Herbicide effectiveness and crop yield responses in directseeded rice: insights into sustainable weed management

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Abstract: Background: Conventional method of rice cultivation has proven to be resource intensive, limiting its long term sustainability. On the contrary direct seeding offers a potential rice establishment method provided its increased susceptibility to weed infestation is taken care of. **Objective**: The principle aim of this study was to evaluate both pre- and post-emergence herbicides for effective weed suppression, while optimizing time window for herbicide application. **Methods**: A comprehensive two year study was conducted to assess the efficacy of new generation pre- and post-emergence herbicides including pendimethalin followed by 2,4-D, penoxsulam, pyrozosulfuron ethyl + pretilachlor, triafamone + ethoxysulfuron, ethoxysulfuron, fenoxyprop p-ethyl along with weed-free and weedy check treatments. **Results:** All herbicides substantially reduced weed biomass by 58–94% at 45 days after sowing.

Pre- mix triafamone + ethoxysulfuron proved most effective against grasses and sedges, followed by penoxulam for sedges and pendimethalin followed by 2,4 -D for broadleaved weeds (BLW). Herbicide application significantly improved yield-related parameters compared to the weedy check. Application of pre-mix triafamone + ethoxysulfuron excelled, yielding 7.3 tons per hectare, a remarkable 383% increase over the weedy check. Key yield attributes such as panicles per square meter (349), grains per panicle (91), and 1,000-grain weight (25 g) were significantly elevated due to the application of triafamone + ethoxysulfuron (60 g a.i ha-1). **Conclusion:** Application of triafamone+ ethoxysulfuron an early post emergence herbicide witnessed significantly greater seed yield which was comparable to weed free situation besides, controlling diverse weed flora with higher weed control efficiency of 87 to 90%.

Keywords: Direct seeding; Herbicides; Rice cultivation; Root growth; Weed flora; Yield

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1. Introduction

Rice, a staple food for more than half of the world's population, is grown in more than100 countries with 90% of the total global production from Asia. India, with a significant share in global rice production, produced 196 million tonnes from 46.4 million hectares (Food and Agriculture Organization of the United Nations, 2023). Rice cultivation globally encompasses diverse ecologies marked by presence or absence of water. This diversity manifests in a rich and varied weed flora in rice fields. To mitigate the impact of weeds, the traditional practice of cultivating rice through transplanting, involving submergence to suppress weed competition, has been widely adopted in Asia. In India, the conventional transplanting method is preferred for its effective weed control and minimal yield loss, driven by the advantage of age and growth of rice seedlings, presence of standing water which prevents light to reach weed seeds (Chauhan 2012). However, the labour-intensive and water-demanding nature of traditional transplanting poses sustainability challenges for rice production. In response to these challenges, alternative methods such as direct seeding have gained prominence over the past two decades, offering advantages such as reduced water requirements, lower labour requirement, early maturity, and comparable yields to the transplanted crop. Nevertheless, direct-seeded rice (DSR) is associated with a significant yield penalty, ranging from 50% to 90%, primarily attributed to intense weed competition (Chauhan, Johnson, 2011; Chauhan, Opena, 2012). Unlike the transplanting method, DSR lacks the early head start and weed suppression achieved through flooding (Jehangir et al., 2022; Chauhan 2012; Zhao et al., 2006). Over the years efforts towards sustainable weed control have explored various approaches, with manual and chemical weeding being common practices. The introduction of new generation pre- and post-emergent broad-spectrum herbicides has opened avenues for DSR, alleviating concerns related to labor and water shortages, besides providing early weed free start to the crop (Matloob et al., 2014), hence ensuring competitive edge and weed control efficacy (Khaliq, Matloob 2011). Chemical weed control has successfully replaced labour- intensive, back breaking, and mechanical weed control,

making DSR cultivation more feasible and economical. DSR is facilitated by the application of herbicides, including pre-emergence herbicides options like pendimethalin, oxadiazon, oxadiargyl, pretilachlor, and post-emergence herbicides such as cyhalofop–butyl, bispyribacsodium, penoxsulam, fenoxaprop, azimsulfuron, 2,4-D, metsulfuron-methyl, triafamone + ethoxysulfuron (Singh, Singh 2012; Mahajan, Singh 2013; Khaliq., et al., 2014; Mishra et al., 2016; Arthanari 2023). However, the optimal time window for herbicide application, tailored to specific crop environments, remains crucial for effective weed suppression. Against this backdrop, a field experiment was conducted at the Mountain Research Centre for Field Crops, SKUAST-Kashmir, to evaluate the response of rice and associated weed flora to new herbicide molecules under dry-seeded conditions in a temperate ecological setting. This study aims to contribute valuable insights into the sustainable management of weeds in DSR, offering practical solutions for enhancing productivity in rice cultivation systems.

