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RESPONSE OF *Amaranthus viridis* PLANT FUNCTIONAL TRAITS TO NPK 12:12:17 AND NPK 15:15:15 FERTILIZERS

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ABSTRACT

A paradigm shift from the prevailing reliance on chemical methods to alternative weed-control approaches is necessary to achieve sustainable weed management. However, the understanding of weed biology explaining "how" and "why" remains insufficient in facilitating this shift. This study employed a trait-based approach — examined the number of leaves, number of inflorescences, and height — to investigate the growth and developmental patterns of *Amaranthus viridis,* a weed species in the tropics, in response to NPK fertilization. The experiments were carried out in three sets of weeds — wild population (untreated and not transplanted; n = 6), NPK 15:15:15 (transplanted and fertilized with NPK 15:15:15 from March 2020 to September 2020; n = 30), and NPK 12:12:17 (transplanted and fertilized with NPK 12:12:17 from May 2021 to September 2021). The NPK treatment sets comprised five treatments, including one untreated control, with six replications for each treatment. Pearson's correlation coefficient (*r*) and linear regression (R^2) in three models were estimated using leaves, inflorescences and height as dependent and independent variables. In Model 1, the number of leaves was the dependent variable and plant height was the independent variable; Model 2 included the number of inflorescences as the dependent variable and the number of leaves as the independent variable, whereas the number of inflorescences as the dependent variable and number of leaves and height as the independent variables were used in Model 3. All models exhibited a significantly positive correlation and R^2 ($p < 0.01$). Specifically, Model 3, examining the interactions of inflorescence with leaf numbers and plant height, demonstrated higher values for both *r* and R^2. In conclusion, this study reveals the distinct patterns of functional traits in *A. viridis* in response to fertilizers and within wild populations, providing predictive models applicable to diverse data types, with implications for understanding inherent growth and responses of weed species for sustainable weed management practices, particularly in collaboration with smallholder farmers.

Keywords: *Amaranthus viridis,* herbicides, plant functional traits, weed biology, weed management

INTRODUCTION

Weeds are recognized as the primary pests hindering crop establishment and growth, resulting in varying yield losses depending on the crop type (Gharde *et al.* 2018), constituting the principal constraint in crop production (Orke 2006), causing an average yield loss of 28% (Vilà *et al.* 2021). Therefore, weed management is critical for ensuring food security and environmental sustainability (Yaduraju & Rao 2013). The emergence of weed science as a distinct discipline is relatively recent, dating back less than 100 years, compared to other disciplines, such as plant pathology (Timmons 2005). The evolution of the weed science discipline stemmed from the discovery of the first synthetic herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), in 1941 by Pokorny, which subsequently underscored the importance of weed management.

Tillage and herbicides stand as the primary methods for weed removal from farms (MacLaren *et al.* 2020). However, the overwhelming success of herbicides has shaped the weed science discipline into a "herbicide-centered" discipline. This shift not only poses challenges for diversifying research in weed management but also imposes several concerns related to the environment, such as

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*Corresponding author, email: tongps@utar.edu.my environmental hazards, contamination of natural resources, and the emergence of herbicide-resistant weeds, as well as consumer health. Herbicide residue contaminations are detected in soil as well as in surface and groundwater (Allinson *et al.* 2017; Sun *et al.* 2017). Another major concern associated with "herbicide-centered" weed management is food safety. Numerous studies have highlighted herbicide residues in fruits and vegetables, raising apprehensions among importing countries and consumers (Amjad *et al.* 2013; Matt *et al.* 2013). Kim *et al.* (2017) revealed the existence of various direct and indirect routes of chemical exposure. Therefore, the persistent use of herbicides is also an alarming concern for toxic exposure to humans and other non-target organisms.

