



RESEARCH ARTICLE

Assessing the utility of GreenSeeker® based Normalized Difference Vegetation Index (NDVI) for weed variability differentiation in Bt Cotton

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Abstract

An experiment was conducted to assess the applicability of GreenSeeker® based Normalized Difference Vegetation Index (NDVI) for weed variability identification in Bt cotton. Various weed management practices consisting of combinations of hand weeding, inter-cultivation, pendimethalin, quizalofop-ethyl, pyriproxyfen-Na including a weedy check and weed free check were used as treatments. Temporal and spatial variability of weed densities was noticed due to treatment effects in respective plots. NDVI value was calculated using a handheld GreenSeeker® for crop plants as well as weeds. All statistical comparisons were tested at the 0.05 level of significance ($p < 0.05$) using appropriate statistical methods. Results indicated that apart from weed free check the treatment with pre-emergence application of pendimethalin 678 g a.i. ha⁻¹ + one hand weeding at 20 DAS and inter-cultivation two times at 45 and 60 DAS recorded significantly less weed population than other treatments at 20 (2.90 m⁻²), 40 (3.95 m⁻²), 60 DAS (2.49 m⁻²) and at harvest (4.02 m⁻²). It also recorded significantly higher NDVI for cotton at 60 (0.72) and 75 DAS (0.73). The NDVI values calculated for Bt cotton could not differentiate the effect of weed management practices in early stage and showed significant difference only at 60 DAS. Whereas NDVI calculated over weed plants showed higher value with increase in density of weeds. The study identifies the limitations of solely relying on GreenSeeker® based NDVI for weed variability identification. The future research can investigate if other indices from multispectral or hyperspectral data improve weed differentiation.

Introduction

Cotton (*Gossypium* sp.) is a valuable commercial cash crop, a major source of raw material for the textile industry and its fibers are used to make various products, including clothing, home textiles and industrial materials. Cotton fulfills the global need for fiber and contributes approximately 16-24 % to the worldwide production of edible oil (1). India has the largest area of cotton cultivation in the world, accounting for 41 % of the total. Cotton cultivation in India covers an area of 12.37 million hectares, resulting in a production of 31.11 million bales and productivity of 428 kg of lint per hectare. However, due to lower productivity, India's contribution to global cotton production is only 25.4 %. (2). The cotton crop faces both biotic and abiotic stresses throughout its growth cycle. Several diseases, insect pests, weeds and abiotic factors like drought stress, waterlogging and heat stress largely reduce cotton yield. Among various factors, weeds

have a particularly detrimental impact on seed cotton yield. Due to its wide spacing and relatively slow growth, cotton is especially susceptible to significant weed infestations during its initial growth stages (3). Generally, yield losses in cotton may be up to 30-40 % due to weed infestation. A complete season of weed competition in cotton could potentially lead to yield losses of up to 90 % (4, 5). A range of broad-spectrum pre-emergence and post-emergence herbicides is available for use in cotton cultivation. These herbicides contribute to providing the crop with a relatively improved weed-free environment during its early growth stages. In cotton fields, traditional weed management practices, including the use of chemical herbicides, mechanical tillage and mowing, are widely utilized. However, these approaches often lead to higher production costs and present environmental challenges (6). Weed management is carried out through the uniform application of herbicides across the entire field, without considering the actual density of weeds

present. Global herbicide consumption has reached 1.48 million tonnes, with India accounting for 6.34 thousand tonnes (7). This practice often results in excessive spraying in areas without any weed growth leading to the wastage of herbicides pollution in the agricultural ecosystem.

In response to these challenges, precision weed management (PWM) has emerged as a promising solution for sustainable and effective weed control. Site-specific weed management (SSWM), which involves targeting weed control based on their density, spatial distribution and typology, offers potential benefits, including reduced herbicide usage, lower costs and minimized environmental impact (8). Preliminary studies on chemical weed control through SSWM have demonstrated that it is possible to achieve herbicide savings of 45 to 66 % without compromising crop yield, compared to traditional methods of uniform application (9). Accurate weed identification and site-specific application of treatments significantly decrease the environmental footprint associated with herbicides, compared to the current practice of whole-field, indiscriminate treatments (10). The knowledge about the distribution of weeds in the field is necessary to effectively implement SSWM.