2. Material and Methods

2.1 Experimental Site

The field experiment was conducted for two consecutive *Kharif* (May to September) seasons in 2021 and 2022 at the Mountain Research Centre for Field Crops, Khudwani, affiliated with Shere-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K). The geographical coordinates of the site are approximately 33.72°N latitude and 75.09°E longitude, with an altitude of 1,600 meters above sea level. The region is characterised by a cold temperate climate experiencing sub-zero temperatures in winter and warm weather in summer, resulting in a short growing season of 140–150 days for the rice crop. The experimental site had a silty clay loam soil, with a neutral pH of 6.5, low available nitrogen (197 kg ha^{-1}) and phosphorus (9.3 kg ha^{-1}) , and medium availability of potassium (214 kg ha $^{-1}$).

2.2 Experimental Design

The experiment was laid out in a randomized block design with three replications. Each exeperimental unit had an area of 8.4 m^2 . It encompassed eight treatments, namely, pendimethalin applied as pre-emergence (PE) 2 days after sowing (DAS) at the rate of 825 g a.i ha-1, followed by 2,4-D at 750 g a.i ha $^{-1}$ at 30 DAS, penoxsulam early post-emergence herbicide (EPOE) at 22.5 g ha-1 at 20 DAS, pyrozosulfuron ethyl + pretilachlor used as PE at $30g + 750 g$ a.i ha⁻¹, triafamone + ethoxysulfuron as early post-emergence EPOE herbicide at 60 g a.i ha-1 at 20 DAS, ethoxysulfuron at 18.7 g a.i ha⁻¹, and fenoxyprop p-ethyl as PE at 500 ml ha-1. Weed-free treatment was maintained through repeated manual weeding, while the weedy check treatment received no control measures, either manual or

herbicidal. A 1-meter buffer zone was maintained between treatments to eliminate any cross-effects of varying water levels and herbicide treatments.

2.3 Planting Material

The newly released *Indica* rice variety, Shalimar Rice-4, was chosen as the test crop due to its high yield potential $(8.0 \text{ t} \text{ ha}^{-1})$, widespread farmer preference, and a maturity period of 140 days.

2.4 Crop Management Practices

The experimental plot was dry ploughed using a disc plough, followed by two tilling operations to achieve a fine tilth. Rice seeds were directly sown at a rate of 50 kg ha⁻¹, with a seeding depth of 2 cm and row and plant spacing of 20 cm x 10 cm, respectively. Nutrients were applied at the rate of 120:60:30N: P_2O_5 : K₂O kg ha⁻¹. One-third of nitrogen, along with the entire quantity of phosphorus and potassium in the form of urea, DAP, and MOP, were applied as basal, and the remaining nitrogen was split into two halves and applied at 20 and 40 DAS. The field was irrigated soon after sowing to expedite germination. After emergence (6–8 days postsowing), no irrigation was provided for the next 10 days to facilitate weed growth. Subsequent irrigation was applied intermittently during vegetative growth, and a shallow 3 cm water level was maintained from booting to milking stages, followed by complete drainage ten days prior to harvest.

2.5 Root Studies

Roots of five randomly sampled rice plants occupying an area of 200 cm2 each at 45 and 90 DAS were used for measuring root volume using the displacement technique (Mishra and Ahmad, 1987) and dry weight after keeping them in oven at 60 °C till constant weight was achieved. The resulting figures were then averaged to get root volume and dry weight per plant.

2.6 Weed studies

Weeds were systematically sampled in 1.0 m² quadrants randomly placed within each experimental plot at 45 and 90 DAS. The collected weeds were carefully severed near ground level, subsequently enumerated, and taxonomically identified. The identified specimens were then categorized into three distinct groups: broad-leaved weeds (BLW), grasses (G), and sedges (S). Following categorization, the weeds underwent a two-step drying process, commencing with sun-drying, followed by oven-drying at 60 °C until a constant weight was attained. Weed density and dry matter were measured and expressed as the number per square meter (m^2) and grams per square meter $(g m^2)$, respectively.