These environmental and health-related challenges underscore the need for a broader perspective in weed management beyond a reliance on herbicides, such as a paradigm shift towards vegetation management on farms by understanding weed ecology, biology, and genetics (Chauhan *et al.* 2017; Clements *et al.* 2014). Furthermore, several aspects of weeds and their interactions remain poorly understood (Jordan *et al.* 2016). For example, the effects of fertilization on weed growth and development remain elusive (Little *et al.* 2021). Fertilizers act as a selection pressure in the competitive dynamics between crops and weeds. Therefore, strategic weed management is crucial to ensure crops outcompete weeds. Desired timing for weed control is when weeds and crop are still in symmetrical competition level, and not in asymmetrical competition where weeds outcompete crops (Little *et al.* 2021). Despite the urgency for effective weed management, the lack of detailed information on weed biology has resulted in weak predictions of weed species on farms; specifically, the "how" and "why" aspects have not been fully explored (Ward *et al.* 2014). These knowledge gaps limit the science-based strategies in the application of weed management practices, particularly in developing countries (Chaney & Baucom 2012; Chauhan *et al.* 2017).

Several weed biology articles specifically have been published in Canada and Australia since the 1970s (Cavers *et al.* 2013; Groves & Panetta 2014); however, the species information is limited to comprehensive literature reviews, offering limited new quantitative information (Ward *et al.* 2014). Additionally, understanding the growth and development of weed species can serve as a basis for integrating different weed control methods (Chaney & Baucom 2012). The parameters determining plant growth include plant height, stem thickness, and biomass, whereas parameters such as the number of leaves and number of inflorescences determine plant development (Dambreville *et al.* 2015).

The Amaranthaceae family consists of 70 species, of which a few are vegetables and some are weeds. Slender amaranth (*Amaranthus viridis* L.) is widely distributed, being native to 33 countries and an introduced species in 98 countries (POWO 2022). *Amaranthus viridis* is an annual broadleaf weed with a C_4 photosynthetic pathway and is found on farms and in open habitats, such as roadsides. The weed species utilizes seed propagation mechanisms. *Amaranthus viridis* has the second highest mean field density and high relative abundance among other weed species found in smallholder maize farms in Malim Nawar, Malaysia, per field surveys conducted in 2017, 2018, and 2020 (Tong & Lim 2022). The high mean density and relative abundance of this weed species reflect the degree of difficulty in its control (de Mol *et al.* 2015).

This study aimed to investigate the functional relationships between traits (number of leaves, number of inflorescences, and plant height) of *A. viridis* in wild populations and their growth responses to NPK 15:15:15 and NPK 12:12:17. The present study demonstrates how enhanced knowledge of weed biology could lead to practical advances benefiting farmers; thus, our study will provide a foundation to initiate the development of science-based weed management technologies.

MATERIALS AND METHODS

Experimental design

The present study evaluated wild *A viridis*. A total of 30 seedlings (5–20 cm high) from a single location in Kampar, Malaysia was used to ensure similar batch of seeds (Hanzawa & Kalisz 1993; Kirkpatrick 1984). The seedlings were transferred to polybags (20 cm × 15 cm × 30 cm; L × W × H) filled with soil from the site from where *A. viridis* were obtained, with one seedling planted in a polybag. The soils used were tailing parent material from the site where seedlings were found, which was a pale brown to brown upper layer of 30–70 cm. The soil composition included sandy clay to clay, weak fine subangular blocky, friable texture, overlaying brown silty clay to clay, olive-brown to brown coarse sandy clay loam with some clay balls.

Seedlings were planted in a Randomized Complete Block Design (RCBD) with six blocks for five treatments in a shade house, maintained at a relative light intensity of 50% in the north-south direction. Each treatment comprised six replicates. The five treatments included a control without fertilization and four sets fertilized with different dosages (1, 2, 4, and 8 g per polybag) of Behn Meyer Nitrophoska® Green NPK 15-15-15+2S, produced by Eurochem Agro (the manufacturer's details are not known). Fertilizer was applied one month after transplanting and subsequently on a weekly basis throughout the study period from March to September 2020. Additionally, six plants in the wild population were studied without any treatment and were not transplanted. The number of leaves, number of inflorescences, and height (cm) of each plant were recorded weekly. Plant organs were considered to reflect the functional characteristics of growth, competitive ability, and reproduction (Garnier & Navas 2012). The experiment was repeated from May to September 2021 for five months with another set of 30 plants. The fertilizer was changed to AgroBridge NPKMg 12-12-17-2+TE, a muriate of potash (MOP) based fertilizer imported from Europe (the manufacturer's details are not known). The duration of both experiments was similar to the maize planting cycle or longer to understand plant responses to fertilizer treatments. These fertilizers were chosen based on their common use by smallholders in maize planting, as reported in weed surveys conducted by Tong & Lim (2022).

