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Full Length Article

Germination and Growth Response of Rice and Weeds to Herbicides under Aerobic Conditions

ABDUL KHALIQ[1] AND AMAR MATLOOB
Department of Agronomy, University of Agriculture, Faisalabad, Pakistan
[1]Corresponding author's e-mail: khaliquaf@gmail.com

ABSTRACT

Studies were conducted to evaluate the germination and growth response of dry-seeded rice (DSR) and associated grassy (jungle rice) and sedge (purple nutsedge) weeds to different pre- and post-emergence herbicides. Seeds of rice and jungle rice (30 each) and tubers (10) of purple nutsedge were sown in individual pots. Pendimethalin, acetochlor, butachlor, oxadiargyl, and pretilachlor were applied as pre-emergence (1 day after sowing; DAS) at 1137, 125, 1200, 80 and 625 g a.i. ha^{-1}, respectively, were applied, while ethoxysulfuron ethyl, penoxsulam, and bispyribac sodium were applied as early post-emergence (15 DAS) at 30, 15 and 30 g a.i. ha^{-1}, respectively. A control treatment without herbicide was maintained for comparison. The tested herbicides depicted differential suppression of germination and seedling growth and accounted for variable seedling mortality of the test species. Acetochlor, butachlor and pendimethalin were effective against jungle rice while ethoxysulfuron ethyl was most efficient in controlling purple nutsedge. Pre-emergence herbicides application also reduced rice germination to a significant extent and caused significant seedling mortality. Nevertheless, irrespective of pre- and post-emergence, all herbicides had detrimental effects on rice seedling growth manifested in the form of reduced elongation of root and shoot and lower leaf and root score and dry biomass. The application timing of pre-emergence herbicides in aerobic rice needs to be adjusted so as to minimize the damage to rice crop.

Key Words: Herbicide phytotoxicity; Germination/seedling growth; Mortality; Dry biomass

INTRODUCTION

Weed communities in DSR are floristically diverse, and higher in abundance than transplanted rice system (Rao *et al*., 2007; Mahajan *et al*., 2009). Recalcitrant weed flora in DSR is difficult to tackle with traditional rice herbicides. Generally, most of the rice herbicides require a critical moisture regime and even flooding is inevitable for their efficient activity. Moisture regimes are known to have a direct bearing on herbicide effectiveness and phytotoxicity by regulating their movement (absorption, adsorption, leaching & translocation) and metabolism in plants. Studies, however, report that herbicides can reduce rice growth as well (Oosterhuis *et al*., 1990; Bollich *et al*., 2000). Recently, Chauhan and Johnson (2011) reported reduction in shoot biomass of rice treated with oxadiazon and bispyribac sodium that was greater under saturated conditions than under aerobic environments. Many of the herbicides are applied at a comparatively advanced stage of growth in transplanted rice (35-40 days after sowing; DAS). In contrast to this, in DSR herbicides are usually applied either after sowing (0-3 DAS) or at 15-25 DAS as post emergence. Timing of application is an important determinant of herbicide efficacy and selectivity due to size differential and other morphological differences (Singh & Singh, 2004), and hence needs to be more precisely identified in DSR than transplanted rice (Rao *et al*., 2007). Owing to sensitivity of management for stand establishment and weed control, DSR has often been considered as a "knowledge-intensive" practice (Johnson & Mortimer, 2005). Unwise herbicide usage can lead to injury, growth stunting and mortality of the crop itself (Snipes & Street, 1987; Oosterhuis *et al*., 1990). Even, if mortality is not observed, injury and stunted growth of seedling can hamper normal stand establishment which is otherwise pivotal for making DSR a success. It is imperative that many of the presently available herbicides molecules may not be useful in DSR simply because those may not satisfy above ends. Therefore, efficacy of such rice herbicides needs to be re-evaluated under aerobic soil conditions, and modifications in application time frame are to be made to select appropriate herbicides for efficient weed management in this rice system. Information regarding herbicide phytotoxicity and rice growth reduction is also crucial to farmers if these are to be used as a tool in DSR on large scale. A number of herbicides varying in mode of action, target weeds, time and method of application are locally available to rice farmers in Pakistan. Jungle rice (*Echinochloa colona* [L.] Link) and purple nutsedge (*Cyperus rotundus* L.) are obnoxious grassy and sedge weeds of DSR (Caton *et al*., 2010) that pose serious

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threat to DSR early in the growing season. Besides their aggressively and competitiveness, these weeds are also troublesome by virtue of their intensified seed bank in DSR fields. Taking into consideration the necessity of chemical weed control for sustainable DSR production, the present study was undertaken to investigate the effectiveness of some pre and post emergence herbicides for controlling jungle rice and purple nutsedge, and to assess their influence on rice growth as well for their field appraisal.