Various impact indices, including weed control efficiency (WCE), weed index (WI), weed management index (WMI), and herbicide efficiency index (HEI), were computed employing the subsequent formulae. Additionally the dry matter of weeds recorded at 45 DAS was used for quantifying the indices such as WMI and HEI.

$$
WCE = \{(X-Y)/ X\} \times 100 \tag{1}
$$

Where

X = Weed dry weight in weedy check

Y = Weed dry weight in treated plot

$$
WI = \{ (Y_{WF} - Y_{T}) / Y_{WF} \} \times 100
$$
 (2)

Where

 Y_{WF} = Yield from weed-free plot Y_{T} = Yield from treated plot

 $WMI = \{(Y_T - Y_c)/ Y_c\} / \{(W_c - W_T)/W_c\}$ (3)

Where

 ${\rm Y}_{_{\rm T}}$ = Yield of the treated plot

 Y_c = Yield of control (weedy check) plot

 W_c = Weed dry weight in control (weedy check) plot

W $_{\textrm{\tiny{T}}}$ = Weed dry weight in treated plot

$$
HEI = \{(Y_T - Y_C) / Y_C\} / (W_T / W_C)
$$
 (4)

Where

YT = Yield of the treated plot

 Y_c = Yield of control (weedy check) plot

 W_c = Weed dry weight in control (weedy check) plot

W $_{\textrm{\tiny{T}}}$ = Weed dry weight in treated plot

2.7 Yield Studies

Yield attributes, including the number of panicles per square meter $(m²)$, the number of grains per panicle, and the 1,000-grain weight (g) at harvest, were recorded from ten randomly chosen hills within each treatment. Data pertaining to grain yield and straw yield were documented on a perplot basis in kilograms. The entire plot was systematically harvested, dried, and weighed, with the recorded figures subsequently converted into metric tons per hectare (t ha- ¹) to facilitate the comparative analyses. The influence of herbicides on yield was evaluated utilizing the following formulae.

YOC (
$$
\%
$$
) = $\frac{Yield from treatment - yield from weedy check}{yield from weedy check} \times 100$

$$
RYL (%) = \frac{Yield from weed free plot-yield from treatment plot}{yield from weed free plot} \times 100
$$

Where, YOC = Yield over check RYL = Relative yield loss

2.8 Statistical Analysis

The data on parameters studied during the course of investigation were statistically analysed using OPSTAT

software. However, data regarding density and biomass of weeds reflected high variation hence were subjected to square root transformation prior to conducting the analysis of variance (ANOVA). ANOVA was employed to assess the variability among treatment means, and the least significant difference (LSD) was applied for mean comparisons at the 0.05 probability level. This approach allowed for robust statistical inferences and reliable differentiation of treatment effects on weed biomass at a significance level set at 0.05.

3. Results and Discussion

3.1 Weed flora

The investigation was conducted under natural conditions in directed-seeded rice, where in a diverse population of weeds was systematically observed. Eight distinct weed species (Table 1), categorized into grasses, BLW, and sedges, were identified.

Aeschynomene plants exhibited varying occurrences across different herbicide treatments, with noteworthy contributions to the total dry matter production (Table 1). Specifically, *Aeschynomene* was recorded in plots treated with penoxsulam, pyrozosulfuron ethyl + pretichlor, and ethoxysulfuron, with respective contributions of 34.0%, 3.0%, and 1.5% of the total dry matter production.

The presence of *Ammania* spp. was significantly affected by herbicide applications. The combination of pendimethalin followed by 2,4-D and pyrozosulfuron ethyl + pretichlor resulted in the complete absence of *Ammania* spp. The distribution of *Ammania* spp. with regard to their contribution to total dry matter production post-herbicide application across other treatments ranked as follows: Fenoxyprop p ethyl > ethoxysulfuron > weedy check > triafamone+ ethoxysulfuron and penoxsulam, with percentage shares of 52.3%, 38.4%, 89.0%, and 2.8%, respectively.

Roripa amphibia was exclusively reported in plots treated with pyrozosulfuron ethyl + pretilachlor (3.5 g m⁻²) and the weedy check (6.6 g m-2).

