Research Article

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Chitosan nanoparticles loaded with mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl to manage weeds of wheat (*Triticum aestivum* L.)

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Abstract: Nanoherbicides are articulated by empowering the potential of nanotechnology for the efficacious delivery of chemical or biological herbicides with the aid of nanomaterial-based herbicide combinations. Therefore, the goal of this work was to investigate the chitosan nanoparticles loaded with mesosulfuron methyl and mesosulfuron methyl + florasulam + (2-methyl-4-chlorophenoxyacetic acid) MCPA isooctyl herbicides as a possible environmentally benign substitute to manage weeds in wheat. Due to intriguing characteristics including biocompatibility, low allergenicity, biodegradability, and nontoxicity, chitosan biopolymers as sustainable chitin derivatives have received intense scrutiny in the biomedical business. The manufactured nanoparticles were characterized by using ultraviolet absorbance, scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FT-IR). The average particle size as revealed by SEM was 40–70 nm in a cluster form with the porous structure. The maximum absorption peaks of both nanoparticles of mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl were 330 and 360 nm. The FT-IR analysis showed an intensive peak at 2θ value of 30.55° for mesosulfuron methyl and 32.79° for mesosulfuron methyl + florasula + MCPA isooctyl, which correspond to the 78 and 198 planes of the anatase phase, respectively. The nanoparticles were sprayed at the third to fourth leaf stages of the targeted weeds. Seven different doses were applied. A total of 100% mortality and visual injury were caused by the chitosan-based nanoparticles of both herbicides at the recommended dose of standard herbicide. The 5-fold lower dose showed the minimum chlorophyll content (5.75%), plant height (2.35 cm), fresh biomass (1.08 g), and dry biomass (0.33 g) of a weed mixture. For the same traits, the herbicide nanoparticles at 10-fold lower dose of commercial herbicides exhibited a similar effect as the recommended dose. Nanoherbicides could recuperate the conventional herbicide effectiveness by enhancing the stability and reducing the toxicity.

Keywords: chitosan nanoherbicides, weed management, chlorophyll content, weed fresh and dry biomass, wheat (*Triticum aestivum* L.)

1 Introduction

Wheat is considered one of the most versatile crops on the planet. It is an important food crop due to its extensive use as a food daily [1]. Therefore, its sustainable production is very crucial for the better livelihood of the community at large. Presently, several climatic and political, agro-physiological, socio-economical, and management factors are

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responsible for low yields of wheat worldwide. Lack of seeding rates, appropriate doses of fertilizers and irrigation, access to resources, as well as the best strategy for pest and weed management are major influencers of wheat farming and productivity [2–4].

Weeds cause approximately 9.5% yield loss of wheat globally [5–7]. *Phalaris minor*, *Avena fatua*, *Chenopodium album*, *Lathyrus aphaca*, *Angalis arvensis*, and *Melilotus indica* are the most common and troublesome ones [8,9]. Since weeds possess competitive and deleterious effects on each growth phase of wheat, it is of prime importance to follow new systems for their management [8]. Preventive measures and biological and chemical approaches were employed for weed control [10]. Currently, the most effective and time-saving method is the chemical control [11]. Herbicides are frequently sprayed on the targeted plants to destroy their structure and impair their function [12]. They significantly decrease the growth and potential seed production of those weeds. Several herbicides are used to control both broad and narrow-leaved weeds in wheat including mesosulfuron methyl and mesosulfuron methyl + florasulam + (2-methyl-4-chlorophenoxyacetic acid) MCPA isooctyl [13]. The wide usage of herbicides increases the chances of weed resistance and farmer's dependence [14]. In addition to the resistance, the hazard caused by herbicides and their persistent toxic effect on the quality of all life aspects after reaching the action site are other major issues related to the chemical control [15,16]. Despite those several herbicide side effects, its use is extremely important in augmenting crop productivity to face all the necessities of food security and sustainability of human populations [17]. Thus, developing a more environmentally friendly herbicide application that is grounded on an innovative technology and a progressive mechanism of action is a must. Today, the agriculture industry is further hastening its revolution, driven by new technologies such as nanotechnology. Nanotechnology proposes stimulating techniques for preventing the herbicide misuse as well as harmless and effective delivery [17]. This technology of exploiting nanomaterials promises the enhancement of the present practices through the amelioration of management procedures. The nanostructured herbicide could considerably diminish the herbicide consumption rate and assure increasing crop productivity [18]. The new-fangled strategy of nanoherbicides is used to battle the complications of the conventional ones. A controlled release mechanism is manufactured in nanostructured formulations comprising an extensive variety of polymeric and metallic nanoparticles (NPs) [18]. One of the most effective methods for lowering the concentration and related negative effects of herbicides applied in the fields is to nanoencapsulate them in polymeric shells. By using nanotechnology, several issues associated with the conventional usage of pesticides can be resolved and minimized. Degradable and certain synthetic polymers are frequently used to package pharmaceutical and veterinary drugs as well as other active substances, yet some studies have labeled these materials as agrochemicals [18]. The competence of nanoherbicides assists in eradicating weeds before developing resistance through extraordinary penetration, bioavailability, solubility, reduced risk of oxidation, and site-specific targeting [19]. Furthermore, nanoherbicides afford short- and long-term protection against phytotoxicity and the lethal doses at which the weed might acquire resistance [20]. Chitin, a naturally occurring polymer contained in the shells of shrimp and other crustaceans, is deacetylated to produce chitosan, a linear polysaccharide. It is one of the biopolymers that is often used in a variety of industries, including clothing, cosmetics, water treatment, and food processing. The hydrophilic characteristic of chitosan in an aqueous solution is increased by the vast number of useful free primary amino groups present, which facilitates its interaction with medicines, polymers, cells, and NPs. The biomedical sector has also closely examined chitosan biopolymers as sustainable chitin derivatives because of intriguing properties like biocompatibility, low allergenicity, biodegradability, and nontoxicity [9].

