Original Research Article

Synthesis, Characterization of Pendimethalin loaded PVA Nanofiber and its impact on Blackgram Sintesis,

ABSTRACT

Nanotechnology operates at the nanoscale, offering precise manipulation and enhancing materials, applications, and performance across various domains. It holds promise in addressing weed-related losses, with nanofibers emerging as a noteworthy option among diverse nano-formulations. This research focuses on crafting pendimethalin-loaded PV
The nanofiber structure and successful pendimethalin integration were examined using SEM and FTIR analyses. C
gram seeds with pendimethalin-infused PVA nanofibers, the study assessed their impact on growth and germinatic
indicated a rise in fibre diameter post-loading in PVA nanofiber-coated seeds (from 118.3 nm to 332.9
pendimethalin-loaded PVA nanofiber coated seeds (from 339.8 nm to 664.5 nm). FTIR results validated pendimeth
within PVA nanofibers. Germination test demonstrated that herbicide-loaded nanofiber coatings didn't hinder g
growth. In summary, synthesizing herbicide-loaded nanofibers and their seed coating presents a pioneering concept, warranting further exploration for its potential in weed seed germination and control.

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Keywords: Nanotechnology, SEM analysis, FTIR analysis, Polyvinyl alcohol

1. INTRODUCTION

Weeds, deemed as undesirable flora, are known to impede the growth of crops. Weeds are responsible for causing significant reductions in yield and a decline in the quality of crops and grains. Studies have estimated that weeds account for one-third of all agricultural pest-related yield losses [1]. Pulses are valuable protein sources for those relying on grain-based diets or facing financial constraints [2]. Black gram (*Vigna mungo*), an important legume crop in tropical areas and is vulnerable to weed infestations in periods of heavy rainfall, which can result in significant reductions in crop yield. Studies reveal that uncontrolled weeds can decrease black gram yield by 35.2% to -87% [3], [4], [5]. Black gram struggles against weeds, especially in its initial four weeks, making effective weed management crucial for optimal growth [6]. To ensure successful cultivation, timely weed control is essential during the critical crop-weed competition period.
The shortage of labor and rising wages for agricultural workers have prompted farmers to turn to chemical weed control, with herbicides comprising 47.5% of a total 2 mt pesticide usage [7]. However, using herbicides comes with drawbacks such as weed resistance, shifts in weed populations, herbicide accumulation in soil, and harm to subsequent crops. Additionally, these chemicals contribute to environmental pollution [8].Nano-technology offers a potential solution to mitigate weed-related losses. Nano-herbicides present farmers with a promising avenue for managing annual, perennial, and parasitic weeds. They can be incorporated into the soil or applied to weed plants without the need for excessive chemical use, thereby avoiding the accumulation of toxic residues and related environmental concerns [9]. Nanofibers are

a novel technology that is gaining prominence among the wide range of nano formulations available. Nanofibers carrying herbicides excel over regular nano herbicides with precise targeting, gradual release for prolonged impact, and minimal harm to non-target organisms. This innovation enhances efficacy and combats resistance, promising greener and more effective weed control. Through a sophisticated delivery system, it lowers herbicide application rates, curbing weed resistance, decreasing environmental pollution, and minimizing CO_2 emissions. Given the promising capability of electro-spun nanofibers to precisely deliver active ingredients, our study focused on encapsulating pendimethalin herbicide. The aim of this study was to synthesize herbicide-loaded nanofiber coating on black gram seeds and its effect on germination and growth.

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2. MATERIAL AND METHODS

2.1. Materials

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Black gram seeds were purchased from the Department of Pulses, TNAU, Coimbatore. Polyvinyl alcohol having a molecular weight of 1,15,000 g/mole was procured from M/s. Astron Chemicals, located in Naroda, Ahmedabad. Pendimethalin technical material was purchased from Sigma Aldrich Chemicals Pvt. Ltd., Bangalore. Black gram seeds, Polyvinyl alcohol, and Pendimethalin are the required materials for this study.