Statistical analyses

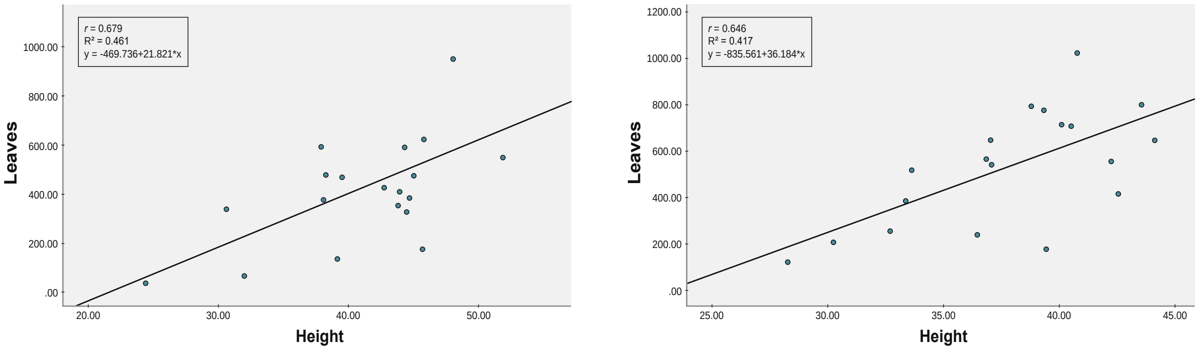
The Shapiro–Wilk normality and Q-Q plot tests were performed to determine whether a variable had a normal distribution. The null hypothesis of normal population distributions was accepted when $p > 0.05$ and rejected otherwise. If the normality assumption was met, the Pearson correlation coefficient (r) and linear regression (R^2) were assessed to analyze the relationship between the number of leaves, number of inflorescences, and plant height. Three models were analyzed to estimate the correlation between the traits: Model 1, where the number of leaves was used as the dependent variable and plant height as the independent variable; Model 2, where inflorescence was the dependent variable and the number of leaves served as the independent variable; and Model 3, where the number of inflorescences was the dependent variable and the number of leaves and plant height were the independent variables. The gradient (β) was tested for significance. A significant relationship was observed when the gradient was not zero ($p < 0.001$). SPSS version 20.0 was used for statistical analyses.

RESULTS AND DISCUSSION

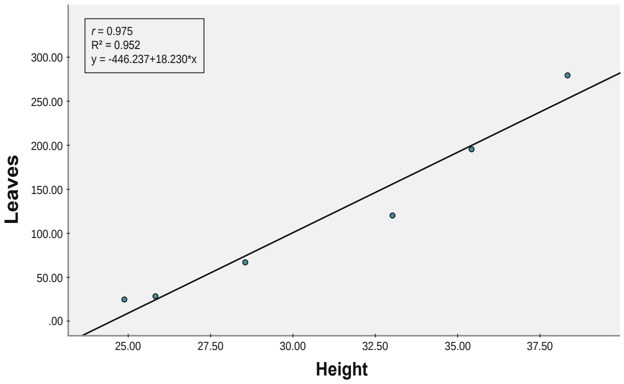
The null hypothesis of population normality was retained for *A. viridis* in wild populations and under the fertilizer treatments NPK 15:15:15 and 12:12:17 when $p > 0.05$. These values were normally distributed.

Model 1: Dependent variable: number of leaves, independent variable: plant height

The number of leaves and plant height were significantly positively correlated ($r = 0.679$) for *A. viridis* under NPK 12:12:17 ($b = 21.821$, $SE_b = 5.900$, $\beta = 0.679$, $t = 3.698$, $p = 0.001$; Figure 1A). The slope coefficient for the leaves was 21.82, suggesting that a 1 cm increase in plant height increases the number of leaves by 21.82. The R^2 value was 0.461, indicating that 46.1% of the variation in the number of leaves could be explained by the model containing only plant height.