Chitosan has been used in a variety of agricultural applications, including seed coating, fertilizers, and nutrients, as well as to enhance the plant growth, frost protection, and self-protective mechanisms [9,21,22]. The ability of agrochemicals including chitosan matrix to serve as a protective reservoir for the functional compounds, shielding the substances from their surroundings, and observing their abilities allows them to be used as efficient delivery vehicles for plant modification [23]. The use of chitosan NPs in the creation of novel delivery systems with enhanced bioavailability, higher specificity and sensitivity, and decreased toxicity has received particular attention [9,24]. The release system developed in NP-based herbicides significantly aids early weed control with a strong potential of eliminating resistance, maintaining the activity of their functioning components, and elongates their liberation over longer times [22,23]. Thus, it was suggested that nanoherbicides aid in the weed control more efficiently even at 10-fold lower dose in comparison with the conventional ones [25–27]. It has also been documented that nano-atrazine at a 10-fold lower dose created comparable effects on maize weeds as the suggested dose of commercial atrazine [28,29].

Therefore, the aim of the current study was to investigate the chitosan NPs loaded with mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl herbicides as a possible environmentally benign substitute to manage weeds in wheat.

2 Materials and methods

2.1 Weed seeds collection

Seeds of two grassy weeds (*Phalaris minor* L. and *Avena fatua* L.) and four broad leaves ones (*Chenopodium album* L., *Lathyrus aphaca* L., *Angalis arvensis* L., and *Melilotus indica* L.) developed in wheat were procured from the Agronomic Research Area, College of Agriculture, University of Sargodha, Pakistan. Seeds were washed, dried, and stored at room temperature in paper bags.

2.2 Chemical synthesis of chitosan-based mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl

The ionic gelification technique was used to prepare the NPs [30].

2.3 Chemicals

The following chemicals were utilized throughout the experiment: chitosan (MW: 27 kDa, degree of deacetylation: 75–85%), tripoly phosphate, clodinofop propargyl, and fenoxaprop-*p*-ethyl.

2.4 Characterization of chitosan-based NPs of mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl

Characterization of chitosan-based NPs of mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl was validated using several techniques. The distribution of size, composition, and NP morphology was recorded by scanning electron microscopy (SEM) (Scanning Electron Microscopy FEI brand model Inspect S50). For the type of NPs, the X-ray diffraction (XRD) (PAN analytical X-pert powder, with Cu-Kα as X-ray source) was employed. Scanning at 2θ with a scan speed of $1° \cdot min^{-1}$ and step size of 0.02° was conducted [20] and was studied using Fourier transform infrared spectroscopy (FT-IR) spectrometer (Thermo-Nicolet 6700) with the potassium bromide disk technique. FT-IR spectroscopy was performed to study the binding characteristics of NPs and investigate the functional group sites on the NPs [31].

2.5 Herbicidal activity of chitosan-based NPs of mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl

To optimize the doses of herbicide NPs, pot experiments were conducted during the winter season of 2020–2021 in the Agronomic Research Area, College of Agriculture, University of Sargodha, Pakistan. The experimental design was a factorial arrangement in a complete randomized block design with three replicates. Five seeds of each weed under investigation were planted in 20 cm × 16.5 cm pots filled with peat moss for germination. For each treatment, a total of 12 weed seedlings were maintained after emergence. At leaf stage 3–4, the NPs of mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl were sprayed on the targeted weeds in seven different doses, i.e., D_0 = weedy check, D_1 = normal herbicide at the recommended dose, D_2 = nanoherbicide at the recommended dose of normal herbicide, D_3 = 05-fold lower dose of nanoherbicide, D_4 = 10-fold lower dose of nanoherbicide, D_5 = 15-fold lower dose of nanoherbicide, and D_6 = 20-fold lower dose of nanoherbicide. Two weeks after the treatment, the chlorophyll content was recorded. The visual injury (%) was determined by visual observation of pots, and the average injury that occurred to plants in pots of each replicate was also determined. Mortality (%) was recorded by observing the total number of plants in each pot, and the average killed ones are determined following the method presented in [32]. Also, the average plant height (cm) of the plants was noted. Average fresh biomass (g per pot) was recorded using a digital balance. Dry biomass (g per pot) was calculated after drying at 70°C for 48 h [32].

2.6 Statistical analysis

The Statistics Software (Statistix version 8.1, Tallahassee, FL, USA) was employed to analyze the data. The means were compared using the highest significant difference (HSD) at the level of probability of 5%.

3 Results

3.1 Chlorophyll content (%)

The results depicted in Table 1 presented that different doses of chitosan-based broad-spectrum herbicides significantly affect the chlorophyll content of the weed mixture when compared with the weedy check. At the standard dose of normal herbicide, the maximum toxic effect caused 100% mortality and 45.49% chlorophyll content with no application of chitosan-based NPs (control) in the weed mixture. The minimum content of chlorophyll (5.75%) was perceived when a 5-fold lower dose of the broad-spectrum herbicide-loaded NPs (D_3) was applied. Also, applying conventional herbicides at the recommended dose (D_1) showed a statistically comparable effect on the chlorophyll content as the 10-fold lower dose of chitosan-based herbicide-loaded NPs (D_4) (10.43% and 9.66%, respectively). The herbicide NPs also showed a statistically significant effect on the chlorophyll content (%) of a mixture of weeds. However, the maximum chlorophyll content (19.38%) was examined with NPs of mesosulfuron methyl + florasulam + MCPA isooctyl and minimum (16.87%) with mesosulfuron methyl. The interaction effects of various doses × broad-spectrum herbicides were likewise found to be significant. The maximum chlorophyll content (46.03%) was exhibited under control, while the minimum (5.54%) was with the 5-fold lower dose of mesosulfuron methyl.