2.2 Methods

2.2.1 Preparation of Polymer (PVA) solution

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Nanofibers were developed using PVA (polyvinyl alcohol) polymer. Different concentrations of polymer solution such as 7,8,9,10 (w/v) were prepared by dissolving 7,8,9 and 10g of PVA in 100 ml distilled water. The solution was stirred constantly using a magnetic stirrer (Tarsons Spinot Digitals) at 120 rpm under room temperature until a clear homogenous solution was obtained. These solutions were electro-spun at a constant voltage of 15kV with a flow rate of 0.6ml per hour with a constant tip-to-collector distance of 15cm. The fibers were characterized using a Scanning Electron Microscope.

2.2.2 Preparation of Pendimethalin stock solution

400 ppm stock solution of pendimethalin was formulated by dissolving 10mg of the compound in 25ml of acetone solvent. Sequential dilutions were then performed, initially obtaining a 100ppm solution from the stock. Further serial dilutions were performed using the 100ppm solution to get the working standard concentrations of 25 ppm, 50 ppm, and 75 ppm, establishing a diverse range of concentrations for subsequent experimental evaluation**.**

2.2.3 Optimizing the concentration of the polymer solution for synthesizing the nanofiber

The optimal concentration for making the nanofiber is based on the viscosity of the polymer solution and the formation of smooth fibers with any formation of beads.

2.2.4 Preparation of the electro-spun mixture for the loading of pendimethalin

The electro-spun mixture was prepared by mixing different concentrations of pendimethalin from the working standard solutions with the optimized polymer solution. PVA solution of 20% was mixed with the pendimethalin standards in the following way as explained below in order to obtain the required concentration of 10% PVA solution and pendimethalin of 25, 50, and 75 ppm (Table 1).

Table 1. Electro-spun mixture for the loading of herbicide

S.No	Pendimethalin stock solution	Pendimethalin concentration (ppm)	Quantity of Pendimethalin stock solution added (ml)	Quantity of 20% PVA added (ml)	The total quantity of solution used for electrospinning (ml)
1	A	50 ppm	5 ml	5 ml	10ml
2	B	100 ppm	5 ml	5 ml	10ml
3	C	150 ppm	5 ml	5 ml	10 ml

The fiber was formed with coaxial method of electrospinning using two 5ml syringe pumps. In syringe pump 1, the syringe is filled with a solution containing pendimethalin, and in syringe pump 2, the syringe is filled with a PVA polymer solution. In syringe pumps 1 and 2, the flow rate is maintained at 0.4ml/hr and 0.6ml/hr respectively. Voltage is maintained constantly at 25kv with a tip-to-collector distance of 15cm. The formed Nanofibers loaded with herbicide are characterized using an SEM instrument.

Fig. 1. Co-axial method of electrospinning

2.2.5 Characterization of Nanofiber

2.2.5.1 Scanning Electron Microscope

The sample surface is imaged via a Scanning Electron Microscope, utilizing a beam of high-energy electrons in a raster scanning pattern. In order to facilitate sample imaging, samples are mounted onto a specimen stub with the use of carbon tape and then placed within the specimen chamber. The surface morphology of herbicide-loaded nanofibers was analyzed by an SEM instrument available at the Centre for Agricultural Nanotechnology (Quanta 250, FEI, and the Netherlands).

2.2.5.2 Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is a robust analytical method utilized for studying molecular vibrational patterns. It relies on the principle that molecules absorb infrared light at specific frequencies corresponding to their chemical bond vibrations. FTIR exposes a sample to a wide infrared spectrum and measures absorbed light intensity against wavelength. This technique reveals the sample's functional groups, aiding in identifying compounds and uncovering structural details. FTIR was applied to confirm the presence of herbicide molecules in PVA nanofibers.

2.2.6 Effect of nanofiber encapsulated pendimethalin coating on black gram seeds

The seeds were coated with a polyvinyl polymer solution at 4ml/hr as the flow rate, using 15kv voltage and 18rpm as speed, and further by using the co-axial coating technique, black gram seeds were coated with the e-spun solution in a vertical electrospinning unit. This method involved two 10ml syringes: one held a solution of pendimethalin in acetone, while the other contained a PVA polymer solution. These syringes were positioned at pumps 1 and 2, each maintaining flow rates of 2ml/hr and 4ml/hr respectively. The applied voltage was 15kV, and the seed coating drum revolved at 18rpm. The consistent coating rate was 5ml/kg of seeds. Subsequently, the coated seeds were subjected to assessment for parameters like germination percentage, vigor index, dry matter production, root length, and shoot length of the resulting seedlings, employing a roll paper towel method.