(A) NPK 12:12:17 (B) NPK 15:15:15



(C) Wild plants

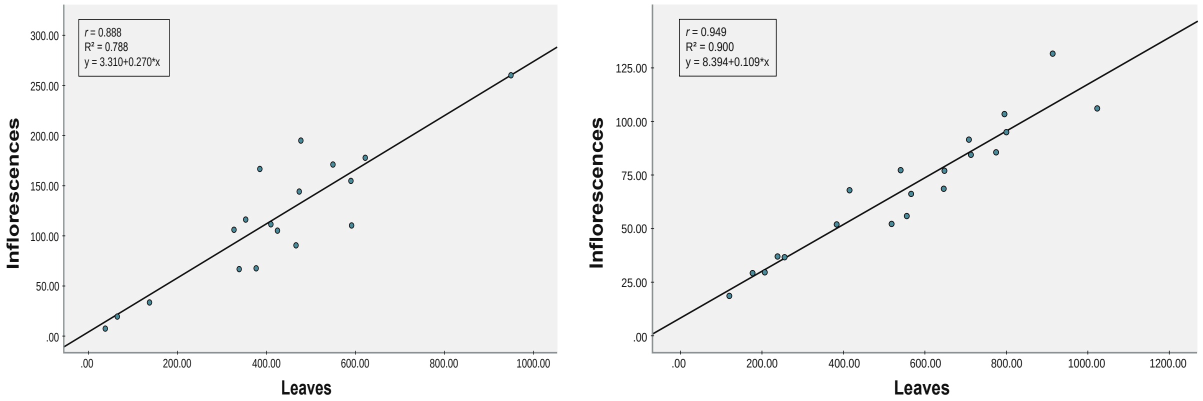
Figure 1 *Amaranthus viridis* studied under (A) NPK 12:12:17, (B) NPK 15:15:15 and (C) wild plants with the number of leaves as the dependent variable and plant height as the independent variable

Pearson's correlation coefficient (r = 0.646) revealed a significant (b = 36.184, SE_b = 10.383, β = 0.646, t = 3.485, $p < 0.001$) positive relationship between the number of leaves and plant height for *A. viridis* under NPK 15:15:15 (Figure 1B). An increase in height by 1 cm led to an increment of 36.18 leaves, and R^2 suggested that 41.7% of the variation in the number of leaves was accounted for by height.

Concordantly, a positive relationship between plants and the number of leaves (r = 0.975) was observed for the wild population (Figure 1C). Plant height significantly predicted the number of leaves (b = 18.422, SE_b = 2.015, β = 0.975, t = 8.864, p = 0.001). The model explained 95.2% of the variance in the number of leaves, and a 1 cm in height increment speculated to increase the number of leaves by 18.23.

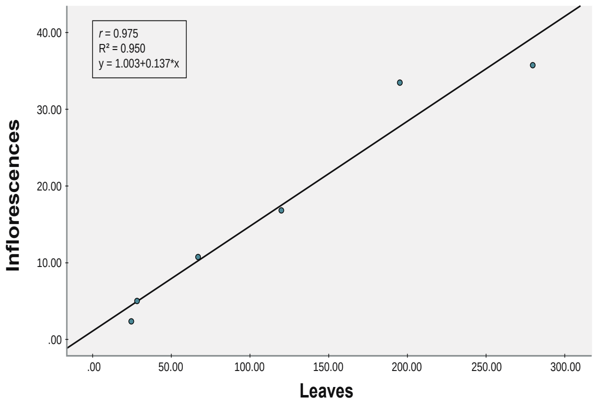
Model 2: Dependent variable: number of inflorescences, independent variable: number of leaves

Assessment using Model 2 revealed a positive correlation (r = 0.888) between the numbers of inflorescences and leaves for *A. viridis* under fertilizer NPK 12:12:17 (Figure 2A). Linear regression analysis revealed a significant relationship (b = 0.270, SE_b = 0.035, β = 0.888, t = 7.706, $p < 0.001$). The slope coefficient for the leaves was 0.270, which indicated an increase in the number of inflorescences by 0.270 for each leaf. The model revealed that leaves accounted for 78.8% (R^2 = 0.788) of the variance in inflorescences.