3.2 Visual injury (%)

The different doses of broad-spectrum herbicides caused a significant influence on visual injury to the weed mixture compared with the weedy check (Table 1). A total of 100% of the plants were injured when broad-spectrum herbicide NPs were used at the recommended commercial herbicide dosage. No injury was documented under control (100% alive plants). Similar injury to the weed mixture was observed for the 10-fold lower dose and the recommended dose of normal herbicide (87.00% and 83.83%, respectively). The NPs of the two herbicides exhibited a significant influence on the visual injury of the weed mixture. The maximum visual injury (70.76%) was recorded with the application of nano mesosulfuron methyl and the minimum (65.05%) with mesosulfuron methyl + florasulam + MCPA isooctyl NPs. The interactive effect of the herbicide NPs and their different doses (nano herbicides × doses) was found significant. Meanwhile, the application of NPs of mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl at the normal herbicides recommended dose resulted in 100% injury to weeds as compared to the control (0% visual injury).

3.3 Mortality (%)

The NPs of the two broad-spectrum herbicides caused a significant effect on the mortality percentage of the weed

Table 1: Effect of nanoparticles of mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl on chlorophyll contents (%), and visual injury (%) of mixture of weeds

Doses of nanoparticle of herbicides	Chlorophyll contents (%)			Visual injury (%)		
	Mesosulfuron methyl	Mesosulfuron methyl + florasulam + MCPA isooctyl	Mean	Mesosulfuron methyl	Mesosulfuron methyl + florasulam + MCPA isooctyl	Mean
D_0	45.23^{NS}	45.74	45.49^a	0.00^f	0.00^f	0.00^E
D_1	9.29	11.57	10.43^c	86.33^{ab}	81.33^b	83.83^B
D_2	No weed	No weed	**No weed**	100.00^a	100.00^a	100.00^A
D_3	5.54	5.97	5.75^d	91.33^{ab}	90.66^{ab}	91.00^{AB}
D_4	8.99	10.33	9.66^{cd}	87.00^{ab}	87.00^{ab}	87.00^B
D_5	14.72	18.97	16.84^b	76.00^{bc}	60.00^{cd}	68.00^C
D_6	17.43	23.72	20.57^b	54.67^d	36.33^e	45.50^D
Mean	16.87^B	19.38^A		70.76^A	65.05^B	
HSD at 5%	**Doses = 4.03, herbicides = 1.55 Doses × herbicides = ^{NS}**			**Doses = 9.88, herbicides = 3.40 Doses × herbicides = 16.13**		

D_0 = weedy check, D_1 = normal herbicides at recommended dose, D_2 = nanoparticles of herbicides at recommended dose of normal herbicide, D_3 = 05-fold lower dose of nanoparticles of herbicides, D_4 = 10-fold lower dose of nanoparticles of herbicides, D_5 = 15-fold lower dose of nanoparticles of herbicides, D_6 = 20-fold lower dose of nanoparticles of herbicides.
In the same column, means with the same letter did not significantly differ at the 5%.

mixture as presented in Table 2. When applied at the recommended dose of normal herbicides, nanoherbicides resulted in maximum mortality (100%). The reduction in the dose of NPs resulted in a decrease in the mortality of weeds, and minimum mortality (0%) was observed under control. A similar mortality effect was exhibited with the application of nanoherbicides at the 10-fold lower dose and suggested dose of the standard herbicide (83.33% and 80.55%, respectively). Between the two herbicides NPs, maximum (68.25%) and minimum (62.70%) mortality were observed by the mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl, respectively. The interaction effect of NPs × various doses was also significant. The 100% mortality was shown with NPs of mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl at the suggested dose of standard herbicide and 0% under control. This 100% mortality of the weed mixture observed with the application of the NPs of two herbicides may have resulted from the excessive penetration of the herbicides into weeds due to the nanosized particles, which killed all weeds compared to the control.

3.4 Plant height (cm)

The influence of using the herbicides NPs on the plant height of the weed mixture is illustrated in Table 2. The results revealed that different doses of NPs affected the plant height. The minimum height (2.35 cm) for the weed mixture was noted by applying the NPs at a 5-fold lower dose, and the maximum (9.14 cm) was recorded for the control. The taller plants (7.43 cm) and shorter ones (7.33 cm) were noticed with the NPs of mesosulfuron methyl + florasulam + MCPA isooctyl and mesosulfuron methyl, respectively. The interaction effects of doses and NPs were significant. The shorter plants (4.63 cm) of the mixture were observed with the application of the mesosulfuron methyl NPs at a 5-fold lower dose of standard herbicide, and the taller ones (9.15 cm) were observed for the weedy check with the application of the same NPs. This study found that exposure to NPs of herbicides at the approved dose of conventional herbicides affected the height of the surviving plants.