The NDVI, one of the earliest remote sensing analytical products used to simplify the complexities of multi-spectral imagery, is now the most popular index used for vegetation assessment (11, 12). The fractions of emitted NIR and red radiation reflected from the sensed area provide information about photosynthetic efficiency, productivity potential and potential yield (13). Recent advances in precision agriculture technologies have led to the development of ground-based optical remote sensors (or crop canopy sensors) that calculate the NDVI reading directly unlike deriving NDVI values from traditional methods *i.e.* using Spectroradiometer and GreenSeeker® (Trimble Inc.). Optical sensors have their own source of light energy and allow for the determination of NDVI at specific times and locations throughout the growing season without the need for ambient illumination or flight concerns. Current weed management strategies in cotton fields predominantly rely on uniform herbicide applications, often leading to excessive chemical use, increased costs and environmental concerns. While precision weed management (PWM) and SSWM have shown promise, their implementation requires accurate weed identification and mapping. Many of the researchers used GreenSeeker® based NDVI alone for agronomic decision making such as, N management in Cotton (14) and Maize (15). Kiranmai (16) used NDVI for water management in maize. Existing studies primarily focus on NDVI for crop health assessment, yet its effectiveness in distinguishing between crops and weeds, is not well-established. In this context an experiment was conducted with the objective of assessing the applicability of GreenSeeker® based NDVI for weed variability identification, which can be used as a principle for site specific weed management.

Materials and Methods

The experiment was conducted at C-75 Block of the main Agricultural Research Station, University of Agricultural Sciences, Dharwad. The research site is geographically positioned at 15° 30' 6" N latitude and 74° 59' 13.2" E longitude, with an altitude of 678 m above mean sea level. Experiment was laid out on well drained, medium deep black soil taxonomically belonging to *Vertic Inceptisols* with a depth ranging from 2-3 m. Ajit-155 cotton hybrid was selected for the experiment, which was planted on July 25, 2022. The crop was sown at a row spacing of 90 cm and a plant-to-plant spacing of 60 cm within a row. Each plot measured 9 m × 6 m, providing a sufficient area for treatment applications and data collection. The experiment was laid out in randomized complete block design with seven treatments replicated three times. The treatments were T₁: Pre-emergence application of pendimethalin 678 g *a.i.* ha⁻¹; T₂: Pre-emergence application of pendimethalin 678 g *a.i.* ha⁻¹ + one hand weeding at 20 DAS and inter-cultivation two times at 45 and 60 DAS; T₃: Post-emergence application of quizalofop-ethyl 37.5 g *a.i.* ha⁻¹ + pyriithiobac-Na 75 g *a.i.* ha⁻¹ at 30 DAS; T₄: Post-emergence application of quizalofop-ethyl 37.5 g *a.i.* ha⁻¹ + pyriithiobac-Na 75 g *a.i.* ha⁻¹ at 30 DAS + inter-cultivation two times at 45 and 60 DAS; T₅: Pre-emergence application of pendimethalin 678 g *a.i.* ha⁻¹ + post-emergence application of quizalofop-ethyl 37.5 g *a.i.* ha⁻¹ + pyriithiobac-Na 75 g *a.i.* ha⁻¹ at 30 DAS; T₆: Weed free check and T₇: Weedy check. Temporal and spatial variability of weed densities was noticed due to treatment effect in respective plots.

NDVI (17) was used in this study. This index is sensitive to the presence of green vegetation and correlated well to biomass of the crop. NDVI can be defined by following equation,

$$NDVI = \frac{NIR - R}{NIR + R}$$

where, NIR and R are the reflectance in the near infrared and red region respectively. The fractions of emitted NIR and red radiation reflected from the sensed area provide information about photosynthetic efficiency, productivity potential and potential yield (13). NDVI readings were recorded from a height of 0.75 -1 m above crop canopy using GreenSeeker® on cloud free days between 9 am to 12:30 pm (Fig. 1). Upon pulling the trigger, the sensor turns on, emits brief bursts of red and infrared light and then measures the amount of each type of light that is reflected at the sensor. The sensor continues to sample the scanned area as long as the trigger remains engaged. The sensor displays the measured value in terms of an NDVI reading on its LCD display screen. NDVI readings range from 0 to 1. Higher the reading, healthier the plant. Five plants from the net plot were selected randomly and tagged for the purpose of recording observations. Based on observations recorded on five tagged plants, average value per plant was calculated for Cotton. The GreenSeeker® gives an average value of NDVI for the vegetation if the trigger is held for a period. The NDVI of the weeds was recorded by directing the instrument to inter row spaces at the above specified height irrespective of the weed species. Five such readings were recorded from the net



Fig. 1. Handheld GreenSeeker®.

plots. Readings were taken at 15, 30, 45, 60 and 75 DAS for both crops and weeds.