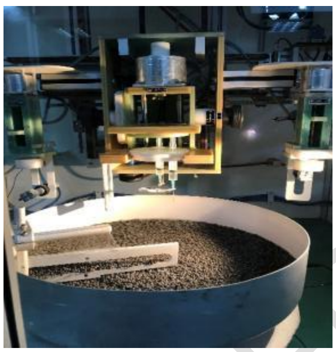


Fig. 2. Vertical electrospinning unit for coating seeds with fibre

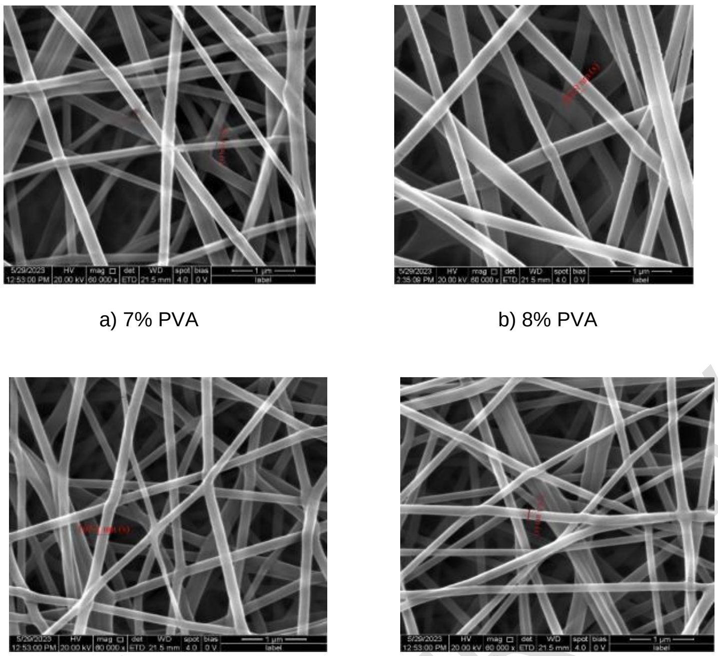
2.3 Statistical analysis

The Completely Randomized Block design was utilized in conducting the experiment, wherein the statistical analysis of data was performed and subsequently interpreted at a probability level of 5%.

3. RESULTS AND DISCUSSION

3.1 Development and Characterization of PVA nanofiber

The Nanofibers were developed using polyvinyl alcohol polymer solution at different concentrations of 7,8,9,10 % which have a diameter range from 231.6nm to 313.5nm, 182.7 to 261.2, 143.2 to 210.3, and 115.3 to 158.5 respectively without the formation of any beads (Fig. 3). The optimized concentration for the loading of herbicide was 10% based on some literature in which an increase in PVA concentration raised viscosity, leading to greater chain entanglement. This counteracted surface tension and ultimately led to the formation of smooth and uniform electro-spun nanofibers without bead formation [10]. Reduced viscosity resulted in a higher occurrence of beads due to elevated surface tension and the polymer's low charge density, causing the formation of droplets or bead structures [11].



a) 7% PVA b) 8% PVA

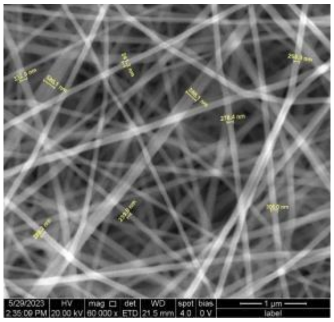
c) 9% PVA d) 10% PVA

Fig.3. SEM morphology of PVA nanofiber

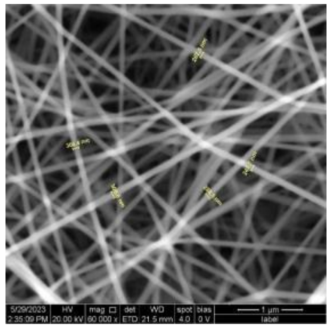
3.2 Encapsulation of Pendimethalin in PVA nanofiber and characterization

