Chapter 4

Experiment -1

4.1 PREPARATION AND CHARACTERIZATION OF RICE HUSK NANOPARTICLES AS 2,4-D HERBICIDE CARRIER

4.1.1 INTRODUCTION

Rice husk (RH), an agro-industrial waste is known for its excellent adsorption capacity. It is granular in structure, insoluble in water with high chemical stability and mechanical strength as well possess functional groups like carboxyl, hydroxyl and amidogen which accounts for its adsorption property (Bansal et al., 2009). RH was reported by a number of researchers for the removal of major water pollutants such as heavy metals and dyes (Bansal et al., 2009; Chowdhury et al., 2011) from aqueous environment. In this current study, an attempt was made to use nano-sized rice husk for adsorbing 2,4-D herbicide, with an aim to check its potential as a herbicide carrier.

4.1.2 MATERIALS AND METHODS

4.1.2.1 CHEMICALS

2,4-Dichlorophenoxyacetic acid (2,4-D) was purchased from Sigma-Aldrich., Bangalore, India. All the other chemicals used in this study were purchased from HiMedia Limited, Mumbai, India and were of analytical reagent grade. The stock solution was prepared by dissolving 2,4-D in methanol where the required concentration was prepared by diluting the stock solution.

4.1.2.2 PREPARATION OF RICE HUSK NANOPARTICLES

Rice husk was procured from a rice mill in Vellore, India, for nanoparticle synthesis. The procured rice husk was sieved through 68-75 µm sieve and the collected microparticles were subjected to a mechanical method reported by Anton et al (2008), with minor modifications for obtaining rice husk nanoparticles or nano-sized rice husk (nRH). For the rice husk nanoparticle (nRH) preparation, about 1 g l⁻¹ of rice husk microparticles were suspended in distilled water which was set at a pH of 3.0 using 0.1 mol l⁻¹ of disodium phosphate/citric acid solution. The suspension was agitated at 10,000 rpm for about 30 min, after which the residue was left to dry in a hot air oven at 60 °C for 4 h, in order to obtain the rice husk nanoparticles (nRH) or nano-rice husk sorbent.
4.1.2.3 PREPARATION OF RICE HUSK BASED NANOFORMULATION (DnRH)

The rice husk based 2,4-D nanoformulation was prepared via adsorption phenomenon. For the nanoformulation preparation, experiments were conducted by adding about 1 g of n-RH to 2,4-D solution at varying weight ratios (0.05, 0.10 and 0.15) in methanol and the percentage of adsorption was determined at regular intervals. The samples collected at regular intervals were centrifuged and the supernatant was filtered prior to 2,4-D analysis in high pressure liquid chromatography (HPLC). The amount of 2,4-D adsorbed on to the n-RH carrier was calculated by subtracting the concentration difference between the initial and equilibrium solutions. A control experiment without the n-RH sorbent was also run in parallel. After attainment of equilibrium, the methanolic solutions were centrifuged and the pellet was dried at 60°C, to obtain the 2,4-D nanoformulation (DnRH), which was then vortexed for 20 s before storage in air-tight containers. All the experiments were performed in triplicates and the statistical analysis of the results employed analysis of variance (ANOVA) and the Bonferroni post-test (GraphPad Prism version 5.0 software).

4.1.2.4.1 PHYSICOCHEMICAL STABILITY

The synthesized n-RH nanoparticles were characterized for size and stability using dynamic light scattering (DLS) (Model: Horizon JUNO 10G-HO) and zeta potential for over a period of 90 days. For size and zeta potential measurement, the n-RH nanoparticles and DnRH nanoformulation were diluted in deionized water in the ratio of 1:1000 (v/v) and were analysed in triplicates at a fixed angle of 90°. Higher values (expressed in mV) indicated greater nanoparticle stability.

4.1.2.4.2 INFRARED SPECTROSCOPY AND X-RAY DIFFRACTION ANALYSIS

The samples were also scanned in the spectral range of 400-4000 cm\(^{-1}\) with a 4 cm\(^{-1}\) resolution in an ALPHA-T, Bruker spectrometer. The nRH nanoparticles and DnRH nanoformulation were analysed for functional group modification using KBr pellet method. The X-ray diffraction (XRD) patterns of nRH and DnRH nanoformulation were also recorded using a Phillips PW1830 X-ray diffractometer with CuK\(\alpha\) radiation (\(\lambda =1.54\ \text{Å}\)), operated at 40 kv and 30 mA.

4.1.2.4.3 MORPHOLOGICAL CHARACTERIZATION

The nRH nanoparticle and 2,4-D nanoformulation (DnRH) were dispersed in deionised water and examined for morphology via Scanning electron microscopy coupled with EDAX (VEGA 3 TESCAN) and Transmission Electron microscopy (Tecnai, G2 20 Twin). The dispersed nanoparticles were coated onto carbon grid for SEM analysis and in
copper grid for TEM characterization. The mean particle size of the nanoparticles were calculated using the ImageJ2 software.

4.1.2.5 QUANTIFICATION OF 2,4-D

For residual 2,4-D analysis, the supernatant collected at regular intervals was filtered with a 0.22 µm microporous membrane prior to analysis on reverse phase HPLC (C18 column, particle size of 5 µm) coupled with a UV detector at 230 nm, where methanol: water (70:30, pH 2.0) was used as mobile phase. The percentage of 2,4-D adsorbed onto the silica nanoparticle was calculated using standard formulas

\[ A_d(\%) = \frac{C_0 - C_t}{C_0} \times 100 \]

Where, \( C_0 \) and \( C_t \) are the concentration (mg/L) of the solution at initial as well as time ‘t’, respectively; \( A_d \) is the percentage of 2,4-D removal.

4.1.2.6 ADSORPTION ISOTHERMS AND KINETICS

In order, to understand the interaction between the 2,4-D molecules and n-RH sorbent, the obtained equilibrium data was fitted with the widely used isotherms models *viz*. Langmuir (Langmuir, 1918), Freundlich (Freundlich, 1906), Temkin (Temkin and Pyzhev, 1940) and D-R (Dubinin, 1960) isotherm were utilized. Among these, the first three models draws the logarithmic equilibrium concentration in the liquid and solid phase (Dehghani et al., 2014). The Langmuir isotherm is used for describing the monolayer sorption into the surface of the n-RH sorbent with an assumption of finite number of identical sites. On the other hand, the Freundlich isotherm is based on heterogeneous surface adsorption whereas the Temkin isotherm takes into account the adsorbent-adsorbate interactions. The Langmuir, Freundlich, Temkin and D-R isotherm models are given by the following equations

**Langmuir:**

\[ \frac{c_e}{q_e} = \frac{1}{q_{max}} + \frac{c_e}{q_m} \]

**Freundlich:**

\[ \log q_e = \log q_{m} + \frac{1}{n} \log C_e \]

**Temkin:**

\[ q_e = B q_{m} \ln C_e \]

**D-R:**

\[ \ln q_e = \ln q_{m} - K_D \varepsilon^2 \]

Where, \( q_e \) (mg/g) and \( q_m \) (mg/g) are the amount of 2,4-D adsorbed per g of n-RH and amount required for monolayer formation; \( C_e \) (mg/L) is the 2,4-D concentration of the solution at equilibrium; \( K, K_t \) and \( B \) are the adsorption constants; \( K_f \) (L/kg) and \( 1/n \) are the adsorption coefficient and adsorption constant; \( \varepsilon \) is the Polanyi potential with the formula \( (\varepsilon=RT \ln(1+1/C_e)) \); \( K_D \) is the sorption energy. The D-R isotherm was also applied
to find out the type of sorption of process taken place, where the mean energy of sorption, $E$ was calculated for analysing the sorption process.

Mean sorption energy: $E = (-2K)^{-1/2}$

The kinetic models were also examined as the physical and/or chemical characteristics of the sorbent as well as mass transport process, in order to understand the mechanism of adsorption. So, to determine the mechanism of 2,4-D adsorption onto n-RH nanoparticle, the kinetic models such as pseudo first order (Ho, 2004), pseudo second order (Ho, 2006) and intraparticle diffusion (Weber and Morris, 1963) models were exploited using the equations

Pseudo first order model, $\log(q_e - q_t) = \log q_e - \left(\frac{K_1}{2.303}\right) t$

Pseudo second order model, $\frac{t}{q_t} = \frac{1}{K_2q_e^2} + \frac{t}{q_e}$

Intraparticle diffusion model, $q_t = K_{id}(t)^{0.5} + C$

Where $q_t$ and $q_e$ are the amount of 2,4-D adsorbed at time ‘t’ and at equilibrium (mg/g), $K_1$ (min$^{-1}$), $K_2$(g/mg/min) and $K_{id}$ (mg/g/min) are the pseudo first order, pseudo second order equation and intraparticle diffusion rate constant.