3.5 Fresh biomass (g)

The influence of the manufactured chitosan-based NPs on fresh biomass of the weed mixture is shown in Table 3. The results illustrated that the fresh biomass was affected by various doses of nanoherbicides. The maximum fresh biomass (6.48 g) was recorded at D_0, while increasing the nanoherbicides doses reduced the fresh biomass. The minimum fresh biomass (0.58 g) was documented at the

Table 2: Effect of nanoparticles of mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl on mortality (%) and plant height (cm) of the mixture of weeds

Doses of nanoparticle of herbicides	**Mortality (%)**			**Plant height (cm)**		
	Nanoparticles of mesosulfuron methyl	**Nanoparticles of mesosulfuron methyl + florasulam + MCPA isooctyl**	**Mean**	**Nanoparticles of mesosulfuron methyl**	**Nanoparticles of mesosulfuron methyl + florasulam + MCPA isooctyl**	**Mean**
D_0	0.00^{f}	0.00^{f}	0.00^{E}	9.15^{NS}	9.13	9.14^{A}
D_1	83.33^{ab}	77.77^{b}	80.55^{B}	6.83	7.10	6.97^{AB}
D_2	100.00^{a}	100.00^{a}	100.00^{A}	No weed	No weed	**No weed**
D_3	88.89^{ab}	88.88^{ab}	88.88^{B}	4.63	4.77	4.70^{B}
D_4	83.33^{ab}	83.33^{ab}	83.33^{B}	6.70	6.97	6.83^{AB}
D_5	72.22^{bc}	55.55^{cd}	63.88^{C}	7.99	8.08	8.03^{A}
D_6	50.00^{de}	33.33^{e}	41.66^{D}	8.47	8.57	8.52^{A}
Mean	68.25^{A}	62.70^{B}		7.33^{NS}	7.43^{A}	
HSD at 5%	**Doses = 10.53, herbicides = 3.62** **Doses × herbicides = 17.18**			**Doses = 2.97, herbicides = 1.14** **Doses × herbicides = ^{NS}**		

D_0 = weedy check, D_1 = normal herbicides at recommended dose, D_2 = nanoparticles of herbicides at recommended dose of normal herbicide, D_3 = 05-fold lower dose of nanoparticles of herbicides, D_4 = 10-fold lower dose of nanoparticles of herbicides, D_5 = 15-fold lower dose of nanoparticles of herbicides, D_6 = 20-fold lower dose of nanoparticles of herbicides.
In the same column, means with the same letter are not significantly different at the 5%.

Table 3: Effect of nanoparticles of mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl on fresh biomass (g) and dry biomass of mixture of weeds

Doses of nanoparticle of herbicides	Fresh biomass (g)			Dry biomass (g)		
	Nanoparticles of mesosulfuron methyl	Nanoparticles of mesosulfuron methyl + florasulam + MCPA isooctyl	Mean	Nanoparticles of mesosulfuron methyl	Nanoparticles of mesosulfuron methyl + florasulam + MCPA isooctyl	Mean
D_0	6.44^a	6.52^a	6.48^A	1.88^a	1.90^a	1.89^A
D_1	0.91^e	1.26^e	1.08^D	0.28^{ef}	0.38^{ef}	0.33^D
D_2	No weed	No weed	**No weed**	No weed	No weed	**No weed**
D_3	0.55^e	0.58^e	0.57^D	0.16^f	0.17^f	0.16^D
D_4	0.89^e	0.93^e	0.91^D	0.27^{ef}	0.29^{ef}	0.28^D
D_5	1.62^{de}	2.59^{cd}	2.10^C	0.49^{de}	0.79^{cd}	0.64^C
D_6	2.90^{bc}	3.87^b	3.39^B	0.86^c	1.18^b	1.02^B
Mean	2.22^B	2.63^A		0.66^B	0.78^A	
HSD at 5%	**Doses = 0.63, herbicides = 0.24**			**Doses = 0.18, herbicides = 0.07**		
	Doses × herbicides = 1.04			**Doses × herbicides = 0.08**		

D_0 = weedy check, D_1 = normal herbicides at recommended dose, D_2 = nanoparticles of herbicides at recommended dose of normal herbicide, D_3 = 05-fold lower dose of nanoparticles of herbicides, D_4 = 10-fold lower dose of nanoparticles of herbicides, D_5 = 15-fold lower dose of nanoparticles of herbicides, D_6 = 20-fold lower dose of nanoparticles of herbicides.
In the same column, means with the same letter are not significantly different at the 5%.

5-fold lower dose of the NPs. Similar effects were detected with the normal herbicide recommended doses and the 10-fold lower dose of NPs, which were recorded as 1.08 and 0.91 g, respectively. The NPs of mesosulfuron methyl + florasulam + MCPA isooctyl and mesosulfuron methyl showed a significant effect on the fresh biomass with maximum (2.63 g) and minimum (2.22 g) fresh biomass for both loaded NPs, respectively. The interactive effect of herbicide-loaded NPs and their different doses were further significant. The addition of 5-fold lower dose of mesosulfuron methyl resulted in minimum fresh biomass (0.57 g), while the maximum fresh biomass (6.52 g) was observed under control (no dose application).