(A) NPK 12:12:17

(B) NPK 15:15:15



(C) Wild plants

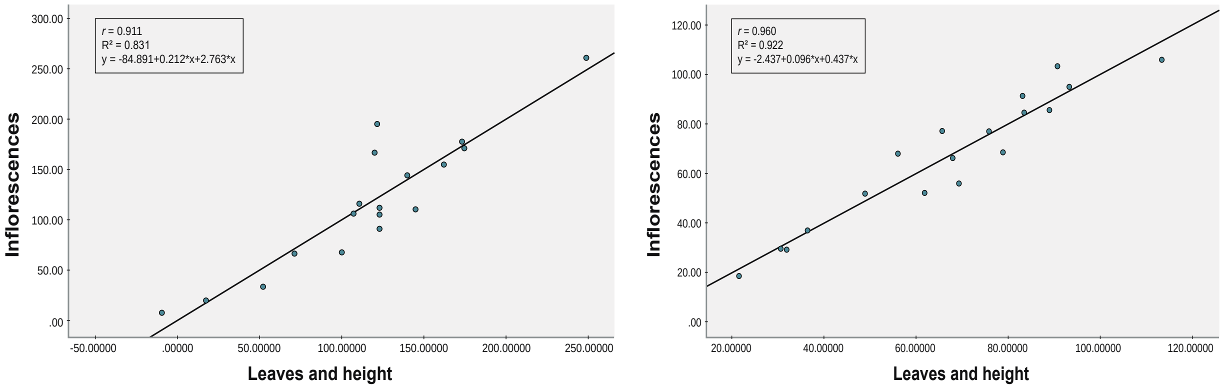
Figure 2 *Amarantus viridis* studied under (A) NPK 12:12:17, (B) NPK 15:15:15 and (C) Wild plants, with inflorescence as the dependent variable and leaves as the independent variable

For treatments under NPK 15:15:15, a significant ($b = 0.109$, $SE_b = 0.009$, $\beta = 0.949$, $t = 12.708$, $p < 0.001$) positive correlation ($r = 0.949$) was observed (Figure 2B). The slope coefficient for inflorescences was 0.109, signifying that the number of inflorescences increased by 0.109 for each additional leaf. The R^2 value indicated that 90% of the variation in inflorescences could be explained by the number of leaves.

The correlation coefficient ($r = 0.975$) between leaves and inflorescences in wild plants was robust and statistically significant ($b = 1.003$, $SE_b = 0.016$, $\beta = 0.975$, $t = 8.745$, $p = 0.001$; Figure 2C). Specifically, each additional leaf corresponds to a notable increase of 0.137 inflorescences. The proportion of variance in the number of inflorescences explained by the number of leaves was 95%.

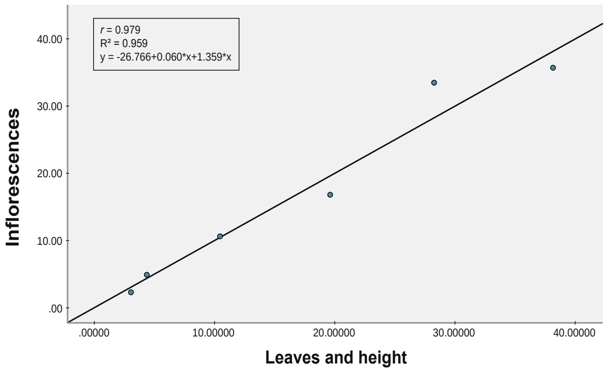
Model 3: Dependent variable: number of inflorescences, independent variable: number of leaves and plant height

The scatterplot revealed a strong linear relationship between the number of inflorescences and leaves and height combined, with an *r*-value of 0.911 (Figure 3A), which was statistically significant [$b = 0.212$ (leaves), 2.763 (height), $SE_b = 0.044$ (leaves), 1.415 (height), $\beta = 0.696$ (leaves), 0.282 (height), $t = 4.810$ (leaves), 1.952 (height), $p < 0.001$]. Specifically, each inflorescence is associated with an increase of 0.212 leaves and 2.763 cm in height. Furthermore, the combined effect of leaves and height accounts for 83.1% of the variance in inflorescences.