4.1.3 RESULTS

4.1.3.1 PREPARATION OF nRH BASED 2,4-D NANOFORMULATION

The nRH nanoparticles synthesized following the method of Anton et al (2008) were chosen for nRH based 2,4-D nanoformulation (DnRH). The adsorption of 2,4-D onto nRH nanoparticles was estimated at various ranges of n-RH to 2,4-D ratios viz. 1:0.05, 1:0.10 and 0.15 (% weight) at regular time intervals. From the results (Fig. 4.1.1), it was noticed that the adsorption (%) of 2,4-D rapidly increased at the initial hours and reached equilibrium within 90 mins. At an n-RH to 2,4-D ratio of 1:0.10 (%), the sorption was found to be quick and rapid until the equilibrium was obtained. In the present study, for the further preparation of DnRH nanoformulation an optimal nRH to 2,4-D concentration of 1:0.10 (%) and contact time of 90 mins was considered, based on the results obtained.
Fig. 4.1.1 Adsorption of 2,4-D onto nRH nanoparticles at various n-RH to 2,4-D ratios at different time intervals. Statistical analysis was employed was analysis of variance (ANOVA) and the Bonferroni post-test, (p<0.005)

4.1.3.2 ADSORPTION ISOTHERMS AND KINETICS

The conformity of the adsorption data fitted with the adsorption data of 2,4-D adsorption onto n-RH nanoparticle is depicted in table 4.1.1. Based on the equilibrium data, the Langmuir isotherm model fitted best (Fig. 4.1.2) with the adsorption data with a high regression coefficient ($R^2$) of 0.998 higher than that of Freundlich isotherm (0.996) and Temkin isotherm (0.931) regression coefficient. Based on the $R^2$ value, the isotherm models conformity could be given as Langmuir> Freundlich> Temkin isotherm model. Thus, the adsorption data was found to be in good conformity with Langmuir isotherm. Similar conformity of 2,4-D adsorption data to Langmuir isotherm was also observed by other researchers (Aksu and Kabasakal, 2004; Salman and Hameed, 2010).
Table 4.1.1 Sorption isotherm parameters of 2,4-D on n-RH nanoparticle

<table>
<thead>
<tr>
<th>Langmuir isotherm</th>
<th>Freundlich isotherm</th>
<th>Temkin isotherm</th>
<th>D-R isotherm</th>
</tr>
</thead>
<tbody>
<tr>
<td>$q_m$ (mg/g)</td>
<td>$K_L$ (L/mg)</td>
<td>$R^2$</td>
<td>$K_f$</td>
</tr>
<tr>
<td>24.75</td>
<td>0.0012</td>
<td>0.998</td>
<td>2.871</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$K_L$ (L/mg)</th>
<th>$R^2$</th>
<th>$B_T$</th>
<th>$K_L$ (L/mg)</th>
<th>$R^2$</th>
<th>$q_m$ (mg/g)</th>
<th>$B$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.298</td>
<td>0.931</td>
<td>0.0084</td>
<td>98.49</td>
<td>0.002</td>
<td>0.990</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4.1.2. Pictorial representation of isotherm data of DnRH nanoformulation (a) Langmuir, (b) Freundlich, (c) Temkin and (d) D-R isotherm

From the D-R isotherm model, an $E$ value of 15.81 kJ mol$^{-1}$ which falls under the ion-exchange reaction range of 8-16kJ mol$^{-1}$ was achieved. So, the type of sorption involved on 2,4-D adsorption onto the nRH nanoparticle was found to be a chemical
sorption process. The regression coefficients of the kinetic parameters of the used models are tabulated in Table 4.1.2.

**Table 4.1.2 Kinetic parameters of sorption for 2,4-D sorption onto n-RH nanoparticle**

<table>
<thead>
<tr>
<th>Initial 2,4-D concentration (mg/L)</th>
<th>Pseudo First order kinetics</th>
<th>Pseudo Second order kinetics</th>
<th>Intraparticle Diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (q_e) (mg/g)</td>
<td>(K_1)</td>
<td>(R^2)</td>
</tr>
<tr>
<td>50</td>
<td>50.5</td>
<td>0.0214</td>
<td>0.794</td>
</tr>
<tr>
<td>100</td>
<td>112.201</td>
<td>0.0201</td>
<td>0.8805</td>
</tr>
<tr>
<td>150</td>
<td>147.91</td>
<td>0.017</td>
<td>0.7291</td>
</tr>
</tbody>
</table>

From the results, the pseudo second order model was found to fit best the adsorption data with higher regression coefficient than the pseudo first order model. The pseudo second order model did appear linear but failed to achieve the criteria of producing the sync between the experimental and calculated \(q_e\) value, whereas the pseudo second model achieved both the criterion and so was considered as best fit. On the other hand, the intraparticle diffusion kinetic model was utilized for understanding the limiting factors for 2,4-D adsorption onto the n-RH adsorbent. The results (table 4.1.2) revealed that the intraparticle diffusion is not the only rate limiting step. As the plot was not found to pass through the origin, although regression coefficient was found >0.99. This suggests that the adsorption of 2,4-D onto the n-RH sorbent is a combination of film diffusion and intraparticle diffusion process (Fig. 4.1.3), with the domination of the latter.
4.1.3 PHYSICOCHEMICAL CHARACTERIZATION OF THE NANOPARTICLES

The morphology, size and composition of the prepared rice husk nanoparticles (nRH) and DnRH nanoformulation were characterized.

4.1.3.3 PHYSICOCHEMICAL STABILITY

The size and surface charge of the n-RH nanoparticle and DnRH nanoformulation was observed for a period of 90 days. The n-RH nanoparticles prepared via the mechanical method was found to have an average hydrodynamic particle size of 42.1 nm (Fig. 4.1.4). It was also noticed to be of uniform size and so, could result in a water
dispersible formulation. When evaluated for stability over time, the nRH and DnRH nanoformulation showed stability for about 60 days, after which an increase in the size of the nRH and DnRH was noticed. In case of zeta potential analysis, a negative value of 28.8 mV was recorded (Fig. 4.1.5) at the 0\textsuperscript{th} day, indicating the moderate stability of the nRH nanoparticle. But the surface area decreased over time and so based on the results, the nRH and DnRH nanoparticle was found to be stable only for period of 30 days.

![Fig. 4.1.4 Stability of nRH nanoparticle and DnRH nanoformulation in terms of mean average size for about 90 days](image-url)

**Fig. 4.1.4 Stability of nRH nanoparticle and DnRH nanoformulation in terms of mean average size for about 90 days**
4.1.3.3 FOURIER TRANSFORM INFRARED AND XRD ANALYSIS

The IR studies carried out for the n-RH nanoparticle revealed the presence of various groups on its surface which are described in detail in Table 4.1.3. The band stretching in the range of 1410-1520 cm\(^{-1}\) indicated the presence of angular deformation of amide N-H groups. The vibrations in the range of 900-1200 cm\(^{-1}\) represented the involvement of C=O bonds of hydroxyl, phosphate, sulfonate groups and cyclic structure of polysaccharides (Fig. 4.1.6).
Different regions of the spectra showed changes in the intensity in case of n-RH after 2,4-D adsorption. A peak in the region of 3000 to 3500 cm\(^{-1}\) corresponds to the N-H from amino group and also a bonded OH group stretch, where clear shifts and stretching were found after n-RH adsorbent contact with 2,4-D. A weak peak at 2918.30 and 2848.86 cm\(^{-1}\) in n-RH adsorbent denoted the involvement of C-H stretch of the alkanes. The peak shifts in the region of 1400 to 1600 cm\(^{-1}\) attributed to the amide (–CO–) group and (–NH–) indicating the binding of 2,4-D molecules on to the surface of n-RH adsorbents. Also, small shifts were noticed in the region of 1076 cm\(^{-1}\) corresponds to the –CO- group vibrations in the 2,4-D molecule. The above indications demonstrate the role of functional groups such as amino, carboxylic, hydroxyl and carbonyl groups in the 2,4-D biosorption process. These results were in accordance with that of the kinetic results supporting the involvement of chemisorption process during the adsorption of 2,4-D on to the n-RH sorbent.
Table 4.1.3 FT-IR spectrum of nRH nanoparticle and DnRH nanoformulation

