenness in the vegetation cover. In the case of herbicide aqueous solution-dispersed broad leaf weeds, the regeneration percentage was found to be 59, 17, and 43% higher as compared to polymer−herbicide gel formulation dispersed *A. arvensis*, *C. album*, and *A. conyzoides*, respectively. The formulated polymer-embedded herbicide when sprayed on the broad leaf weeds permits the release of the active ingredient of the herbicide (carfentrazone-ethyl) for the specific site even at a lower dose of the herbicide with increased retention due to the sustained nature of polymer−herbicide conjugate formulation. Therefore, the broad leaf weeds, which have become a menace in the agricultural fields, resulting in reduced crop yield, can be managed through release technology, employing polymer−herbicide conjugate gel formulation, which not only enhances the herbicidal bioefficacy but also helps in achieving the desired effect at lower herbicide concentration.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c01782.

Optimization of dose; formulation retention analysis at 0.25 and 0.5% w/v; quantitative mortality data of weeds; and regeneration data weeds (PDF)

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Notes

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