3.2.1 Scanning Electron Microscope (SEM)

The fibre diameter tends to be increased after the loading of pendimethalin. The PVA fibre diameter was in the range of 115.3nm to 158.5nm. The diameter of the fibre after the loading of pendimethalin at different concentrations of 25, 50, and 75 ppm ranges from 245.6 to 305.4nm, 291.8 to 445.3nm, and 230.0 to 445.3nm (Fig.4). The fibre diameter of PVA-coated seeds and pendimethalin-encapsulated PVA-coated seeds ranges from 331.2 to 472.1 and 515.6 to 970.3 respectively. This observation finds validation in the research by [12], wherein *Methylobacterium* was incorporated into electro-spun PVA nanofibers. The morphology of electro-spun fibres containing microbial cells (*Methylorubrum aminovorans*) demonstrated an enlargement in fibre diameter attributed to the incorporation of these microorganisms. The morphology of PVA electro-spun fibres loaded with hormones displayed notable changes. GA_3 loading led to an increase in diameter from 131 to 266 nm, while IAA loading resulted in a diameter increase from 106 to 275 nm [13].

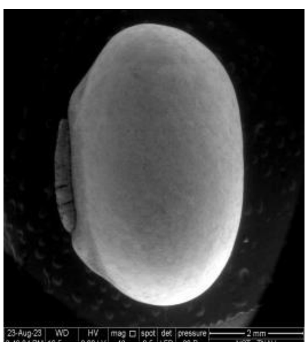


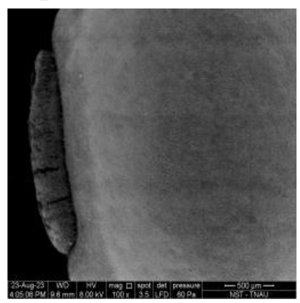
a) 75ppm of Pendimethalin loaded PVA Nano fibre



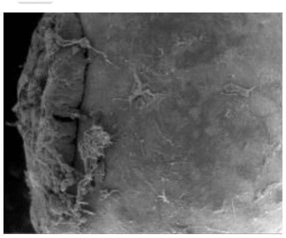
b) 25ppm of Pendimethalin loaded PVA Nano fibre

Fig.4.SEM images of Pendimethalin loaded PVA Nanofiber

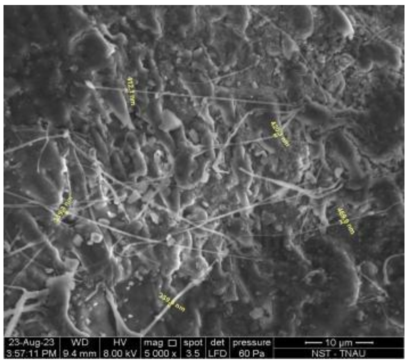




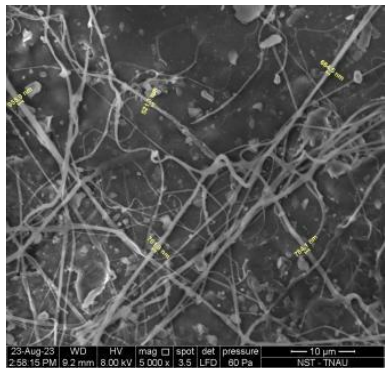
a) Surface morphology of control seed



b) Surface morphology of PVA Nanofiber coated seed



c) SEM image of PVA coated seed



d) SEM image of Pendimethalin loaded PVA coated seed

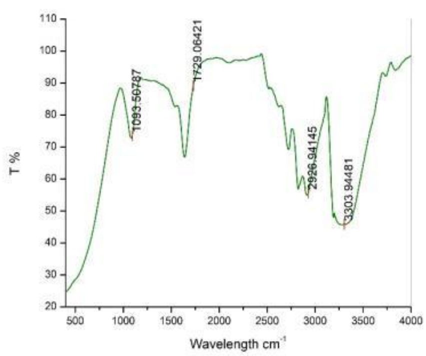
Fig.5. Surface morphology of control seed, PVA coated and Pendimethalin loaded PVA coated seeds