Data on weed density and NDVI values were subjected to Analysis of Variance (ANOVA) using RStudio (version 4.3.1). Mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at a significant level of $p < 0.05$. Weed count data were transformed using square root transformation ($\sqrt{(x+1)}$) to stabilize variance before analysis

Results and Discussion

Weed density as influenced by weed management practices

The dominant weed flora of the experimental area includes grassy weeds like *Cynodon dactylon* (L.) Pers., *Cyperus rotundus* L., *Digitaria sanguinalis* L. (Scop.), *Eleusine indica* (L.) Gaertner. etc. and broad leaf weeds viz. *Alternanthera sessilis* (L) DC., *Digera arvensis* Forssk., *Commelina benghalensis* L., *Parthenium hysterophorus* L., *Phyllanthus niruri* L., *Euphorbia geniculata* Orteg. etc. The weed free check treatment recorded the significantly lowest total weed population among all the treatments at all stages (Table 1). Among other weed management practices, the treatment with pre-emergence application of pendimethalin 38.7 CS@1.75 L ha⁻¹ + one hand weeding at 15-20 DAS and inter-cultivation two times at 45 and 60 DAS recorded significantly less weed population than

other treatments at 20 (2.90 m⁻²), 40 (3.95 m⁻²), 60 DAS (2.49 m⁻²) and at harvest (4.02 m⁻²). However, at harvest (4.57), it was statistically comparable with post-emergence application of quizalofop-ethyl 5 EC + pyriithiobac-Na 10 EC each @0.75 L ha⁻¹ at 30 DAS + inter-cultivation two times at 45 and 60 DAS.

Spectral characteristics of Bt cotton

The NDVI values at 15 DAS did not exhibit significant differences, likely due to the small size of both cotton and weed plants, resulting in limited vegetation coverage. During the early stages of crop growth, when the green leaf area is small, NDVI results are very sensitive to soil background effects. This can make it difficult to accurately identify and quantify weed presence. However, as the cotton crop grew and its canopy, biomass and colour diverged due to distinct weed management treatments, the variance in NDVI became more significant. At 30 DAS, Bt cotton plants in weedy check recorded significantly higher NDVI (0.65) and it was comparable with pre-emergence application of pendimethalin 38.7 CS @1.75 L ha⁻¹ (0.60) and post-emergence application of quizalofop-ethyl 5 EC + pyriithiobac-Na 10 EC each @ 0.75 L ha⁻¹ at 30 DAS + inter-cultivation two times at 45 and 60 DAS (0.58) (Table 2). Notably, the weed-free check (0.78) displayed higher NDVI values compared to the weedy check (0.63) at 60 DAS, signifying the adverse impact of weed competition on growth and visual characteristics in the latter.

Weeds often compete with cotton plants for crucial resources such as nutrients, water and sunlight. This competitive pressure can stress cotton plants, diminishing their vitality and potentially leading to lower NDVI readings. Furthermore, certain weeds can negatively influence the health of cotton plants through mechanisms like allelopathy or by providing a habitat for pests and diseases. These factors could further exacerbate the stress on cotton plants, leading to a potential reduction in their NDVI values (18). This dynamic could potentially explain the lower NDVI values observed in cotton plants under weedy conditions.

While significant differences in NDVI values were observed among some treatments, it is important to note that relying solely on GreenSeeker® based NDVI for spectral characterization might not fully capture the effects of various

Table 1. Total weed density (m⁻²) in Bt cotton at different stages of crop growth as influenced by weed management practices