<table>
<thead>
<tr>
<th>Wavenumber (cm(^{-1}))</th>
<th>nRH nanoparticle</th>
<th>DnRH nanoformulation</th>
<th>Assignment of functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>3342.64, 3302.13, 3172.90</td>
<td>3267.41, 3211.48</td>
<td>-OH stretching</td>
<td></td>
</tr>
<tr>
<td>2918.30, 2848.86</td>
<td>-</td>
<td>C-H stretching of alkanes</td>
<td></td>
</tr>
<tr>
<td>2337.72</td>
<td>2335.80</td>
<td>NCO stretching</td>
<td></td>
</tr>
<tr>
<td>1627.92, 1710.86</td>
<td>1631.78</td>
<td>C=O</td>
<td></td>
</tr>
<tr>
<td>1512.19</td>
<td>1517.99</td>
<td>C=C stretching vibration of alkenes and aromatic functional groups</td>
<td></td>
</tr>
<tr>
<td>1462.04, 1427.32</td>
<td>1467.83</td>
<td>-CH(_2) and -CH(_3) stretching</td>
<td></td>
</tr>
<tr>
<td>1074.35</td>
<td>1149.57</td>
<td>Asymmetric stretching of Si-O-Si stretch of SiO(_4) tetrahedran</td>
<td></td>
</tr>
<tr>
<td>891.11</td>
<td>795.74</td>
<td>Symmetric Si-O bending, Cl stretch</td>
<td></td>
</tr>
<tr>
<td>412.77, 460.99</td>
<td>518.85, 557.43, 570.93</td>
<td>-Si-H, -CHOH stretching, Si-O-Si stretching</td>
<td></td>
</tr>
</tbody>
</table>

The X-ray diffraction pattern of nRH and DnRH nanoformulation are presented in Fig. 4.1.7. A peak at 2\(\theta\) of 22° was noticed in nRH nanoparticle, indicating the presence of silica, corresponding to JCPDS no: 96-721-6267. The crystal structure was found to be monoclinic and amorphous in nature. The broadness of the peak indicated that the rice husk was present in nanosize. In case of DnRH nanoformulation, decrease in the broadness of the peak was noticed which could be due to the adsorption of 2,4-D onto nRH, thus resulting in aggregation of the nanoparticles.
4.1.3.3.3 MORPHOLOGICAL ANALYSIS

With the help of SEM analysis, the cell surface of the nRH nanoparticle was found as irregular squares with smooth surface individually (Fig. 4.1.8a). This could be due to the carbon coating of the samples for SEM analysis, which would have resulted in irregular nanoparticles visualization. The EDAX spectrum of nRH nanoparticle depicted the presence of C, O and Si. The 2,4-D adsorption was found to induce changes on to the morphological and surface characteristics of the adsorbent and thereby could have resulted in aggregate formation (Fig. 4.1.8b). This aggregation might affect the surface properties of the nRH nanoparticle and reduced its access to 2,4-D molecules. The EDAX spectrum of DnRH nanoformulation showed the presence of C, O, Si and Cl revealing 2,4-D on to the surface of n-RH nanoparticle (Fig 4.1.9 a,b).
Fig. 4.1.8 Scanning electron microscopic image of (a) nRH nanoparticle and (b) DnRH nanoformulation

Fig. 4.1.9 Energy dispersive (EDAX) spectrum of (a) nRH nanoparticle and (b) DnRH nanoformulation

The morphology (Fig. 4.1.10) of the nRH nanoparticles and DnRH nanoformulation was examined by transmission electron microscopy (TEM). Individual
spheres of the nRH nanoparticles in the size of about 65.71 nm of dense and solid structures were visualized. Increase in the size of the nRH nanoparticles were noticed after 2,4-D adsorption (DnRH nanoformulation), which appeared of size about 105.20 nm. The noticed increase in size could be due to the adsorption of 2,4-D on to the surface of the nRH nanoparticles, which could have caused aggregation.

Fig. 4.1.10 Transmission electron microscopic image of (a) nRH nanoparticle and (b) DnRH nanoformulation
Hence, using the rice husk nanoparticle, a 2,4-D nanoformulation (Fig. 4.1.11) was synthesized for the first time and was evaluated for herbicidal activity in further experiments.
4.2 PREPARATION AND CHARACTERIZATION OF BIOGENIC SILICA NANOPARTICLES TO SERVE AS 2,4-D HERBICIDE CARRIER

4.2.1 INTRODUCTION

Silica nanoparticles could be a valuable source for producing newer formulations of easily water dispersible pesticides. Although silica nanoparticles could be synthesized via chemical or biogenic method, the latter is often pointed out as safe, less toxic, biocompatible and eco-friendly (Shedbalkar et al. 2014; Bayat et al. 2015). As the living plant adsorbs silicic acid from the soil and accumulates around its cellulose micro compartments, the acid chemical treatment of RH makes it possible to extract amorphous silica with high surface area (Davarpanah and Kiasat, 2013) and increased biocompatibility, at a low cost.

4.2.2 MATERIALS AND METHODS

4.2.2.1 BIOGENIC SILICA NANOPARTICLE SYNTHESIS

The biogenic silica nanoparticles (bnSi) were synthesized from RH following the method of Athinarayanam et al. (2014), with modifications. Briefly, RH of about 10 g was mixed with 50 ml of 0.1 M HCl with magnetic stirring. The mixture was then autoclaved at 120 °C under 15 lbs for 2 h. For removing the hydrochloric acid, the autoclaved RH-HCl mixture was washed twice with Milli-Q water and the residue was calcinated using muffle furnace at 700 °C for 1 h. The colour change of the acid treated residue from brown to white was taken as an indication of biogenic silica nanoparticles formation (bnSi).

4.2.2.2 PREPARATION OF SILICA NANOPARTICLE BASED 2,4-D NANOFORMULATION (DbSi)

2,4-D was loaded onto the bnSi by adsorption technique, where 1g of bnSi was added in 20 mL of methanol at ambient temperature and pH 5.0, where 2,4-D was included in different weight ratios of bnSi to 2,4-D such as 1:0, 1:0.15, 1:0.20 and 1:0.25, respectively. A control experiment without the bnSi nanoparticle was also run in parallel. The solutions was kept under agitation and aliquots of samples were taken for 2,4-D analysis at definite intervals, until it reaches equilibrium. The amount of 2,4-D in the solution was analysed using HPLC using the protocol described earlier. The obtained equilibrium data was also fitted with the isotherm and kinetic models for understanding the mechanism of 2,4-D adsorption onto the bnSi nanoparticles. After which, the solution
was centrifuged for separating the 2,4-D loaded bnSi, which yielded a biogenic silica based 2,4-D nanoformulation, named as DbSi nanoformulation.

4.2.2.3 CHARACTERIZATION OF THE NANOPARTICLES

4.2.2.3.1 PHYSICOCHEMICAL STABILITY

The physicochemical stability of the bnSi nanoparticles and DbSi nanoformulation was measured in terms of size and stability using dynamic light-scattering technique (DLS) and zeta potential using a zeta sizer instrument, JUNO 10G-HO, Nanoparticle Analyser SZ-100, for a period of 90 days.

4.2.2.3.2 INFRARED SPECTROSCOPY AND X-RAY DIFFRACTION ANALYSIS

The samples were scanned in the spectral range of 400-4000 cm⁻¹ with a 4 cm⁻¹ resolution in an ALPHA-T, Bruker spectrometer. The bnSi nanoparticles and DbSi nanoformulation were analysed for functional group modification using KBr pellet method. The X-ray diffraction (XRD) patterns of bnSi and DbSi nanoformulation were also recorded using a Phillips PW1830 X-ray diffractometer with CuKα radiation (λ =1.54 Å), operated at 40 kV and 30 mA.

4.2.2.3.3 MORPHOLOGICAL CHARACTERIZATION

The morphological analysis of the synthesized bnSi and DbSi nanoformulation was visualized using Scanning Electron Microscope (SEM) of model, VEGA 3 TESCAN, by dispersing the formulations in deionised water. The size and morphology of the nanoparticles were also characterized using transmission electron microscope (TEM) of Tecnai, G2 20 Twin model. The mean particle size of the nanoparticles was calculated using the ImageJ2 software.

4.2.3 RESULTS

4.2.3.1 PREPARATION OF bnSi BASED 2,4-D NANOFORMULATION

For DbSi nanoformulation preparation, the 2,4-D loading onto the biogenic silica nanoparticle (bnSi) was analysed at various weight ratios of bnSi:2,4-D (1:0.15, 1:0.20 and 1:0.25) and the results are shown in Fig 4.2.1. At all the tested ratios, the adsorption of 2,4-D onto the bnSi nanoparticle was found to be rapid in the initial hours and reached an equilibrium within 240 min, after which no notable adsorption was noticed. The loading of 2,4-D onto the bnSi was found to be 144.36, 196.6 and 230.44 mg/g, for 1:0.15, 1:0.20 and 1:0.25 ratios, respectively.
4.2.3.2 ADSORPTION ISOTHERMS AND KINETICS

The equilibrium data obtained at different 2,4-D loading ratios onto the bnSi were fitted with the isotherm models and the results are presented in Table 4.2.1. Based on the regression coefficient, the isotherm data was found to fit better with the Freundlich isotherm (Fig. 4.2.2) than the Langmuir isotherm followed by the Temkin and D-R isotherm. A Freundlich isotherm’s adsorption intensity ‘n’ was found greater than 1.0, thus revealing the process as favourable. The Freundlich model fit depicts a heterogeneous surface reversible adsorption over the adsorbent. As the Langmuir isotherm assumes a homogenous surface of adsorption with identical adsorption sites, the lower regression coefficient of the isotherm ($R^2=0.994$) confirms a multilayer mode of sorption. Here, in case of 2,4-D adsorption onto the bnSi nanoparticle, the D-R isotherm showed a mean sorption energy (E) of about 11.47 kJ mol$^{-1}$ which implies an ion-exchange reaction as it falls under the range of 8-16 kJ mol$^{-1}$. 