3.2.2 FTIR

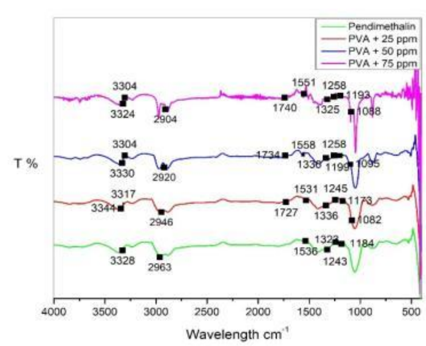
The technical material pendimethalin showed the transmittance peaks at wavelengths of 1184 cm^{-1} and 1243 cm^{-1} (C-N stretch), 1323 cm^{-1} and 1536 cm^{-1} (stretching vibration of NO2 group), 2963 cm^{-1} (C-H stretch) and 3328 cm^{-1} (N-H stretch). The PVA polymer showed transmittance peaks at 1093 cm^{-1} (C-O stretch), 1729 cm^{-1} (C=O stretch), 2927 cm^{-1} (C-H stretch), and 3303 cm^{-1} (O-H stretch). The FTIR spectra of pendimethalin-loaded nanofiber at 25 ppm showed the transmittance peaks at 1082 cm^{-1} (C-O stretch), 1727 cm^{-1} (C=O stretch), 2946 cm^{-1} (C-H stretch) and 3317 cm^{-1} (O-H stretch), 1173 cm^{-1} and 1245 cm^{-1} (C-N stretch), 1336 cm^{-1} and 1531 cm^{-1} (stretching vibration of NO_2 group), 2946 cm^{-1} (C-H stretch) and 3344 cm^{-1} (N-H stretch). PVA nanofiber loaded with pendimethalin @50 ppm have the transmittance peaks at 1095 cm^{-1} (C-O stretch), 1734 cm^{-1} (C=O stretch), 2920 cm^{-1} (C-H stretch) and 3304 cm^{-1} (O-H stretch), 1199 cm^{-1} and 1258 cm^{-1} (C-N stretch), 1336 cm^{-1} and 1558 cm^{-1} (stretching vibration of NO_2 group), 2920 cm^{-1} (C-H stretch) and 3330 cm^{-1} (N-H stretch). Pendimethalin loaded PVA nanofiber @75 ppm exhibited the transmittance peaks at 1088 cm^{-1} (C-O stretch), 1740 cm^{-1} (C=O stretch), 2904 cm^{-1} (C-H stretch) and 3304 cm^{-1} (O-H stretch), 1193 cm^{-1} and 1258 cm^{-1} (C-N stretch), 1325 cm^{-1} and 1551 cm^{-1} (stretching vibration of NO_2 group), 2904 cm^{-1} (C-H stretch) and 3324 cm^{-1} (N-H stretch). The appearance of peaks at 1193,1558,2946,3330 cm^{-1} corresponds with the functional groups C-N, NO_2, C-H, and N-H, respectively which demonstrates the loading of pendimethalin molecules in PVA nanofibers. These results are in accordance with the study of [14] in which they prepared the pendimethalin microcapsules through microfluidic technology and also from [15] in which they analyzed the FTIR spectra of the pendimethalin compound for their controllable release and bioactivity.

Table 2. **FTIR spectrum of Pendimethalin loaded PVA Nanofibre**

Functional group	PVA nanofibre (cm^{-1})	Pendimethalin compound (cm^{-1})	PVA nanofibre+Pendimethalin @25ppm(cm^{-1})	PVA nanofibre + Pendimethalin @50ppm (cm^{-1})	PVA nanofibre + Pendimethalin @ 75ppm (cm^{-1})
O-H stretch	3303		3317	3304	3304
C-H stretch	2927	2963	2946	2920	2904
C=O stretch	1729		1727	17341	1740
C-O stretch	1093		1082	1095	1088
N-H		3328	3344	3330	3324
NO2		1536	1531	1558	1551
		1323	1336	1336	1325
C-N		1243	1245	1258	1258
		1184	1173	1199	1193



FTIR spectra of PVA nanofiber



FTIR spectra of PVA nanofiber

Fig .6. FTIR spectrum

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FTIR spectra of pendimethalin and pendimethalin-loaded PVA nanofiber