3.6 Dry biomass (g)

The different doses of broad-spectrum herbicide-loaded NPs produced a statistically significant influence on the dry biomass of the weed mixture (Table 3). The minimum dry biomass weight (0.16 g) was observed with a 5-fold lower dose of NPs, and the maximum (1.89 g) was shown under control. The recommended dose of normal herbicides and the 10-fold lower dose of the loaded NPs produced a nonsignificant effect on dry biomass (0.33 and 0.28 g, respectively). Herbicide-loaded mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl NPs significantly influenced the dry biomass. The mesosulfuron methyl + florasulam + MCPA isooctyl-loaded NPs was less toxic compared to the mesosulfuron methyl loaded ones reflecting the maximum (0.78 g) and the minimum (0.68 g) dry biomass, respectively. The interaction of various doses and nanoherbicides was similarly significant. The 5-fold lower dose of mesosulfuron methyl results in the minimum (0.16 g) and mesosulfuron methyl + florasulam + MCPA isooctyl at 0 g a.i ha^{-1} produced the maximum dry biomass (1.90 g) of the weed mixture.

3.7 SEM

The shape and surface morphology of the chitosan-based NPs of mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl were studied using SEM. Figures 1 and 2 demonstrated that the average particle size was 40–70 nm in a cluster form with the porous structure. This could be because of the existence of some toxic compounds. The size range of the NPs was 40 and 70 nm, respectively.

3.8 Fourier transform infrared spectroscopy

FT-IR analysis was used to examine the physical and chemical compatibilities of the mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl herbicide-loaded NPs

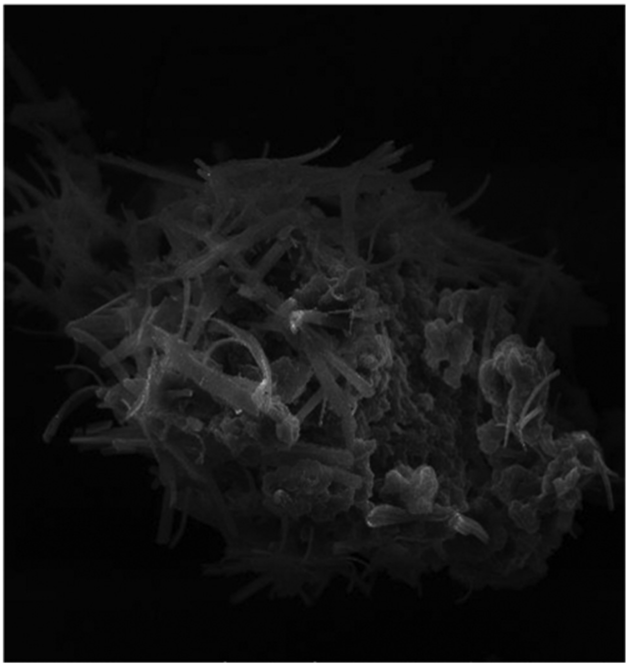

Figure 1: SEM and SEM-EDX micrograph of chitosan-based mesosulfuron methyl.

made of chitosan. The FT-IR spectra of the nanoherbicides were illustrated in Figures 3 and 4. The main functional groups in the FT-IR region were between 649 and 2,923 cm^{-1}. Free and

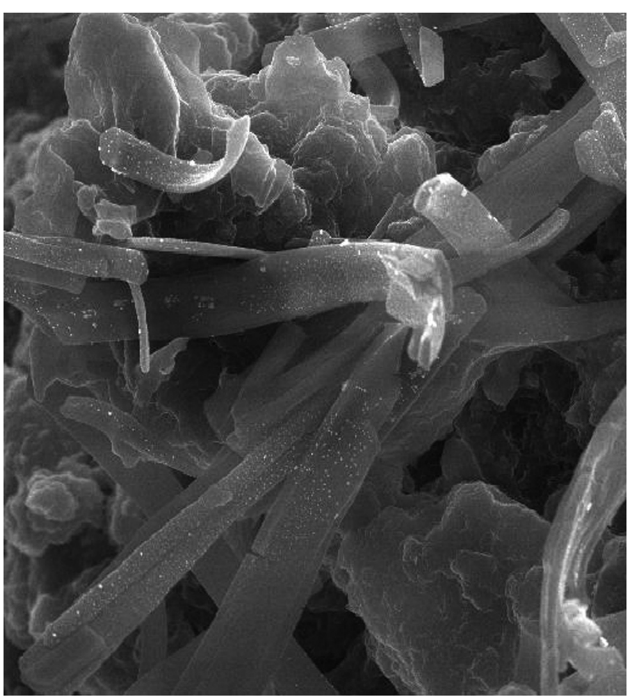

Figure 2: SEM and SEM-EDX micrograph of chitosan-based mesosulfuron methyl + florasulam + MCPA isooctyl.

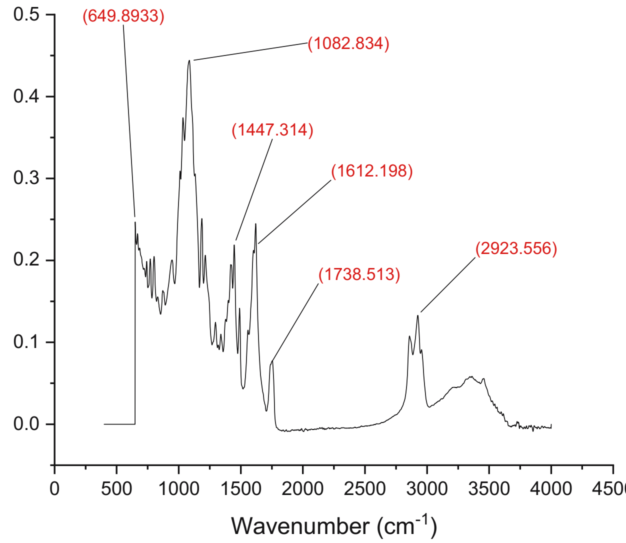

Figure 3: FT-IR of chitosan-based mesosulfuron methyl.