Cyprus difformis dominated the overall weed flora, with the highest dry matter reported in non-treated weedy check plots (224 g m⁻²), followed by 97.3 g m⁻² in pendimethalin followed by 2,4-D, 48.0 g m⁻² in pyrazosulfuron ethyl + pretichlor, and 12.67 g m⁻² in fenoxyprop p ethyl. The treatments with triafamone+ ethoxysulfuron and penoxsulam recorded the complete absence of *Cyprus difformis*.

Cyprus iria was found in significant quantities in plots treated with pendimethalin followed by 2,4-D (31.3 g m⁻²), followed by the weedy check (8.0 g m-2), ethoxysulfuron (3.7 g m⁻²), and fenoxyprop p ethyl (1.1 g m⁻²).

Echinochloa colona exhibited maximum dry matter production in the weedy check (118.8 g m⁻²), followed by ethoxysulfuron (70.9 g m⁻²). However, the application of triafamone+ ethoxysulfuron resulted in complete control of *Echinochloa colona*.

Eclipta alba was entirely absent in plots treated with penoxulam, while minimal dry matter production of 0.6 g $m⁻²$ was reported in triafamone + ethoxysulfuron. Other herbicide treatments yielded varying weed dry matter productions: 13.4, 11.9, 9.1, 4.2, and 3.5 g m-2 for fenoxyprop p ethyl, ethoxysulfuron, weedy check, pyrozosulfuron ethyl + pretichlor, and pendimethalin followed by 2,4-D, respectively.

Polygonum plebegium was reported exclusively in plots treated with fenoxyprop p ethyl (2.7 g m⁻²) and the weedy check (27.5 g m⁻²), and was absent all other treatments.

3.2 Weed Control

In the DSR fields, a diverse weed flora was observed across various treatments, and the weed population dynamics were influenced by different weed management strategies (Table 2). In comparison to the weedy check, the treatments employing herbicides exhibited lower densities of grasses, BLW, and sedges. Notably, treatments with pyrozosulfuron + pretilachlor were predominantly infested by grassy weeds, while BLW weeds dominated the weed flora in other treatments. Plots treated with pendimethalin followed by 2,4-D were primarily infested with sedges. Strikingly, the application of triafamone+ ethoxysulfuron resulted in the complete absence of grasses and sedges, with penoxulam showing the presence of a few grasses at 45 DAS, but complete control was achieved as the days from sowing progressed.

Our study indicated that total weed dry matter ranged from 1.4 to 9.0 and 1.4 to 11.9 gm-2 among herbicide-treated plots at 45 and 90 DAS, respectively (Table 3). The weedy check exhibited significantly greater dry matter at 45 DAS (10.7 gm-2), followed by pyrozosulfuron ethyl + pretilachlor (9.2 gm^2) and pendimethalin fb 2,4-D (9.2 gm^2). At 90 DAS, the highest dry matter (11.9 gm^{-2}) was observed in the weedy check. Notably, triafamone+ ethoxysulfuron and penoxulam treatments recorded significantly lower dry matter production at both 45 DAS (1.4 and 4.0 gm⁻²,

4 Adv Weed Sci. 2024;42:e020240004 respectively) and 90 DAS (1.4 and 1.8 gm⁻², respectively) (Table 3). Regardless of the number of days from sowing, the plots treated with triafamone + ethoxysulfuron and penoxulam showed absence of grassy and sedge weeds, while BLW was efficiently controlled by pendimethalin fb 2, 4-D at both 45 and 90 DAS.

3.3 Yield and yield attributes

Weed control treatments exerted a significant impact on yield attributes and overall yield. In comparison to the weedy check, all other treatments demonstrated higher values for yield attributes and grain yield. Specifically, treatments with triafamone+ ethoxysulfuron produced the highest number of paniclesm- 2 (349), grains panicle⁻¹ (91), and 1,000-grain weight (25.5 g), closely followed by penoxulam-treated plots. Although the weed-free treatment yielded the highest grain yield $(7.66 \text{ t} \text{ ha}^{-1})$, the triafamone+ ethoxysulfuron treatment was not significantly different, recording a yield of 7.30 t ha⁻¹. The yield superiority among herbicide treatments ranged from 115% to 383%, with triafamone+ ethoxysulfuron and penoxulam demonstrating the maximum increment, while the lowest increase was observed with fenoxyprop p-ethyl (Table 4).The magnitude of relative yield reduction on account of weed interference ranged from 4.1% to 80.0% (Table 4). Maximum yield decrement, amounting to 80% was documented in weedy check. While the plots resorted to application of triafamone+ ethoxysulfuron resulted in comparatively lower yield penalty of 4.6% followed by penoxulam with 12.4%.