(A) NPK 12:12:17

(B) NPK 15:15:15



(C) Wild plants

Figure 3 *Amaranthus viridis* studied under (A) NPK 12:12:17, (B) NPK 15:15:15 and (C) wild plants with inflorescence as the dependent variable and leaves and height as the independent variable

The Pearson correlation coefficient of 0.960 indicated a strong linear relationship between inflorescence and both height and the number of leaves (Figure 3B), with a significant relationship (b = 0.096 (leaves), 0.437 (height), SE_b = 0.010(leaves), 0.540 (height), β = 0.910 (leaves), 0.074 (height), t = 9.935 (leaves), 0.809 (height), $p < 0.001$). For each inflorescence, the number of leaves was increased by 0.096 and the height by 0.437 cm. Both leaves and height collectively explained 92.2% of the variation in the number of inflorescences.

Additionally, a robust correlation of 0.979 was observed between inflorescences and both leaves and height (Figure 3C). Our findings affirmed that the number of leaves and plant height significantly predicted the number of inflorescences [b = 0.078 (leaves), 1.359 (height), SE_b = 0.085 (leaves), 1.650 (leaves), β = 0.449 (leaves), 0.557 (height), t = 0.820 (leaves), 1.609 (height), $p = 0.008$], with 95.9% of the variance in the number of inflorescences was accounted for by leaves and height.

Plant height is an indicator of organ growth, growth rate, and leaf number (Dambreville *et al.* 2015; Kunstler *et al.* 2016) and indicates reproductive maturity in herbaceous plants (Garnier & Navas 2012). Members of *Amaranthus* can grow up to a maximum height of 2.2 m (Martínez-Núñez *et al.* 2019). Concordant with our findings, those of previous studies have demonstrated that *A. retroflexus* shows a linear increase in height throughout its life cycle (Li *et al.* 2015; Little *et al.* 2021). Leaves play a crucial role in resource acquisition, while inflorescences are pivotal for reproduction. A higher leaf number indicates better growth rates and exhibits a strong correlation with other plant traits (He *et al.* 2020). The number of inflorescences is a reliable indicator for plants using seed production strategies, which facilitates the prediction of seed numbers (Chaney & Baucom 2012). Each inflorescence of *A. viridis* contained an average of 347 seeds, with a seed length of 1.25 ± 0.15 mm.

Growth and developmental parameters exhibit interconnectedness throughout the plant life cycle (Dambreville *et al.* 2015; He *et al.* 2020). The scatterplots generated using Models 1, 2, and 3 revealed strong positive relationships between the dependent and independent variables. The independent variables in these three models significantly predicted the dependent variables, with the latter demonstrating an increase corresponding to the rise in the independent variable(s). Moreover, weeds in wild populations exhibited growth and development patterns mirroring those of weeds studied under NPK 15:15:15 and NPK 12:12:17. This finding contradicts the hypothesis of Lavorel and Garnier (2012), which posited distinct trait responses for wild plants and farm weeds. Wild and arable weeds largely share ecological strategies, including reproduction (Bourgeois *et al.* 2019). Previous studies have shown that trait-based approaches can overcome the challenges posed by the heterogeneity within and between weed species (Gaba *et al.* 2017; Lavorel & Garnier 2002). In this study, the analysis of three models aimed to explore the functional relationships between traits revealed that the findings could guide the selection of the model for a more comprehensive understanding and accurate prediction of other weed species while considering data availability for certain traits.