**Fig 4.2.1 Adsorption of 2,4-D onto bnSi nanoparticles at various bnSi to 2,4-D ratios at different time intervals. Statistical analysis was employed was analysis of variance (ANOVA) and the Bonferroni post-test, (p<0.005)**
Fig. 4.2.2. Pictorial representation of isotherm data of DbSi nanoformulation
(a) Langmuir, (b) Freundlich, (c) Temkin and (d) D-R isotherm

Table 4.2.1 Sorption isotherm parameters of 2,4-D loading on biogenic silica nanoparticle

<table>
<thead>
<tr>
<th>Langmuir isotherm</th>
<th>Freundlich isotherm</th>
</tr>
</thead>
<tbody>
<tr>
<td>( q_m ) (mg/g)</td>
<td>( K_L ) (L/mg)</td>
</tr>
<tr>
<td>153.84</td>
<td>0.00026</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temkin isotherm</th>
<th>D-R isotherm</th>
</tr>
</thead>
<tbody>
<tr>
<td>( B_T )</td>
<td>( K_L ) (L/mg)</td>
</tr>
<tr>
<td>0.0091</td>
<td>2.806</td>
</tr>
</tbody>
</table>
Following the isotherm models, the equilibrium data of 2,4-D loading onto the bnSi nanoparticles was also fitted with the adsorption kinetics for understanding the 2,4-D sorption mechanism. The uniformity between the theoretical and experimental values of equilibrium data and an associated high regression coefficient suggested the process of 2,4-D adsorption onto the bnSi nanoparticle as a chemisorption process (table 4.2.2). These results were in accordance with the D-R isotherm results confirming a chemisorption process.

**Table 4.2.2 Kinetic parameters of sorption for 2,4-D onto bnSi nanoparticle**

<table>
<thead>
<tr>
<th>Initial 2,4-D weight ratio (%)</th>
<th>Pseudo First order kinetics</th>
<th>Pseudo Second order kinetics</th>
<th>Intraparticle Diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>q_e (mg/g)</td>
<td>K_1 (g/mg/min)</td>
<td>R^2</td>
</tr>
<tr>
<td>0.150</td>
<td>107.59</td>
<td>0.0071</td>
<td>0.916</td>
</tr>
<tr>
<td>0.200</td>
<td>180.71</td>
<td>0.0059</td>
<td>0.981</td>
</tr>
<tr>
<td>0.250</td>
<td>195.43</td>
<td>0.0066</td>
<td>0.948</td>
</tr>
</tbody>
</table>

The intraparticle diffusion kinetics for 2,4-D sorption onto the bnSi nanoparticle was also evaluated by the plot between q_e and t^{0.5}. From the plot, three stages of 2,4-D diffusion such as initial fast uptake, then medium phase followed by a slow phase was noticed and also the plot did not pass through the origin (Fig. 4.2.3). This shows that intraparticle diffusion was not the rate limiting step in case of 2,4-D adsorption onto the bnSi nanoparticles. So, from the isotherm and kinetic data obtained, the adsorption of 2,4-D onto the bnSi was found to be a heterogeneous multilayer process via chemisorption mechanics. This mechanism could be the reason for increased 2,4-D uptake as well for the herbicides strong bonding onto the surface of bnSi nanoparticles, to yield DbSi nanoformulation.
4.2.3.3 PHYSICOCHEMICAL CHARACTERIZATION OF THE NANOPARTICLES

The morphology, size and composition of the bnSi nanoparticles and DbSi nanoformulation were characterized for better understanding of the properties of the nanoparticles.

4.2.3.3.1 PHYSICOCHEMICAL STABILITY

The bnSi nanoparticles showed a hydrodynamic particle size of 54.1 nm and a negative zeta potential value of 21.5 mV (Fig. 4.2.4a), whereas the DbSi nanoformulation exhibited a hydrodynamic particle of 79.0 nm and negative potential of 49.7 mV. When the stability of the nanoparticles were evaluated over a period of 90 days, the bnSi and DbSi nanoformulation showed an enhanced stability for over 60 days (Fig. 4.2.5). After
which the stability was found decreased in terms of size as well surface charge, due to the particle aggregation.

Fig. 4.2.4 Stability of bnSi nanoparticle and DbSi nanoformulation in terms of mean average size for about 90 days
4.2.3.3 Fourier Transform Infrared and XRD Analysis

The FT-IR spectra of the bnSi nanoparticle and DbSi nanoformulation are depicted in Table 4.2.3, where, characteristic peaks at 1047.35 cm$^{-1}$ and 400-600 cm$^{-1}$ which corresponds to O-Si-O asymmetric stretch and Si-O bending vibrations were exhibited. The peak shifts in the region of 1020-1050 and 2300 to 2500 cm$^{-1}$ corresponds to the presence of C-H bending of alkanes and C-N stretch of aliphatic amines and –OH groups in the silica. Among the peaks, shifts noticed in the range of 680-850 cm$^{-1}$ corresponds to C-Cl functional group representing the alkyl halides. This could be due to the loading of 2,4-D onto the bnSi nanoparticle (Fig. 4.2.6).
Table 4.2.3 FT-IR spectrum of bnSi nanoparticle and DbSi nanoformulation

<table>
<thead>
<tr>
<th>Wavenumber (cm(^{-1}))</th>
<th>Assignment of functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>bnSi nanoparticle</td>
<td>DbSi nanoformulation</td>
</tr>
<tr>
<td>2164.13</td>
<td>2333.87, 2158.35</td>
</tr>
<tr>
<td>1641.42</td>
<td>1732.08</td>
</tr>
<tr>
<td>-</td>
<td>1367.53</td>
</tr>
<tr>
<td>1047.35</td>
<td>1047.35</td>
</tr>
<tr>
<td>565.14, 460.99, 447.49</td>
<td>557.43, 455.20</td>
</tr>
</tbody>
</table>

Fig. 4.2.6 FT-IR analysis of (a) nRH nanoparticles and (b) DnRH nanoformulation

The X-ray diffraction data of bnSi and DbSi nanoformulation are presented in Fig 4.2.7. The peak at 2\(\theta\) of 22° indicated the typical characteristic silica peak, which
corresponds to the cristobalite phase (JCPDS no: 96-900-1579). The broadness of the peaks confirmed that the synthesized bnSi is in nanoscale size and was found to be in tetragonal crystal structure with space group of P (\((41, 21, 2 (92))\)). The crystallinity of the bnSi was not found reduced, even after the incorporation of 2,4-D onto the bnSi’s surface. This indicates that the addition of the 2,4-D herbicide on to the bnSi nanoparticles, did not alter the crystallinity of the nanoparticle (Fig. 4.2.2) and so the silica nanoparticle could be exploited as a effectual herbicide carrier.

![X-ray diffraction of bnSi nanoparticles and DbSi nanoformulation](image)

**Fig. 4.2.7 X-ray diffraction of bnSi nanoparticles and DbSi nanoformulation**

**4.2.3.3.3 MORPHOLOGICAL CHARACTERIZATION**

The bnSi and DbSi nanoformulation was further characterized for surface morphology by using SEM-EDAX, where homogenous suspensions of bnSi nanoparticles were visualized (Fig. 4.2.8), in support with the XRD spectra obtained. When the DbSi nanoformulation was visualized, the tetragonal surfaces appeared clumsy and attached, which could be due to the surface adsorption of 2,4-D onto the bnSi nanoparticle. The EDAX spectra (Fig. 4.2.9) of bnSi nanoparticle showed the presence of C, O and silica elements, whereas the presence of C,O, Si and Cl was noticed in case of DbSi nanoformulation. These results confirm the adsorption of 2,4-D onto the surface of bnSi nanoparticles.
Fig. 4.2.8 Scanning electron microscopic image of (a) bnSi nanoparticle and (b) DbSi nanoformulation

Fig. 4.2.9 Energy dispersive (EDAX) spectrum of (a) bnSi nanoparticle and (b) DbSi nanoformulation
The TEM analysis depicted the presence of small spherical structure of bnSi nanoparticles (Fig. 4.2.10) and whose morphology was found clumped in case of DbSi nanoformulation. The size of the bnSi nanoparticle was calculated to be about 21.03 nm, whereas the DbSi nanoformulation had an average size of 40.17 nm.
Hence, using the biogenic silica nanoparticle, a 2,4-D nanoformulation (Fig. 4.2.11) was synthesized for the first time and was evaluated for herbicidal activity in further experiments.
4.3 STUDIES ON THE SUSTAINED RELEASE, LEACHING BEHAVIOR AND BIOEFFICACY OF THE 2,4-D NANOFORMULATIONS

4.3.1 INTRODUCTION

Sustained release are advantageous over the conventional herbicide formulations as larger rates of application are required to compensate the losses via various common pesticide dissipation pathways. The sustained release formulations were formulated in order to maintain to prolong the bioavailability of the herbicide for longer periods and thereby reducing the associated environmental and economic problems (Pereira et al., 2014). The additional benefits of sustained release formulations are reduced leaching and phytotoxicity, and less contamination of the environment.