3.4 Weed Indices

WCE, an indicator of weed control, exhibited variability among herbicide treatments, ranging between 16% and 86.6% at 45 DAS and 18.5% to 90.4% at 90 DAS compared to the weedy check. Triafamone + ethoxysulfuron, closely followed by penoxulam, consistently demonstrated greater

DAS: days after sowing; PRE: pre-emergence; POE: post emergence, EPOE: Early post emergence. Data in parenthesis represent original values

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WCE over the two-year period. Conversely, pendimethalin *fb* 2,4-D exhibited the lowest values of WCE at 45 DAS (9.0%) and 90 DAS (18.5%). Notably, WCE tended to increase as the crop approached maturity. Among herbicides, triafamone+ ethoxysulfuron recorded the least WI value of 4.7 and a higher HEI of 29.3, while fenoxyprop p-ethyl (3.7) closely followed by ethoxysulfuron (3.8) and triafamone+ ethoxysulfuron (4.4) exhibited lower weed management indices (WMI) (Figure 1).

3.5 Root Dry weight and volume

Root dry weight ranged from 0.74 to 3.75 and 1.41 to 3.89 g per plant¹ at 45 and 90 DAS, respectively (Table 5). Triafamone+ ethoxysulfuron recorded the highest root dry weight (3.75 g plant⁻¹) at 45 DAS, while the weedy check had the lowest value (0.74 g plant⁻¹). At 90 DAS, triafamone+ ethoxysulfuron again exhibited the maximum root dry weight, closely followed by the weed-free treatment. Root volume increased from 45 to 90 DAS, with the highest root volume

DAS: days after sowing; PRE: pre-emergence; POE: post emergence; EPOE: Early post emergence; YOC: Yield over check; RYL: relative yield loss

W1: Pendimethalin *fb* 2 4-D; W2: Penoxsulam; W3: Pyrazosulfuron ethyl + pretilachlor; W4: Triafamone+ ethoxysulfuron; W5: Ethoxysulfuron; W6: Fenoxyprop- p- ethyl; W7: Weed free W8: Weedy check

Figure 1 - Impact of herbicide treatments on weed indices in direct seed rice

observed in triafamone+ ethoxysulfuron-treated plots. The increment in root dry weight and volume ranged from 3.7% to 47.5% and 18% to 45%, respectively, as the crop progressed from 45 to 90 DAS. Notably, rice grain yield demonstrated a positive correlation with both root dry weight and volume at 45 and 90 DAS as indicated by coefficients of determination (R2) ranging from 0.74 to 0.90 (Figure 2a-d).

4. Discussion

The observed diversity in weed flora across various treatments underscores the importance of tailored weed management strategies. Weed flora demonstrated variable responses to different herbicides. Notably, the herbicide treatments involving triafamone+ ethoxysulfuron and penoxulam demonstrated superior efficacy in controlling a

majority of weed species. Both herbicides are acetolactate synthase (ALS) inhibitors working on the principle of halting the flow of assimilate supply to sink thereby inhibiting weed growth (Aranthari et al., 2023; Divine et al., 1990; Shaner et al., 1991). Furthermore, the advantages associated with triamafone+ ethoxysulfuron can be accounted for its greater availability encompassing both foliar and root pathways. *Ammania* and *Eclipta*, although in limited numbers, were the only weed species observed in treatments employing the application of triafamone+ethoxysulfuron.This observation can be potentially attributed to its greater phenotypic plasticity and persistent seed bank encouraging multiple-year germination (Caton et al., 1997).

Our findings regarding penoxulam align with Ghosh et al. (2016), indicating its limited effectiveness against

Table 5 - Effect of herbicide treatments on root dry weight and volume of rice plant under direct seeded condition (Pooled over two uppre

DAS: days after sowing; PRE: pre-emergence; POE: post emergence; EPOE: Early post emergence

Figure 2 - Relationship between root dry weight 45 DAS (a-b), root volume (c-d) and yield at 45 and 90 DAS

Echinochloa crusgalli when applied at 15 DAS. However, this contrasts with the earlier reports of Lassiter et al. (2006), who documented greater vulnerability of *Echinochloa* spp. to penoxulam application. The observed discrepancies highlight the variability in weed responses to herbicides owing to time of application.