Treatments	20 DAS	40 DAS	60 DAS	At harvest
T₁: Pre-emergence application of pendimethalin 678 g a.i. ha ⁻¹	3.68 ^d (12.53)	5.47 ^b (29.02)	6.09 ^c (36.20)	6.04 ^b (35.55)
T₂: Pre-emergence application of pendimethalin 678 g a.i. ha ⁻¹ + one hand weeding at 20 DAS and inter-cultivation two times at 45 and 60 DAS	2.90 ^e (7.46)	3.95 ^d (14.67)	2.49 ^e (5.23)	4.02 ^c (15.29)
T₃: Post-emergence application of quizalofop-ethyl 37.5 g a.i. ha ⁻¹ + pyriithiobac-Na 75 g a.i. ha ⁻¹ at 30 DAS	4.93 ^b (23.29)	5.58 ^b (30.15)	6.85 ^b (45.90)	6.83 ^a (45.62)
T₄: Post-emergence application of quizalofop-ethyl 37.5 g a.i. ha ⁻¹ + pyriithiobac-Na 75 g a.i. ha ⁻¹ at 30 DAS + inter-cultivation two times at 45 and 60 DAS	4.65 ^c (20.64)	5.03 ^c (24.33)	3.34 ^d (10.18)	4.57 ^c (20.08)
T₅: Pre-emergence application of pendimethalin 678 g a.i. ha ⁻¹ + post-emergence application of quizalofop-ethyl 37.5 g a.i. ha ⁻¹ + pyriithiobac-Na 75 g a.i. ha ⁻¹ at 30 DAS	3.49 ^d (11.19)	4.03 ^d (15.27)	5.89 ^c (33.75)	5.88 ^b (33.64)
T₆: Weed free check	1.00 ^f (0.00)	1.00 ^e (0.00)	1.00 ^f (0.00)	1.00 ^d (0.00)
T₇: Weedy check	5.36 ^a (27.74)	6.48 ^a (41.04)	7.65 ^a (57.53)	7.32 ^a (52.57)
S.E.m.±	0.09	0.12	0.10	0.20

* Figures in the parenthesis represent original values

Data subjected for transformation using $\sqrt{(x+1)}$, where x is weed count

Table 2. GreenSeeker® based normalized difference vegetation index of Bt cotton as influenced by weed management practices

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS
T₁: Pre-emergence application of pendimethalin 678 g <i>a.i.</i> ha ⁻¹	0.45 ^{ab}	0.60 ^a	0.64 ^{ab}	0.63 ^c	0.59 ^c
T₂: Pre-emergence application of pendimethalin 678 g <i>a.i.</i> ha ⁻¹ + one hand weeding at 20 DAS and inter-cultivation two times at 45 and 60 DAS	0.45 ^{ab}	0.50 ^b	0.63 ^{abc}	0.72 ^{ab}	0.73 ^{ab}
T₃: Post-emergence application of quizalofop-ethyl 37.5 g <i>a.i.</i> ha ⁻¹ + pyrithiobac-Na 75 g <i>a.i.</i> ha ⁻¹ at 30 DAS	0.49 ^{ab}	0.54 ^b	0.58 ^{bc}	0.65 ^{bc}	0.59 ^c
T₄: Post-emergence application of quizalofop-ethyl 37.5 g <i>a.i.</i> ha ⁻¹ + pyrithiobac-Na 75 g <i>a.i.</i> ha ⁻¹ at 30 DAS + inter-cultivation two times at 45 and 60 DAS	0.45 ^{ab}	0.58 ^{ab}	0.54 ^c	0.71 ^{abc}	0.68 ^b
T₅: Pre-emergence application of pendimethalin 678 g <i>a.i.</i> ha ⁻¹ + post-emergence application of quizalofop-ethyl 37.5 g <i>a.i.</i> ha ⁻¹ + pyrithiobac-Na 75 g <i>a.i.</i> ha ⁻¹ at 30 DAS	0.46 ^{ab}	0.51 ^b	0.55 ^{bc}	0.70 ^{abc}	0.67 ^b
T₆: Weed free check	0.50 ^a	0.51 ^b	0.53 ^c	0.78 ^a	0.80 ^a
T₇: Weedy check	0.48 ^{ab}	0.65 ^a	0.70 ^a	0.63 ^c	0.57 ^c
S.E.m.±	0.02	0.03	0.03	0.03	0.02

weed management practices on plants. NDVI values can be influenced by factors beyond weed management practices, such as lighting conditions, stress conditions due to factors other than weed (insect pests and diseases, moisture stress etc.), soil type and moisture content and the presence of crop residues on the soil surface during the initial stages (19). Even at 60 DAS, the NDVI values among some treatments remained comparable, despite significant variations in growth and yield parameters. This can be attributed to the indeterminate nature of cotton, where the vegetative growth of plants continues even after flowering and thereby it may show recovery from the symptoms of weed pressure. The NDVI values of cotton and the weed density in different treatments show strong negative correlation (-0.92) at 60 DAS, indicating that higher weed density reduces crop NDVI values (Fig. 2).