4.3.2 MATERIALS AND METHODS

4.3.2.1 SUSTAINED RELEASE OF 2,4-D IN WATER

To evaluate the sustained release potential of the prepared 2,4-D nanoformulation viz. rice husk based (DnRH) and biogenic silica nanoparticle based (DbSi) in water, about 3.0 g of the nanoformulation was added in 500 mL of distilled water at room temperature with agitation (100 rpm). At definite intervals, about 5 ml of distilled water was withdrawn, and an equivalent amount of distilled water was replaced to the flask. The withdrawn samples were measured for 2,4-D concentration with the help of HPLC. The experiments were done in triplicates and the average mean was expressed. The release experiments were carried out till the attainment of 100% release of the adsorbed 2,4-D from the nanoformulation.

4.3.2.2 SUSTAINED RELEASE OF 2,4-D IN SOIL

In case of sustained release of 2,4-D in soil from the nanoformulation viz. DnRH and DbSi, a thin layer of pesticide free soil (10 g) was placed in a Buchner funnel. The soil was amended with the DnRH and DbSi separately, accounting to 0.5 % and 1.0 % by weight, respectively onto a nylon filter cloth of 0.05 mm mesh size paved in the funnel. For the release tests, about 40 ml of distilled water was sprayed in 8-10 min for nine times with an interval of 1 h in the funnel. The samples collected at regular intervals were extracted with equal amount of methanol prior to 2,4-D concentration analysis with the help of high pressure liquid chromatography (HPLC). For comparison, a control experiment with addition of technical grade 2,4-D was also performed without the
addition of either DnRH or DnSi nanoformulation. All the experiments were performed in triplicates and the statistical analysis of the results employed analysis of variance (ANOVA) and the Bonferroni post-test (GraphPad Prism version 5.0 software). The total percentage of 2,4-D release after nine watering were calculated using the formulae

$$\left( \sum_{i=1}^{n} \frac{M_i}{M_0} \right) \times 100 \%$$

Where, $M_i$ is the amount of herbicide released in mg and $M_0$ is the initial amount of herbicide added to the soil in mg.

4.3.2.3 LEACHING BEHAVIOUR OF NANOFORMULATIONS IN SOIL COLUMN

A soil column (20 cm high) was constructed with the help of five PVC rings, (height and diameter of 4 cm) sealed with waterproof tapes and filled with air dried and sieved, red loam soil. The soil constituted of silt 83%, clay 6% and sand 11%, with a pH 5.9. Each of the column was blocked with filter paper at the end and then filled with soil. The columns were left to drain water for 24 h prior to the 2,4-D nanoformulation (DnRH, DbSi) application. The 2,4-D nanoformulation (DnRH, DbSi) were applied at a concentration equivalent to field application rate (2.5 kg/ha). After application, the column were precipitated by adding 25 ml of water, which is equivalent to 70 mm of water, for 24 and 48 h. The columns were dismantled for quantification of 2,4-D as well as for the evaluation of herbicidal activity in each rings. A control column was also set where the bulk 2,4-D was applied at the same rate as the 2,4-D nanoformulation.

4.3.2.4 BIOACTIVITY OF THE 2,4-D NANOFORMULATIONS

The herbicidal activity of the 2,4-D nanoformulation viz. DnRH and DbSi, was tested on pots (10 cm high with diameter of 12.5 cm) filled with 600 g of plant substrate (soilrite mix). For the tests, fifteen seeds of non-target plant (Brassica sp.) and 10 seeds of target plant (Zea mays) was sown and grown for four days. After four days of growth, the DnRH and DbSi nanoformulation was applied at a concentration equal to the field application rate (2.5 kg/ha). In the control pot, an equal quantity of rice husk nanosorbent or biogenic silica nanoparticles, without 2,4-D was applied to analyse the influence of rice husk or silica nanoparticle on the plant growth. After 24 h, the plants were weighed after washing and drying for dry mass estimation, to evaluate the post-emergence activity of the nanoformulation. In case of pre-emergence activity, the pots were analysed for about 14 days, for seedling emergence (%), root length and shoot length.

4.3.2.5 RELEASE KINETICS FOR 2,4-D NANOFORMULATION
The release pattern of 2,4-D from DbSi nanoformulation was analysed mathematically by fitting with the Korsmeyer-Peppas kinetic model. This semi-empirical model provides further insight into the release pattern mechanism of 2,4-D, which is given by the equation,

\[
\frac{M_t}{M_0} = kt^n
\]

Where, \(M_t/M_0\) is the fraction of 2,4-D released in time, \(t\); \(k\) is the kinetic constant and \(n\) is the exponent which explains the type of release mechanism. The value of \(n \leq 0.43\) indicates the mechanism of Fick’s law, while \(n>0.85\) implies a relaxation process. The values of \(n\) intermediates to the above values (0.43\(<n<0.85\)) suggests a combination behaviour of diffusion and relaxation process.

4.3.2.6 QUANTIFICATION OF 2,4-D IN SOIL

For 2,4-D quantification in soil, about 5 g of soil from each ring was extracted with equal amount of methanol followed by agitation for 20 min at 30 °C. After agitation, the suspension was allowed to settle for 30 min for supernatant removal. The procedure was repeated thrice and the collected supernatant was concentrated in a rotary evaporator before quantification using HPLC.

The herbicidal activity in the columns were also analysed by sowing 10 seeds in each ring at different depths. The seedling emergence (%) was used as indicators for calculating the herbicide presence at all depths of the column. The percentage of growth inhibition was determined using the formulae

\[
\text{Growth inhibition} (\%) = 100 \frac{L_c - L_t}{L_c}
\]

Where, \(L_c\) and \(L_t\) are the heights of the control and test samples at any soil depth.

4.3.3 RESULTS

4.3.3.1 SUSTAINED RELEASE OF 2,4-D IN WATER

4.3.3.1.1 RELEASE OF 2,4-D FROM DN RH NANOFORMULATION

The sustained release pattern of different ratios of n-RH to 2,4-D from the n-RH nanosorbent was checked in water (Fig. 4.3.1). The release of 2,4-D from the n-RH sorbent was slow and the sustained in the range of 4, 6 and 10 days for 2,4-D concentrations at weight ratios of 0.05, 0.10 and 0.15 (%), respectively. Based on the optimum 2,4-D weight ratio of 0.10 (%) in the preliminary experiments, it was worth reporting a sustained release of 2,4-D from the n-RH sorbent for about 6 days without any
polymer coating. In the control experiment, nearly 93.4% of the crude 2,4-D was found to get dissolved in water.

![Graph showing cumulative sustained release of 2,4-D from DnRH nanoformulation at various ratios](image)

**Fig. 4.3.1 Cumulative sustained release of 2,4-D from DnRH nanoformulation at various ratios**

4.3.3.1.2 RELEASE OF 2,4-D FROM DbSi NANOFORMULATION

The sustained release performance of the DbSi nanoformulation and free 2,4-D was compared and the release profile with time as function is shown in Fig. 4.3.2. An initial burst release of 2,4-D less than the free 2,4-D was noticed from the DbSi formulation, which reached a steady stage of release after 5 h. This could be due to the release of loosely bonded 2,4-D on to the surface of the bnSi carrier.

After the burst release, the 2,4-D release rate reached a steady state which might due to the chemical sorption mode of adsorption of 2,4-D onto the bnSi carrier. Thus, the pattern of 2,4-D release from the bnSi resembled a controlled diffusion model. A sustained release of 2,4-D from DbSi nanoformulation was noticed for nearly 22 days. The 2,4-D release profile alteration in the presence of bnSi nanoparticle where prolonged time was required for the complete release of the technical 2,4-D was noted. These results could be advantageous to reduce the usage of herbicide usage for controlling the weeds as well overcomes the associated environmental pollution.
4.3.3.2 MATHEMATICAL KINETICS FOR 2,4-D RELEASE IN WATER

The release pattern of 2,4-D from the nRH nanoparticles was fitted with the Korsmeyer-Peppas (K-P) kinetic model (Fig. 4.3.3a) for obtaining further insight into the release pattern mechanism. From the linearized model equation, a release coefficient (n) value of 0.5 was obtained indicating the 2,4-D release due to diffusion process. The value of release constant (k) was about 0.13 min\(^{-1}\) depicting a faster release rate, respectively. Thus, the present sustained release pattern of 2,4-D from an agro-industrial waste nanosorbent could be advantageous over the other costly polymeric systems and other nanopesticide formulations due to its ecofriendliness and increased bioefficacy for a longer time.
In case of DbSi nanoformulation, a high regression coefficient of 0.99 showed the fitness of the model (Fig. 4.3.3b). A low ‘n’ value of 0.157 obtained from the DbSi nanoformulation displays Fickian diffusion kinetics where the release was found to be caused mainly by diffusion in spherical monolithic matrices. A higher value of ‘k’ indicates faster release, but a low ‘k’ value of 0.12 day$^{-1}$ obtained indicates a slow release phenomenon of 2,4-D loaded on the bnSi carrier. Based on the results obtained, the release mechanism of DbSi nanoformulation was found to be a controlled diffusion process.