esterified carboxyl groups were designated by carbonyl bands in the 649–880 and 1,082–1,320 cm^{-1} regions, respectively. Ether's presence led to the formation of the absorption band at 1,447–1,520 cm^{-1}. Meanwhile, the C–C cyclic bonds in the mesosulfuron methyl were responsible for the band between 1,612 and 1,750 cm^{-1}. The polymeric O–H stretching band was responsible for the wide band between 1,690 and 2,923 cm^{-1}, whereas the carboxyl group's O–H stretching band was visible at 2,800 cm^{-1} [33]. Furthermore, the FT-IR spectra of the mesosulfuron methyl + florasulam + MCPA isooctyl noticeably revealed the main functional groups in the mesosulfuron

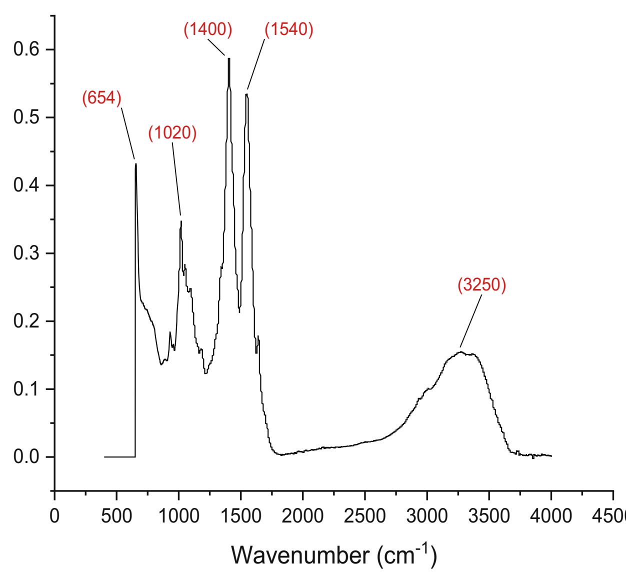

Figure 4: FT-IR of chitosan-based mesosulfuron methyl + florasulam + MCPA isooctyl.

methyl + florasulam + MCPA isooctyl FT-IR region between 654 and 3,250 cm^{-1}. In the areas of 654–810 and 1,020–1,270 cm^{-1}, respectively, carbonyl bands identified free and esterified carboxyl groups. Ether was responsible for the absorption band between 1,400 and 1,510 cm^{-1}, while the C–C cyclic bonds in the mixture of mesosulfuron methyl + florasulam + MCPA isooctyl produced the band between 1,540 and 3,250 cm^{-1}.

3.9 UV-visible (UV-Vis) absorption spectrum

The ultraviolet (UV)-Vis absorption spectrum of the chitosan-based NPs of mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl was presented in Figures 5 and 6, respectively. The peaks of maximum absorption of mesosulfuron methyl NPs were 330 nm. Meanwhile, the maximum absorption peaks of mesosulfuron methyl + florasulam + MCPA isooctyl NPs were 330 and 360 nm. This distinctive signature displayed the NPs formation.

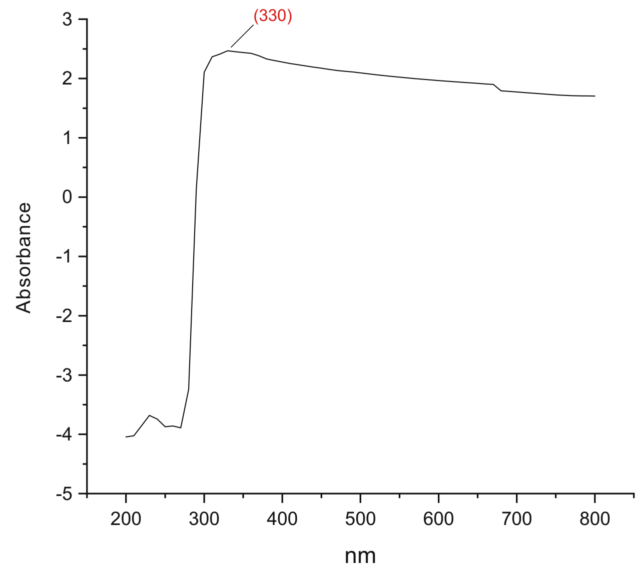

Figure 6: UV-Vis absorption spectrum of chitosan-based mesosulfuron methyl + florasulam + MCPA isooctyl.

3.10 XRD analyses

Crystallinity and crystallite size of the NPs were tested by acquiring the corresponding XRD patterns (Figures 7 and 8). It was perceived that the chitosan-based NPs of mesosulfuron methyl revealed a rigorous peak around 2θ value of 30.55°, which corresponded to the 78 planes of the anatase phase. In addition, various additional minor peaks were further detected at 2θ values of 20.56°, 24.56°, 28.70°, 32.68°, 38.32°, 43.60°, 47.27°, and 56.16°, which corresponded to (68), (63), (57), (38), (42), (40), (32), and (28) planes of the anatase phase. In the case of NPs of mesosulfuron methyl + florasulam + MCPA isooctyl, it demonstrated an intensified peak around 2θ value of 32.79°, which corresponded to the 198 planes of the anatase phase, and various additional ones at 2θ values of 25.66°, 30.03°, 34.48°, 50.10°, and 58.55° corresponding to (143), (165), (142), (83), and (104) planes of the anatase phase.