3.3 Effect of Nanofiber coated black gram seeds on different quality parameters

The pendimethalin-encapsulated nanofiber-coated seeds germinated equally as the non-coated seeds (control). The control recorded a germination percentage of (81%), root length (19.20 cm), shoot length (10.52 cm), dry matter production (124.26 g per 10 seedlings), and vigor index (2115.75). Among the different concentrations of pendimethalin, 75 ppm-coated seeds recorded the germination percentage (80%), root length (19.24 cm), shoot length (10.1cm), dry matter production (123.12 g per 10 seedlings), and vigor index (2111.75) which is on par with the other treated seeds (Table 3 & Fig.8).

The seeds are enveloped in herbicide-infused PVA nanofibers, boasting a larger surface area compared to their uncoated counterparts. This facilitates enhanced water absorption, accelerating the imbibition process and consequently promoting earlier germination compared to untreated seeds. These results are in accordance with [16] who stated that the substantial surface area relative to volume and diminutive particle dimensions enhances the accessibility of encapsulated active components. Similarly, some of the literature reported that seeds coated with PVA nanofibers containing microorganisms and hormones resulted in improved germination, root and shoot length, seedling vigor, dry matter production, plant biomass, root volume, nodule numbers, and nodule weight in legumes [12], [13].

Fig.7. Comparison of Pendimethalin encapsulated PVA nanofiber-coated blackgram seeds with uncoated blackgram seeds

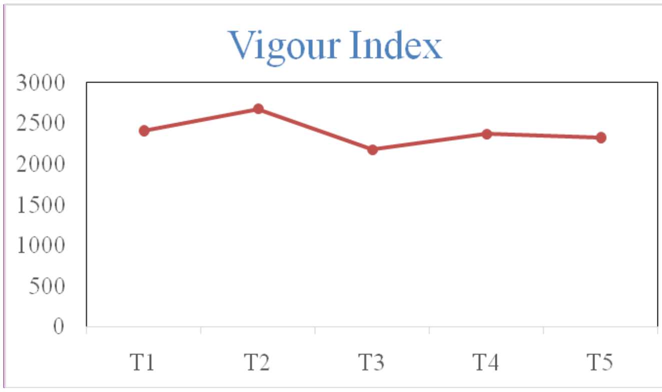
Table 3. Effect of pendimethalin encapsulated nanofiber coating on the quality of black gram

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	DMP (g/10 seedlings)	Vigour Index
Control	81 (62.40)	10.52	19.20	124.26	2115.75
PVA nanofiber-coated seeds	83 (65.29)	11.80	20.35	134.62	2496.50
PVA nanofiber + Pendimethalin @ 25 ppm	80 (62.42)	10.10	19.45	125.65	2112.25
PVA nanofiber + Pendimethalin @ 50 ppm	81 (61.72)	10.20	18.95	124.60	2123.75
PVA nanofiber + Pendimethalin @ 75 ppm	80 (62.75)	10.10	19.24	123.12	2111.75
Mean	81 (62.19)	10.59	19.44	126.45	2192
SEd	4.42	0.119	0.173	3.057	89.34
CD (P=0.05)	13.579	0.253	0.368	6.514	190.383

a) Control

b) 75 ppm pendimethalin + PVA nanofiber coated seeds

Fig.8.Germination of black gram untreated seeds (A) compared with 75 ppm of pendimethalin-loaded PVA nanofiber-coated seeds (B)



Treatments

Fig .9. Vigor Index

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4. CONCLUSION

The use of nanofibers carrying diverse compounds offers a revolutionary edge in agriculture, ensuring precise delivery of active agents like pesticides, fertilizers, and growth regulators. This innovation optimizes resource efficiency, diminishes environmental pollution, and elevates crop productivity through targeted deployment. The study's results underscore that enveloping a specific herbicide with a hydrophilic polymer via nanofibers doesn't impede the germination or growth of black gram seeds; remarkably, it even expedites germination. While the potential of nanofiber-coated pendimethalin seeds is promising, its originality mandates further enhancement, rigorous experimentation, and a thorough assessment of its effects on weed seed germination and control mechanisms.

Comment [S10]: should be supported by data from the results

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