4.3.3.3 SUSTAINED RELEASE OF 2,4-D IN SOIL

4.3.3.3.1 RELEASE OF 2,4-D FROM DnRH NANOFORMULATION

The experiment for analyzing the sustained release of 2,4-D from the DnRH and DbSi nanoformulation was evaluated by amending the formulation in soil (Fig. 4.3.4). The n-RH nanosorbent was checked for its sustained release via a thin soil layer amended with 0.5 % and 1.0 % (wt) of DnRH formulation and the release profiles are shown in Fig 4.3.5.

Fig. 4.3.4 Experimental setup (a) for analysing the sustained release of 2,4-D in soil and (b) top view of the setup
From the results, the control experiment, where the technical grade 2,4-D was applied was found to get released very quick and completely by end of the nine waterings. On the contrary, as hypothesized, the 2,4-D release from n-RH nanosorbent was decreased in the range 0.5 % n-RH followed by 1.0 % n-RH sorbent. This could be due to the potential of n-RH adsorbent in sustaining the release of 2,4-D. Although a very small amount of n-RH was amended in soil, it had played a significant role in slowing down the rapid release of 2,4-D at both the weight ratios tested. Hence, the n-RH nanosorbent could be considered as a good soil amendment for enabling sustained release of herbicides like 2,4-D, with high leaching capacity.

4.3.3.3.2 RELEASE OF 2,4-D FROM DbSi NANOFORMULATION

The DbSi formulation was applied at two different ratios such as 0.5 and 1.0 % (wt). Fig. 4.3.6 shows the release pattern of 2,4-D in soil where the a slower release of 2,4-D was noticed in case of soil applied with DbSi nanoformulation than the free 2,4-D formulation.
The free 2,4-D formulation was found to get released from the soil completely at the end of nine watering’s, whereas the release rate was found regulated in case of the DbSi nanoformulation. The soil amended with 0.5 wt (%) showed restricted 2,4-D release (%) than the soil added with 1.0 wt (%) nanoformulation. This result demonstrates the ability of bnSi in controlling the release of 2,4-D in soil and thus reduce leaching. The reduce leaching of 2,4-D in DbSi amended soil could be due to the competition between the soil particles for 2,4-D adsorption, which reduced the leaching gradually. So, the DbSi nanoformulation could decrease the release rate of the herbicide as well act as a soil amendment simultaneously.

4.3.3.4 BIOACTIVITY OF THE 2,4-D NANOFORMULATION
4.3.3.4.1 BIOACTIVITY OF DnRH and DbSI AGAINST TARGET PLANT

The Brassica sp. chosen as target plant was tested for both post and pre emergence herbicidal activity of the nanoformulation. The pot 1 served as control, where the plants were grown using water. For post emergence activity check, the target plant, Brassica sp., grown for 4 days and was exposed to free 2,4-D and DnRH formulation, where it was found to cause 100% mortality within 1 day (Fig. 4.3.7). The pots 2 and 4 was subjected to free 2,4-D and DnRH nanoformulation and was analysed for post emergence activity for 14 days (Fig. 4.3.8), where seedling emergence was noticed in 2,4-D applied pot alone (pot 2) after 4 days. But for nearly 8 days, no seedling emergence (%) was noticed in pot 4, which was subjected to DnRH nanoformulation. The results illustrates the sustained activity offered by the DnRH nanoformulation in comparison with the free 2,4-D.
The effect of nRH nanoparticle (pot 3) on the plant was also checked and was not found to cause any damage on the plant’s germination index (94%), in both pre-and post-emergence activity (Fig. 4.3.9). The results reveal the enhanced potential of 2,4-D nanoformulation in target species while the non-target plant was not affected at all. This enhanced herbicidal effect in case of 2,4-D nanoformulation could be due to the reduced soil sorption or increased bioavailability of 2,4-D in the soil.
Fig. 4.3.9 Post emergence (a) and pre emergence activity (b) of DnRH nanoformulation against target plant (*Brassica sp.*)

The bioefficacy of the DbSi nanoformulation against target plant (*Brassica sp.*) was also investigated (Fig. 4.3.10). Similar results were found in case of the pot 4, subjected to DbSi nanoformulation, where 100% mortality of the plant was noticed, in case of post emergence activity. The increase in activity in the plants administered with nanoformulation *viz.* DnRH and DbSi formulation could be due to the enhanced their herbicide activity *via* faster penetration. The herbicidal activity in the pots administered with DbSi and free 2,4-D were found similar, which demonstrates that the nanoformulation preparation did not alter the activity of the 2,4-D herbicide.

The pots were also analysed for pre-emergence activity of the herbicide for nearly 14 days. The pot 4 subjected to DbSi nanoformulation did not show any seedling emergence (%) till the end of 14 days, whereas the pot 2, where free 2,4-D was applied showed seedling emergence (%) after 4 days (Fig. 4.3.11). This further reveals the enhanced herbicidal activity of the DbSi nanoformulation for both pre-emergence and post emergence weed control (Fig. 4.3.12).
Fig. 4.3.10 Post emergence herbicidal activity of DbSi nanoformulation in target plant (Brassica sp.)

Fig. 4.3.11 Pre emergence herbicidal activity of DbSi nanoformulation in target plant (Brassica sp.)
4.3.12 Post emergence (a) and pre emergence activity (b) of DbSi nanoformulation against target plant (Brassica sp.)

4.3.3.4.2 BIOACTIVITY OF DNRH and DBSI AGAINST NON-TARGET PLANT

Fig. 4.3.13 Bioactivity of DnRH in non-target plant (Zea mays) A) control, B) free 2,4-D, C) nRH nanoparticle and D) DnRH formulation

The prepared DnRH nanoformulation were checked for phytotoxic effect on the non-target plant (Zea mays). In case of Zea mays, an germination index of 92 % was obtained in case of free 2,4-D and the DnRH nanoformulation. This confirms that the
DnRH nanoformulation does not affect the plant development nor change the activity of the herbicide (Fig. 4.3.13).

![Graph showing dry weight, shoot length, and root length of DnRH exposed non-target plant](image1)

**Fig. 4.3.14** Dry weight (a), shoot length (b) and root length (c) of DnRH exposed non-target plant

The germination index, root length and shoot length of the plants were also recorded and no significant difference was noticed in all the chosen parameters (Fig. 4.3.14). Similar results like that of DnRH nanoformulation was also observed in DbSi formulation exposed Zea mays plants (Fig. 4.3.15).

![Bioactivity of DbSi in non-target plant (Zea mays)](image2)

**Fig. 4.3.15** Bioactivity of DbSi in non-target plant (Zea mays) A) control, B) free 2,4-D, C) bnSi nanoparticle and D) DbSi nanoformulation
Fig. 4.3.16 Dry weight (a), shoot length (b) and root length (c) of DbSi exposed non-target plant

The control experiment where free bnSi was applied without 2,4-D onto its surface showed no effects on the plants studied and was found to have nil effect on the root length and shoot length (Fig. 4.3.16) similar to that of the control plants grown without herbicide application.

4.3.3.5 LEACHING EXPERIMENTS IN SOIL COLUMN

4.3.3.5.1 LEACHING BEHAVIOUR OF DnRH NANOFORMULATION

For understanding the leaching behaviour (Fig. 4.3.17) of the DnRH and DbSi nanoformulation (1.0 % (wt)) against that of the free 2,4-D herbicide, the different formulation of 2,4-D were applied on the top of the column and watered. After 48 h of watering, the amount of 2,4-D at different depths of the column was quantified after extraction with methanol and analysed for residual 2,4-D concentration. The column treated with the nanoformulation viz. DnRH and DbSi exhibited highest 2,4-D concentration on the topmost ring. The concentration gradient was found reduced in the lower segments. The higher retention of 2,4-D in the topmost segment of the column could be due to the presence of colloids in the soil.
Fig. 4.3.17 Experimental setup diagram for analysing the reduced leaching potential of 2,4-D nanoformulation

On the other hand, the concentration of 2,4-D in the column applied with free 2,4-D formulation was higher in case of all the four column segments. The leaching of applied free 2,4-D could be the reason for its presence in all the column segments and as well for its detection in the ground and surface waters (Fig. 4.3.18 and Fig. 4.3.19). This leaching accounts for the reduced herbicidal activity in the topmost column segment applied with free 2,4-D. Hence, the results portrays that application of DbSi nanoformulation on the fields could increase the retention of active ingredient in the topmost soil, reduce repeated application of 2,4-D and prolong its bioactivity for a longer period.
Fig. 4.3.18. Soil column experiment for residual quantification in DnRH nanoformulation treated soil.