Triafamone+ ethoxysulfuron and penoxulam treatments resulted in the complete absence of grasses and sedges. Additionally, the sequential application of pendimethalin followed by 2,4-D demonstrated effective control of BLW. These outcomes are consistent with previous studies reporting successful control of sedges

and grasses with triafomome + ethoxysulfuron application (Yadav et al., 2019). Jehangir et al. (2021) also highlighted the effectiveness of penoxulam against both grasses and sedges up to 60 DAS. Furthermore, sequential application of pendimethalin followed by 2,4-D, demonstrated more effective control of BLWs. This finding is consistent with observations by Mahajan, Chauhan (2013), who reported decreased weed biomass with the sequential application of pendimethalin followed by azimsufuron. Additionally, Dhakal et al. (2019) observed diminished weed biomass in DSR when pendimethalin and 2,4-D were applied sequentially, as opposed to the sole application of pendimethalin.

Higher grain yield in weed-free plots and comparable yields in plots treated with triafamone+ ethoxysulfuron emphasize the significance of effective weed management. Differences in yield among treatments can be attributed to variations in yield attributes at varied weed infestation levels (Jehangir et al., 2021). These results are in line with Menon et al. (2016), who also reported increased yield in plots treated with triafamone+ ethoxysulfuron due to improved WCE and broad-spectrum weed control.

The application of triafamone+ ethoxysulfuron resulted in a significant reduction in weed biomass, highlighting its efficacy. The broad-spectrum nature of triafamone, coupled with its diverse mechanisms of availability through foliage and soil residue activity might have contributed to its absolute weed control (Rosinger et al., 2012). Consistent with Arthanari (2023), our findings support the higher WCE of triafamone + ethoxysulfuron as an EPOE. Moreover, the observed increase in WCE at later stages of the crop may be attributed to the canopy closure, resulting in a smothering effect on weed growth. The lower values of WI and WMI, along with higher HEI values associated with the application of triafamone + ethoxysulfuron, indicate its broad-spectrum weed control efficacy. These findings align with studies by Sen et al. (2020) and Meena et al. (2019), which similarly attribute lower WI values and higher HEI values to the heightened efficacy of herbicides.

Root systems play a crucial role in resource acquisition, influencing the competitive ability of plants, particularly in the acquisition of underground resources. Our study revealed a modest increase in root dry weight from 45 to 90 days, particularly favouring the plots treated with triafamone + ethoxysulfuron, which exhibited a substantial increase in root volume. This can be attributed to the fact that at the earlier stage of plant growth, plants experienced minimal competition from weeds resulting in primary root elongation with good dry wt. thereafter primary and secondary root hairs develop resulting in its greater volume. Conversely, plants experiencing greater competition from weeds might have undergone a different trajectory, resulting in lesser root development.

The observed positive relationship of grain yield with root dry weight and volume up to 90 DAS underscores

the critical role of root growth in ensuring higher grain yield. This aligns with studies emphasizing the competitive advantage of plants in acquiring underground resources through robust root systems (Schaedlera et al., 2020). The study contributes valuable insights into the dynamic interplay between weed management strategies, root development, and crop yield, highlighting the multifaceted nature of sustainable agriculture practices.

5. Conclusions

Our research contributes valuable insights into the complex dynamics of weed management, crop yield, and root development in DSR. We conclude that there are a number of PE and POE herbicide options available for controlling weeds in dry direct seeded rice. Early postemergence application of triafamone + ethoxysulfuron registered significantly greater yield which was comparable to weed free situation besides, controlling weeds with higher WCE of 87 to 90%. Furthermore, additional advantage associated with the same herbicide was its ability to provide opportune window for weed control. Herbicide application also ensured good nutritional environment to the rice crop by establishing robust root system leading to higher yield.

Author's contributions

All authors read and agreed to the published version of the manuscript. IAJ, WR, and AH: conceptualization of the manuscript and development of the methodology: I.A.J., W.R., and A.H.; data collection and curation: A.H.L., and WR.; data analysis: I.A.J, Z.A.S. and E.A.D.; data interpretation: I.A.J, A.H., L.A.S., and S.M.S.; funding acquisition and resources: L.A.S., S.M.S.; and Z.A.S.; project administration: I.A.J., W.R., and A.H.; supervision: I.A.J., and A.H: writing, review and editing.

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