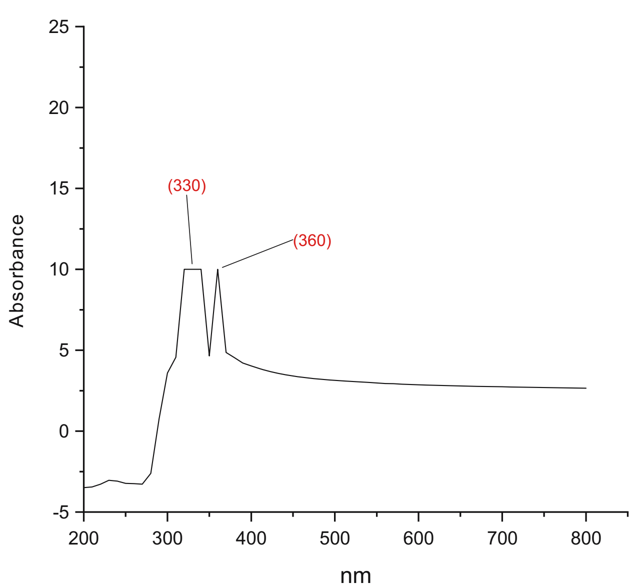

Figure 5: UV-Vis absorption spectrum of chitosan-based mesosulfuron methyl.

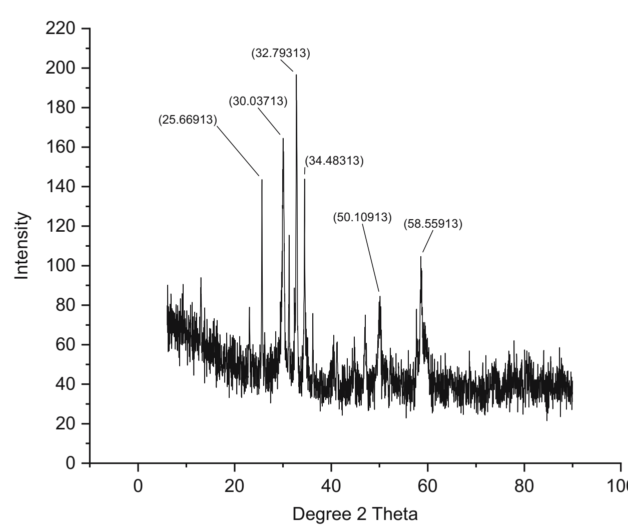

Figure 7: XRD analyses of chitosan-based mesosulfuron methyl.

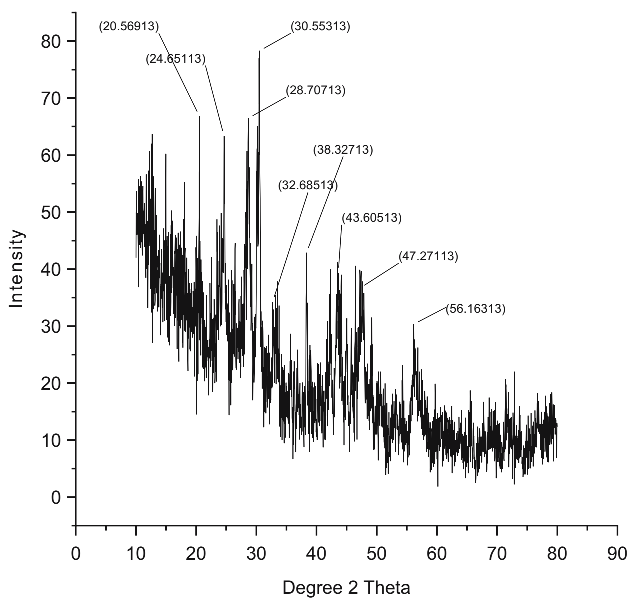

Figure 8: XRD analyses of chitosan-based mesosulfuron methyl + florasulam + MCPA isooctyl.

4 Discussion

As shown by the total growth suppression of a combination of wheat weeds, the application of nanoherbicides of mesosulfuron methyl and mesosulfuron methyl + florasulam + MCPA isooctyl improved herbicide efficacy compared to commercial herbicides at suggested doses. When herbicides are applied at the authorized dose and 10-fold lower doses, similar effects on the development of a combination of weeds were observed. The increased activity of chlorophyllase, the chlorophyll-breaking down enzyme, disruption of chloroplast fine structure, and chloroplast volatility that results in chlorophyll oxidation and reduction of its concentration in plants may be the cause of reduced chlorophyll content of the mixture of weeds after applying the NPs of both broad-spectrum herbicides. Similar outcomes were observed when atrazine-loaded nanocapsules were applied at lower doses, which resulted in a greater reduction in photosystem II activity than the original formulation of atrazine at a similar concentration in *Amaranthus viridis* and *Bidens pilosa* [33]. The findings of this work are consistent with those of ref. [9], who noted the decrease in the chlorophyll concentration with the application of NPs of fenoxaprop-*p*-ethyl and clodinofop propagyl because it was assimilated into cell membrane function via physiological processes including membrane depolarization. In comparison to commercial herbicides, the application of herbicide-loaded NPs regulates weeds at a lower dose, according to the findings of other researchers [9,19].

When nanoherbicides were applied at the acceptable dose of conventional herbicides, a larger harm to the weed mixture was investigated. There was no change in injury under weedy check. The indicated regular herbicide doses and 10-fold lower doses of both nanoherbicides showed similar effects on injury to the combination of weeds. The nanoherbicides applied topically to the weed mixture caused 100% damage, which may be related to depolarization of the membrane and further delay of cell membrane processes [34]. These results are consistent with the findings of ref. [19], which showed that the application of nanoherbicides manages weeds at a lower dose than that of commercial herbicides. Thus, herbicide-loaded NPs can be utilized to lessen herbicide use, increase efficacy, and make it less toxic to the environment. The findings of this work are consistent with those of ref. [9], who mentioned that the increase in visual injury to *P. minor* was observed with applying the NPs of fenoxaprop-*p*-ethyl and clodinofop propagyl.