Fig. 4.3.19. Soil column experiment for residual quantification in DbSi nanoformulation treated soil.
4.3.3.5.2 HERBICIDAL ACTIVITY IN THE SOIL COLUMNS

After analysing the residual 2,4-D concentration in the soil column segments, the herbicidal activity in the segments was analysed with *Brassica* sp. seeds and the germination index was recorded. A germination index of about 95% was achieved all the column segments in case of the column treated with distilled water. In case of the column segments treated with free 2,4-D and nanoformulation (DnRH and DbSi), inhibition in the growth of the plant was noticed in the depth of 0-5 cm, zero growth (Fig. 4.3.20 and Fig. 4.3.21).

![Graph 1](image1)

**Fig. 4.3.20** Herbicidal activity of the DnRH on target plant (*Brassica* sp.) at different soil depths

![Graph 2](image2)

**Fig. 4.3.21** Herbicidal activity of the DnRH and (b) DbSi on target plant (*Brassica* sp.) at different soil depths
Fig. 4.3.22 Pictorial presentation of the herbicidal activity of the DnRH on target plant (*Brassica* sp.) at different soil depths (a) soil treated with bulk 2,4-D (b) with DnRH
Fig. 4.3.23 Pictorial presentation of the herbicidal activity of the DbSi on target plant (*Brassica* sp.) at different soil depths (a) soil treated with bulk 2,4-D (b) with DbSi

On the contrary, in the other column segments of 5-10, 10-15 and 15-20 cm, seedling emergence (%) was found increased in case of DnRH (Fig. 4.3.22) and DbSi (Fig. 4.3.23) formulation than that of free 2,4-D treated column, in the following order: (5-10 cm) < (10-15 cm) < (15-20 cm). This could be due to reduced leaching of the adsorbed 2,4-D from the nRH or bnSi nanoparticle compared to that of the free 2,4-D herbicide. Thus, the use of carriers for herbicide delivery could increase herbicidal activity and reduce their frequent application as well. So, the use of agro-industrial waste based nanocarrier could aid in reducing the environmental pollution without any effect on its bioefficacy. Based on the results on the sustained release, reduced leaching and bioefficacy tests, the DbSi nanoformulation was found to exhibit enhanced activity and so, was chosen for further tests.
4.4 STUDIES ON THE BIOSAFETY OF BIOGENIC SILICA BASED 2,4-D NANOFORMULATION VIA TOXICITY STUDIES

4.4.1 INTRODUCTION

In most of the agricultural countries, the contamination of the environment by agrochemicals has become a great matter of concern (Goujon et al., 2014). It is mandatory to characterize the nanoparticulate systems for their toxicity, in order to evaluate the expected reduction in toxicity of the nanoformulation on human health and environment. In the present study, the DbSi nanoformulation, which exhibited enhanced herbicidal activity in comparison with DnRH nanoformulation, was checked for cytotoxicity and genotoxicity. The in vitro cell cultures could acts an alternative of whole animals for examining the cytotoxicity of effect of 2,4-D, in a rapid and cost effective way, while the higher plants could provide a useful screening and monitoring genetic setup of the changes induced by the environmental pollutants (Goujon et al., 2014). So, the toxicity testes was conducted for attaining a better insight on the alternative way of utilizing 2,4-D herbicide.

4.4.1 MATERIALS AND METHODS

4.4.1.1 CYTOTOXICITY OF THE NANOPARTICLES

The cytotoxicity of the DbSi nanoformulation was determined with the help of MTT or tetrazolium reduction test. The HepG2 cell lines at a density of 1.2 X 10^4 per well and was cultured in a 96 well titer plates with 100 µL of DMEM medium supplemented with 10% Fetal Bovine Serum. After culturing overnight, the cultured cell lines were incubated with DbSi nanoformulation, dummy bnSi nanoparticles and bulk 2,4-D at different concentrations ranging from 0.4 to 2.6 mg/mL for 24 h. After incubation, the medium was removed and 100 µL of fresh medium was added along with 10 µL of 5 mg/ml MTT. After 4 h, the medium was discarded and the formed formazan crystals were dissolved using DMSO and the absorbance was read at 570 nm in a microtiter plate reader (Mossman, 1983). The untreated cells were taken as reference. The experiments were performed in triplicates and the results of cytotoxicity were expressed as cell viability (%).

4.4.1.2 GENOTOXICITY OF THE NANOPARTICLES
For the genotoxicity assay, *Allium cepa* bulbs were procured from the local market and was germinated in 1.7 mg/L of either free 2,4-D or DbSi nanoformulation and as well in the biogenic silica nanoparticles alone. Water was used as the control. The bulbs were germinated till the root length was about 2 cm. After which the roots were removed from the respective solutions, washed with water and were kept in 0.1 N HCl for about 12 minutes at 60 °C. After incubation, the HCl was withdrawn and washed with distilled water thrice followed by 100 µL acetocarmine staining for about 2 min. The stain was washed with distilled water and the slide was prepared by crushing the root tip gently using a cover slip for visualizing at X100 magnification under light microscope for cytological damages (Kumar et al., 2013). The relative mitotic index (RMI) and the chromosomal aberration index (CAI) was calculated using standard formulas

\[
\text{Relative mitotic index (RMI)} = \frac{MI}{MIC}
\]

\[
\text{Chromosomal aberration index (CAI)} = \frac{CA}{TDx(100)}
\]

Where, MI and MIC are the total number of cells in different mitotic phases divided by the total number of the cells in interphase and MI of the control, respectively, while CA and TC are the total number of cells in interphase and the total number of divisions, respectively.

4.4.3 RESULTS

4.4.3.1 CYTOTOXICITY OF THE NANOPARTICLES

The results of cytotoxicity for DbSi nanoformulation with HepG2 cells are shown in Fig. 4.4.1. From the assay, the IC\(_{50}\) value for bulk 2,4-D was estimated to be about 1.7 mg/ml approximately, while the cells treated with DbSi nanoformulation did not exhibit no toxicity virtually at all the concentrations tested below the IC\(_{50}\) value. A slight decrease in the cell viability was noticed in cells exposed to DbSi nanoformulation above the IC\(_{50}\) value. On the other hand, the nanoparticles used for 2,4-D carrier showed cell viability which was approximately above 90% at all the concentrations tested. So, the use of bnSi nanoparticle as herbicide carrier was found to be advantageous and so could be exploited for safe herbicide usage. The decrease in DbSi nanoformulation in comparison with bulk 2,4-D, indicates the ability of the bnSi nanoparticles in reducing the toxicity of the herbicide in association with the nanoparticle.
Fig. 4.4.1 Cytotoxicity results of cellular viability (%) using HepG\textsubscript{2} cell in different concentrations of (a) bnSi (b) DbSi and (c) bulk 2,4-D

4.4.3.2 GENOTOXICITY OF THE NANOPARTICLES

The mitotic index and the chromosomal aberrations noticed in \textit{Allium cepa} upon treatment with bulk 2,4-D, dummy bnSi nanoparticle and DbSi nanoformulation are tabulated in Table 4.4.1.

\textbf{Table 4.4.1 Mean mitotic index recorded in the tested treatments}

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Prophase (%)</th>
<th>Metaphase (%)</th>
<th>Anaphase (%)</th>
<th>Telophase (%)</th>
<th>Mean mitotic index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88.45</td>
<td>4.9</td>
<td>2.3</td>
<td>4.3</td>
<td>93.04±0.2</td>
</tr>
<tr>
<td>2,4-D</td>
<td>80.54</td>
<td>3.1</td>
<td>1.2</td>
<td>2.2</td>
<td>85.6±1.0</td>
</tr>
<tr>
<td>bnSi</td>
<td>85.23</td>
<td>4.5</td>
<td>2.0</td>
<td>4.5</td>
<td>93.54±0.9</td>
</tr>
<tr>
<td>DbSi</td>
<td>81.34</td>
<td>3.8</td>
<td>2.0</td>
<td>4.3</td>
<td>90.89±0.5</td>
</tr>
</tbody>
</table>
Increased chromosomal aberration index was also noticed in case of bulk 2,4-D, while only fewer chromosomal aberrations were noticed, in case of DbSi nanoformulation. This shows the reduction in 2,4-D toxicity when in association with the biogenic silica nanoparticles. At the tested concentration, the bnSi nanoparticles was not found to induce chromosomal aberration and recorded a score equal to that of the control. Upon exposure to DbSi nanoformulation and bulk 2,4-D, different chromosomal aberration features like sticky chromosome, chromosomal breaks, laggard chromosome,
multipolar anaphase etc., were observed (Table 4.4.2). This could be due to the interaction of the nanoparticles with the plants root’s surface or by adsorption, which could have led to cellular toxicity. The chromosomal aberration visualized under microscope are presented in Fig. 4.4.3.