Fertilizers exert the primary selection pressure on arable weeds. The positive correlations observed in this study indicate the co-optimization of *A. viridis*. Under favorable conditions, such as nutrient abundance, weeds strategically enhance both vegetative and reproductive aspects, consequently increasing plant fitness (Li *et al.* 2015; Little *et al.* 2021). An increase in vegetative components, such as height and leaves, promotes the competitive ability of weed species (Hegazy *et al.* 2005). This study discerned that continuous resource availability promotes co-optimization between traits. Weeds continue to grow and produce inflorescences until senescence (Hegazy *et al.* 2005). The response-effect framework can clarify patterns that reflect plant functional responses and aid in grouping species at the community level, such as facilitating the upscaling of individual plant responses to fertilizers (Lavorel & Garnier 2012). The hub trait (i.e., inflorescence) interacts with other traits and has a higher degree of correlation, whereas the mediator trait indicates the betweenness of the two traits which is a single spectrum of traits (He, *et al.,* 2020), suggesting Model 3 was a hub trait, and Models 1 and 2 were mediator traits.

In this study, an assessment of these traits on farms provided insights into aboveground growth and development patterns. Analyzing the growth development patterns in individual plant behavior at the trait level represents the initial phase for species comparison and broader generalization, marking the commencement of a systemic approach to weed control in practice (Little *et al.* 2021). Weeds undergo evolutionary adaptations within the temporal and spatial dimensions of the farm environment. To gain a comprehensive understanding, research efforts could be expanded to include other weed species within the same farm, investigating whether their fundamental biology aligns with that of *A. viridis* or similar species from different locations. Effective weed management practices can be implemented by acquiring detailed information on the growth and development parameters of weed species on farms. Moreover, the review of post-emergence herbicide application, encompassing considerations such as dosage, timing, and frequency at specific heights to minimize seed numbers, could be an integral component of vegetation management on farms.

Biological-intensification weed management involving holistic and sustainable approaches is complex and requires careful consideration and handling of several factors (Gaba *et al.* 2017; Little *et al.* 2021). Research efforts using this framework should actively engage smallholders in validation processes and joint efforts with smallholders to review their herbicide applications are essential. To conclusively demonstrate growth and development patterns, a follow-up program combining trait and response-and-effect approaches is necessary (Gaba *et al.* 2017; Garnier & Navas 2012). Experimental studies have suggested a timeframe of four to six years in the fields, taking into account the pressures the smallholder farmers face from weeds, which significantly influence their decisions and practices (Gaba *et al.* 2016). Therefore, facilitating the understanding of the smallholders regarding the basic concepts and appropriate technologies through continuous learning is crucial (Terlau *et al.* 2019). Furthermore, research on farmer participation is pragmatic for the development of sustainable weed management (Hall *et al.* 2000). Although chemical herbicides remain a central control method, refining their use by adjusting the dosage and application timing of fertilizers can contribute to reducing overall herbicide usage (Bastiaans *et al.* 2000).

LIMITATIONS

This study has some limitations in its examination of the germination rate, vegetative stage from germination to the emergence of the first inflorescence, and overall life span from germination to senescence under different environmental conditions (i.e., wild populations, NPK 15:15:15, and NPK 12:12:17). To ensure practical feasibility, the growth and development of *A. viridis* treated with fertilizers under farm conditions were not compared. Furthermore, this study focused on a single species with a single-density approach instead of investigating intra- and inter-specific weed competition. Another challenge in studying weeds is their plasticity within specific habitats and at different densities.

CONCLUSION

This study demonstrated the patterns of functional attributes of *A. viridis* in response to fertilizers and wild populations. Traits such as the number of leaves, number of inflorescences, and plant height explained individual plant responses. Models 1, 2, and 3, focusing on the interactions among these traits, revealed significant r and R^2. Specifically, Model 3 of the inflorescence to leaf numbers and height showed higher r and R^2 values. This study predicted that *A. viridis* would respond to selection pressures such as fertilizers. These three models serve as valuable tools to predict the dependent variables based on the types of available data. The applicability of these models could be extended to broadleaved species with seeds as the primary reproduction mechanism, offering insights into how the traits of a weed species in the wild and responses to fertilizer contribute to its inherent growth and responses. The findings of this study hold potential for broader application, allowing the exploration of these mechanisms in other species for sustainable weed management with the involvement of smallholders. Overall, this study demonstrates how enhanced knowledge of weed biology and responses to selection pressures such as fertilizers could lead to practical advances benefiting farmers. Nevertheless, future research should delve into weed–crop interactions within agricultural settings and optimal fertilization times by integrating the findings of this study to develop a science-based weed management strategy.

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