Applying herbicide-loaded NPs at the suggested dose of regular herbicides resulted in a 100% mortality of a mixture of weeds, which may be explained by the improved effectiveness of the NPs' penetration into the weeds relative to the control. When regular herbicides at the suggested dose and 10-fold lower doses of both herbicides' NPs were applied, similar effects on mortality to the weed mixture were recorded. In comparison to non-nano formulations, metsulfuron methyl, diuron-loaded carboxymethyl, pectin, and metolachlor-loaded NPs have been shown to have a greater herbicidal effect [11,30,31].

This study showed that exposure to NPs of herbicides at the prescribed dose to regular herbicides affected the height of surviving plants. When standard herbicides were applied at the indicated dose and 10-fold lower doses, a similar effect on the height of a combination of weeds was seen. This might be because there is less photosynthesis and less activity in other metabolic processes, which causes the plants that survive to grow less tall than the control plants. These results are also consistent with [35], who found that the 10-fold dilution of atrazine-loaded poly (ε-caprolactone) (PCL) nanocapsules had similar suppressive effects on *A. viridis* and *B. pilosa* growth characteristics.

Transformation of regular herbicides into NPs amended their performance by increasing the charge-to-mass ratio of the herbicides, which in turn increased penetration, effectiveness, and decreased the fresh biomass of a variety of weeds. The two broad-spectrum herbicides under consideration had statistically identical harmful effects on a mixture of weeds when used at doses up to ten times lower than those used in commercial formulations. The results are corroborated by ref. [34], who showed that atrazine-loaded

PCL nanocapsules were further successful in reducing the growth of the shoot and root of *B. Pilosa* than commercial atrazine at a 10-fold lower dose, which eventually resulted in a decrease in plant biomass [33]. The findings of this work are consistent with those of ref. [9], who noted that the reduction in plant height, fresh biomass, and dry biomass of *P. minor* was observed when NPs of fenoxaprop-*p*-ethyl and clodinofop propagyl were applied. The clustered form with the porous structure and round shape of the produced NPs reported in this work was supported by numerous investigations [36], and it could be because of the harmful substances.

The C–C cyclic bonds in the mesosulfuron methyl were responsible for the band between 1,612 and 1,750 cm^{-1}. The polymeric O–H stretching band was responsible for the wide band between 1,690 and 2,923 cm^{-1}, while the carboxyl group's O–H stretching band was seen at 2,800 cm^{-1} [37,38]. The suitable existence of herbicide in the nanoformulation was confirmed by the FT-IR spectra of the herbicide-loaded NPs of both herbicides under examination. Mesosulfuron methyl NPs have 330 nm maximum absorption peaks. Mesosulfuron methyl + florasulam + MCPA isooctyl had the highest absorption peaks at 330 and 360 nm.

To effectively administer chemical or biological pesticides, nanotechnological preparations or herbicide formulations based on nanomaterials are used to create nanoherbicides. In comparison to conventional herbicides, formulations based on nanomaterials or nanostructures could increase the solubility, boost the efficacy, and decrease the toxicity of the herbicide. Early weed control utilizing NP-based herbicide release systems could lower the risk of herbicide resistance, preserve the active ingredient's action, and extend their slower release [18]. The creation of a particular herbicide molecule enclosed in a NP is directed to attach with certain receptors existed in the root of the aimed weed. The created NP enters the root and transports to perform the effect, which in turn prevents roots from undergoing glycolysis. This action instigates the plant to famish, consequently dyes. These nanoherbicides may also be utilized in rain-fed locations where insufficient soil moisture causes herbicides to evaporate. Weeds can be eliminated with the aid of the controlled release of herbicides via encapsulation.

5 Conclusion

NPs can function as effective carriers and, when combined with herbicides, can create nanoformulations. These nanoformulations aid in solving the primary problem facing the herbicide sector, such as the development of plants that resist herbicides. The ease in preparation of chitosan-loaded herbicide complex contributes with better release characteristics can noticeably alter the herbicide applications. The chitosan nanoherbicides can change the herbicide interaction with soil and effectively avoid the unfavorable consequences of the herbicide.

Overall, the findings of this study showed that mesosulfuron methyl and mesosulfuron methyl + floresulam + MCPA isooctyl NPs based on chitosan may be employed for 100% control of both narrow and broad-leaved weeds. This study also compared the effectiveness of weed control between chitosan-based NP herbicides with no risk of environmental pollution and commercial herbicides at a recommended dose and a 10-fold lower dose.

According to the results of this study, D_1 = normal herbicide at the recommended dose, D_2 = nano herbicide at the recommended dose of normal herbicide, D_3 = 5-fold lower nano herbicide dose, D_4 = 10-fold lower nano herbicide dose, and D_5 = 15-fold lower nano herbicide dose were found to be effective in the management of mixture of weeds. As a result, these doses will be examined in more detail in the field.

It is worth to mention that although nanopesticides may have several advantages, they are currently in the early stages of research. Unfortunately, there are less studies on the assessment of the environmental safety of polymer-based nanopesticides, which is due to the absence of standardized methods to evaluate the environmental risk of nanopesticides for regulatory purposes. There is always a need for a practical evaluation technique by adopting recommendations obtained from the ecological risk assessment of conventional pesticide products.

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