Fig. 4.4.3 Chromosomal aberrations visualized under optical microscope in the *Allium cepa* cells when exposed to DbSi nanoformulation

The results revealed the reduction in the level of environmental damage caused when 2,4-D is applied in the form of DbSi nanoformulation. The non-toxic nature of the DbSi nanoformulation at lower concentrations and its safety to be used as an herbicide
nanocarrier was highlighted. These observations depicts the biological safety and eco-friendliness of 2,4-D when applied in the form of DbSi nanoformulation.
Experiment -5

4.5 STUDIES ON THE FATE OF DBSI NANOFORMULATION AND ITS
ON-FIELD EFFECTIVENESS

4.5.1 INTRODUCTION

Nanotechnology in agriculture has received attention from all corners of the world
but their current state of understanding must be improved for envisioning the vision. As
the understanding of how and to what extent a nanoformulation may influence the active
ingredient is very important for assessing the associated environmental risk (Kah et al.,
2014). In the study, the fate of DbSi nanoformulation was assessed via soil sorption
studies (Grillo et al., 2014) for better understanding. For practical applicability, any
formulation should be effective not only in lab scale but in on-field as well (Kumar et al.,
2014). The present study aims at studying the on-field effectiveness of DbSi
nanoformulation in a maize field (Zea mays) and its ultimate effect on the improvement
of the crop and environmental safety. Therefore, prior to the on-field activity, the DbSi
nanoformulation was investigated of its fate in the environment.

4.5.2 MATERIALS AND METHODS

4.5.1 FATE OF 2,4-D IN SOIL VIA SORPTION STUDIES

For the study, surface soil was collected, air-dried and sieved via a 40 mm mesh,
to remove dirt and stones. The collected soil had no history of pesticide application for at
least for the past 4 years. The soil constituted of silt 83%, clay 6%, sand 11%, with a pH
of 5.9. The herbicide soil sorption studies were performed according to standard
equilibrium test of OECD guidelines 106, where 2,4-D was present in 0.01 mol/L CaCl₂.
For the test, about 1 g of the soil was taken in a flask with 15 mL of CaCl₂ solution
containing bulk 2,4-D and DbSi nanoformulation at equal concentrations. The solution
was agitated at room temperature and at definite intervals, 1 mL of the sample was
withdrawn, centrifuged (2000 rpm, 5 min) and the supernatant was filtered using a 0.22
µm membrane and the 2,4-D concentration was determined using HPLC. The sorption
experiments were performed in triplicates and the difference in the 2,4-D concentration in
the initial solution and the solution at soil equilibrium was determined. The sorption data
was fitted with the sorption kinetic mathematical modelling and the model which
described the model best was obtained based on the regression coefficient ($R^2$).
4.5.2 EVALUATION OF THE ON FIELD EFFECTIVENESS OF DBSI NANOFORMULATION

In order to evaluate the on field effectiveness of the DbSi nanoformulation, four plots of 1 m² equal dimensions (with 1 m separation) were selected and considered for the study. Each of the plot contained ten healthy Zea mays plants, six days of age. The first plot was taken as the reference or the control. The second, third and fourth plot were treated with free 2,4-D, dummy bnSi nanoparticles (without 2,4-D) and DbSi nanoformulation, respectively. At field application rate, about 150 mL of the separately prepared different formulations were sprayed onto the respective plants. After spraying, at regular intervals, the condition of the weeds grown along with the target plant were examined for DbSi bioefficacy.

4.5.3 RESULTS
4.5.3.1 SORPTION BEHAVIOUR: 2,4-D vs. DBSI NANOFORMULATION

The soil sorption kinetics of bulk 2,4-D and DbSi nanoformulation are depicted in Fig. 4.5.1. The 2,4-D sorption was found to be fast, which reached equilibrium within 60 minutes. A small difference in the sorption of the herbicide in comparison with the bulk 2,4-D and DbSi nanoformulation, where less sorption was found in case of the nanoformulation. This explains the fact that the use of bnSi nanoparticles could reduce the problem of sorption of 2,4-D with the soil particles. So, the finding suggests that the use of DbSi nanoformulation could leave more 2,4-D available for the target plant.
4.5.1 Adsorption profile of 2,4-D sorption on soil particles

Fig. 4.5.2 Pseudo first order mathematical kinetic model of soil sorption experiments with DbSi and bulk 2,4-D at different time intervals
Fig. 4.5.3 Pseudo second order mathematical kinetic model of soil sorption experiments with DbSi and bulk 2,4-D at different time

The soil kinetics data was also fitted with the pseudo first order and pseudo second order kinetics and the results are tabulated in table 4.5.1. The results indicated the best fit of the adsorption data with the pseudo second order model. This describes that the sorption of 2,4-D onto the soil is via heterogeneous surfaces and thus increasing the bioavailability of 2,4-D to the target plant (Fig. 4.5.2 and Fig. 4.5.3).
Table 4.5.1 The kinetic model result for the soil sorption of 2,4-D either as bulk 2,4-D or DbSi nanoformulation at room temperature

<table>
<thead>
<tr>
<th>Sample</th>
<th>Kinetics</th>
<th>q_e max (mg/g)</th>
<th>K (mg/g min)</th>
<th>h (mg/g min)</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D</td>
<td>Pseudo first order</td>
<td>1.0874</td>
<td>0.340</td>
<td>-</td>
<td>0.899</td>
</tr>
<tr>
<td></td>
<td>Pseudo second order</td>
<td>2.35</td>
<td>0.284</td>
<td>0.234</td>
<td>0.994</td>
</tr>
<tr>
<td>DbSi</td>
<td>Pseudo first order</td>
<td>1.0038</td>
<td>0.032</td>
<td>-</td>
<td>0.901</td>
</tr>
<tr>
<td></td>
<td>Pseudo second order</td>
<td>1.782</td>
<td>0.3885</td>
<td>0.302</td>
<td>0.997</td>
</tr>
</tbody>
</table>

4.5.3.2 ON FIELD EFFECTIVENESS OF DBSI NANOFORMULATION

The investigation of the on-field activity data is presented in table 4.5.2, where the DbSi nanoformulation was checked for its post emergence activity. The weeds were found appeared after 6 days (Fig. 4.5.4), which were differentiated as dicotyledonous and monocotyledons and the bioactivity of the DbSi nanoformulation was analysed. The DbSi nanoformulation was found superior in bioactivity against the target weeds in compared with the bulk 2,4-D herbicide (Table 4.5.2). No injury was found to the tested non-target plant *Zea mays* (Fig. 4.5.5) when the formulation was applied at field application rate (Table 4.5.3).
Table 4.5.2 Herbicidal activity of either bulk 2,4-D or DbSi nanoformulation upon application in on-filed experiments

<table>
<thead>
<tr>
<th>Type</th>
<th>Scientific name</th>
<th>Common name</th>
<th>Leaf stage</th>
<th>Activity at field application rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Dicotyledonous weeds</td>
<td>Chenopodium album</td>
<td>common lambquait</td>
<td>3-4 L</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>Amaranthus retroflexus</td>
<td>Redroot pigweed</td>
<td>4-6 L</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>Polygonum lapathiofolium</td>
<td>Pale smartweed</td>
<td>4-6 L</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>Ambrosia trifida</td>
<td>Giant Rag weed</td>
<td>4-6 L</td>
<td>0±0</td>
</tr>
<tr>
<td>Monocotyledonous weeds</td>
<td>Stellaria media</td>
<td>Steme</td>
<td>2-3 L</td>
<td>0±0</td>
</tr>
<tr>
<td></td>
<td>Cyperus microiria</td>
<td>Cyprni</td>
<td>2-3 L</td>
<td>0±0</td>
</tr>
</tbody>
</table>
Fig. 4.5.4 On-field activity of DbSi nanoformulation (a) six day grown plants (b) weeds grown alongside (marked red)

Table 4.5.3 Effect of DbSi nanoformulation on *Zea mays* plant in on-field activity

<table>
<thead>
<tr>
<th>Non-target plant</th>
<th>Leaf stage</th>
<th>Activity of treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td><em>Zea mays</em></td>
<td>4-6 L</td>
<td>0±0</td>
</tr>
</tbody>
</table>
Based on the on-field activity evaluation, the DbSi nanoformulation which was found to exhibit good herbicidal activity when compared with bulk 2,4-D, could be exploited for achieving the long lasting dream of the environmentalists viz. increased efficiency at low herbicide application rates.