The variation in NDVI becomes more pronounced at 75 DAS. By this point, the critical period of crop-weed competition has typically passed and any potential yield losses are likely irreversible. It is worth noting that the NDVI values of the cotton plant might have been affected by the presence of weeds as both the crop and weed have similar characters. Since NDVI is derived from the ratio of near-infrared and red-light reflectance, it primarily reflects the amount of live green vegetation in an area. Therefore, when both cotton plants and weeds are present within the same area, NDVI might capture the collective vegetation information without differentiating between them. This is a limitation of NDVI as a standalone

metric, as it might not provide insights into the individual contributions of cotton plants and weeds to the observed values. Advanced sensors (hyperspectral) and image processing techniques like image segmentation can be used to get NDVI of only a particular species and overcome this limitation.

Spectral characteristics of weeds

The weed plants in the weedy check treatment displayed a significantly higher normalized difference vegetation index (Table 3) compared to the other treatments at 15 (0.61), 30 (0.69), 45 (0.74), 60 (0.77) and 75 (0.82) DAS respectively. However, it was on par with pre-emergence application of pendimethalin 38.7 CS @1.75 L ha⁻¹ at 30 (0.64), 45 (0.73), 40 (0.75) and 75 DAS (0.80) respectively and post-emergence application of quizalofop-ethyl 5 EC + pyrithiobac-Na 10 EC each @ 0.75 L ha⁻¹ at 30 DAS (0.53, 0.72, 0.74 and 0.75 at 15, 30, 60 and 75 DAS respectively). Significantly lower NDVI was observed in weed free check at 15 (0.26), 30 (0.35), 45 (0.41), 60 (0.46) and 75 DAS (0.47) respectively.

The NDVI values of weeds and weed density across different treatments exhibit a strong positive correlation (0.98) at 60 DAS, confirming that higher weed populations lead to increased NDVI values in weed-dominated areas. The NDVI values of the weed plants growing in inter row space at all stages of crops displayed higher NDVI values. This can be attributed to the fact that weeds are also plants and their

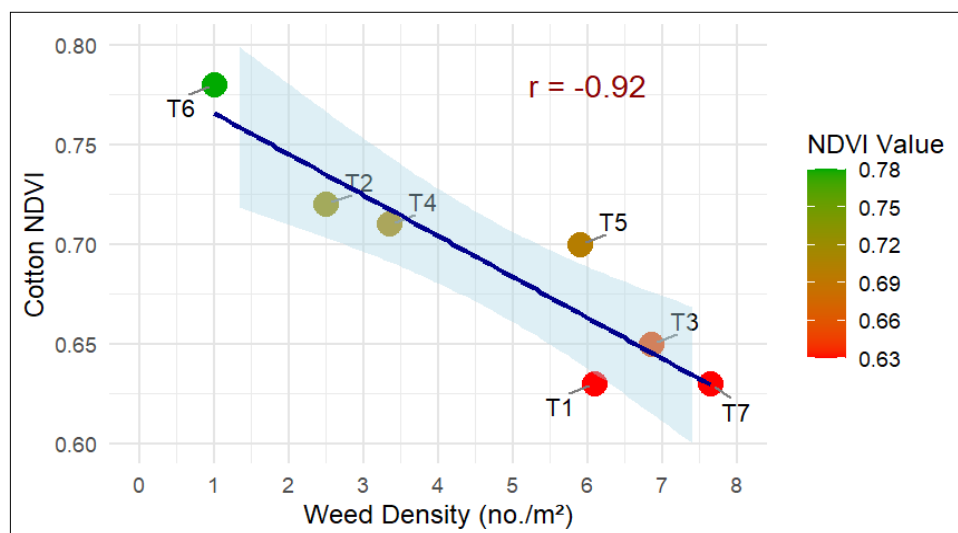
**Fig. 2.** Relationship between weed density and cotton NDVI at 60 DAS.

Table 3. GreenSeeker® based normalized difference vegetation index of weed plants as influenced by weed management practices

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS
T₁: Pre-emergence application of pendimethalin 678 g <i>a.i.</i> ha ⁻¹	0.41 ^b	0.64 ^a	0.73 ^a	0.75 ^a	0.80 ^a
T₂: Pre-emergence application of pendimethalin 678 g <i>a.i.</i> ha ⁻¹ + one hand weeding at 20 DAS and inter-cultivation two times at 45 and 60 DAS	0.24 ^c	0.45 ^b	0.69 ^a	0.50 ^{bc}	0.59 ^b
T₃: Post-emergence application of quizalofop-ethyl 37.5 g <i>a.i.</i> ha ⁻¹ + pyriithiobac-Na 75 g <i>a.i.</i> ha ⁻¹ at 30 DAS	0.53 ^a	0.72 ^a	0.56 ^b	0.74 ^a	0.75 ^a
T₄: Post-emergence application of quizalofop-ethyl 37.5 g <i>a.i.</i> ha ⁻¹ + pyriithiobac-Na 75 g <i>a.i.</i> ha ⁻¹ at 30 DAS + inter-cultivation two times at 45 and 60 DAS	0.53 ^a	0.75 ^a	0.51 ^{bc}	0.55 ^b	0.58 ^b
T₅: Pre-emergence application of pendimethalin 678 g <i>a.i.</i> ha ⁻¹ + post-emergence application of quizalofop-ethyl 37.5 g <i>a.i.</i> ha ⁻¹ + pyriithiobac-Na 75 g <i>a.i.</i> ha ⁻¹ at 30 DAS	0.29 ^c	0.62 ^a	0.46 ^{bc}	0.72 ^a	0.73 ^a
T₆: Weed free check	0.26 ^c	0.35 ^b	0.41 ^c	0.43 ^c	0.47 ^c
T₇: Weedy check	0.61 ^a	0.69 ^a	0.74 ^a	0.77 ^a	0.82 ^a
S.Em.±	0.03	0.03	0.03	0.04	0.03

presence contributes to increased overall greenness and vegetation density, thus leading to higher NDVI values. This correlation aligns with the principle that a higher NDVI value corresponds to denser and healthier vegetation. The weedy check treatments where higher weed population was observed, recorded higher NDVI than weed free check. This observation suggests that NDVI could potentially serve as a tool to estimate weed density in inter row spaces, especially in crops with wider row-to-row spacing. However, it should be noted that practical implementation faces challenges, including the need for accurate modelling to predict weed density and the limitations of using proximal sensors like GreenSeeker® for calculating NDVI over larger areas.

When it comes to weed mapping, the combined presence of crops and weed in the field makes it difficult to differentiate between them solely based on NDVI from aerial platform. Moreover, various stress conditions can indeed affect NDVI values. For example, factors such as water stress, nutrient deficiencies, disease and pest damage can all affect how plants reflect and absorb light, thereby influencing NDVI values (20). In real field conditions, it is quite challenging to determine which specific stress is affecting the NDVI. To precisely assess the impact of weed management practices on cotton growth, it's crucial to employ a comprehensive approach that integrates NDVI with other data sources, such as visual observations, ground truthing and potentially even advanced machine learning techniques. One potential solution could be the use of remote sensing technologies, such as drones or satellites equipped with multispectral cameras. These technologies can capture NDVI data over large areas more efficiently than handheld sensors. The data can then be processed using specialized software to generate NDVI maps, which can provide valuable insights into weed density and distribution across the field.

Conclusion

This study aimed to assess the viability of using GreenSeeker® based NDVI as a decision-making tool for weed management, drawing parallels with its successful applications in nitrogen and irrigation management. The findings underscore the utility of NDVI in gauging overall vegetation density and greenness. However, NDVI's utility for early stage weed differentiation was limited due to overlapping spectral signatures of young cotton and weeds, compounded by soil

background interference. Furthermore, factors other than weed competition introduce variability to NDVI values, necessitating the integration of additional data sources. Visual observations and ground truthing are identified as essential components in spectral characterization to address these limitations. The study acknowledges the intricate challenge of distinguishing between crops and weeds based solely on NDVI, emphasizing the imperative for a holistic approach that incorporates a diverse array of data inputs. In short, while NDVI remains valuable, its effective utilization for weed management requires a comprehensive strategy that accommodates the multifaceted factors influencing vegetation dynamics in real-world field condition. Future research should focus on integrating NDVI with advanced remote sensing technologies, machine learning models and multispectral or hyperspectral imaging to improve weed differentiation and enable more precise site-specific weed management strategies.

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Authors' contributions

AKK carried out the investigation, data curation and formal analysis and drafted the manuscript. MP contributed to methodology, provided resources, supervised the study and validated the findings. AK contributed to methodology, provided resources, supervised the study, validated the findings and participated in manuscript review and editing. GB provided resources, supervised the study, validated the findings and contributed to manuscript review and editing. AAS participated in manuscript review and editing. YK contributed to data curation and manuscript review and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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