MATERIALS AND METHODS

Planting material: Seeds of rice cultivar Super Basmati were obtained for Rice Research Institute, Kala Shah Kaku, Gujranwala, Pakistan. Seeds of jungle rice and tubers of purple nutsedge were collected from Agronomic Research Area, University of Agriculture Faisalabad and were cleaned manually to ensure physical purity. Tubers were sorted out for uniformity of size. Seeds and tubers were surface sterilized with water: bleach solution (10:1) for 15 min. and rinsed with distilled water four times.

Bioassay: Plastic pots (29 × 18 cm; 10 kg capacity) without hole at the bottom were filled with air dried, sieved, well mixed soil (pH of saturated soil paste & electrical conductivity (EC) of the saturation extract were 7.7 & 0.41 dS m^{-1}, respectively). Since it was difficult to sterile the soil in bulk, inherent seed bank in the soil was subject to suicidal germination twice before it was used for bioassay studies. Seeds of rice and jungle rice (30 each) and tubers (10) of purple nutsedge were sown at a depth twice of their length in individual pots. Pendimethalin, acetochlor, butachlor, oxadiargyl, and pretilachlor were applied as pre-emergence (1 day after sowing; DAS) at 1137, 125, 1200, 80, and 625 g a.i. ha^{-1}, respectively, were applied, while ethoxysulfuron ethyl, penoxsulam, and bispyribac sodium were applied as early post-emergence (15 DAS) at 30, 15 and 30 g a.i. ha^{-1}, respectively. These herbicides were sprayed using hand operated atomizer after volume calibration at 2-4 leaf stage (BBCH scale growth stage 12-14) of test species. A control treatment without herbicide application was maintained for comparison. Herbicides were applied at field capacity (-0.03 MPa) and first irrigation was applied three days after herbicide application. The pots were placed in a screen house (a rectangular steel chamber with 2 mm wire mesh & overhead plastic cover to avoid rain water) under natural solar radiation with an average temperature of 35 ± 5°C. The pots were irrigated with a sprinkler mist as and when required to keep the soil moist and maintain aerobic conditions (one third of gravimetric water content) but avoiding water stress.

Germination counts were made on daily basis according to Association of Official Seed Analysts (1990) until a constant count was achieved in pots treated with pre-emergence herbicides. Seed/tuber was considered to be germinated/sprouted when the hypocotyl length exceeded 2 mm. Application of pre-emergence herbicides inhibited germination and caused seedling mortality of test species as well, yet some plants still remained and were used for recording observations. Final germination percentage was calculated as:

$$\text{Germination (\%)} = \frac{\text{No. of seedlings emerged}}{\text{No. of seeds/tuber sown}} \times 100$$

Plants were uprooted 30 DAS (15 days after post emergence spray) after irrigating the pots with water. The roots were washed with mild tap water pressure to remove adhering soil particles and avoiding breakage of roots. Root and shoot lengths were measured thereof with a scale. All roots and shoots from each pot were separated and oven dried at 70°C for 48 h to get dry biomass of root and shoot. Number of leaves and secondary roots were counted manually and averaged. Seedling mortality was calculated as follows:

$$\text{Mortality(\%)} = \frac{\text{No. of seedlings emerged} - \text{No. of seedlings survived}}{\text{No. of seedlings emerged}} \times 100$$

Separate set of experiments were established for each test species. The pots were arranged in a completely randomized design with four replications and repeated in time. Data were pooled as the results of two runs were similar and were analyzed following an analysis of variance using MSTAT-C software (Freed & Scott, 1986). Treatment means were separated using least significant differences (LSD) at $P \leq 0.05$. Sigma Plot 10.0 (Systat Software Inc., Point Richmond, CA) was utilized for graphics of data.

RESULTS

Jungle rice: Pre-emergence herbicides decreased the germination of jungle rice (Fig. 1). Pendimethalin treated pots recorded 30% germination that was 67% less than that observed in control pots. All other pre-emergence herbicides had similar ($P \leq 0.05$) germination inhibition that ranged from 44 to 57%. Both pre- and post- emergence herbicides significantly suppressed seedling root and shoot growth and caused mortality of jungle rice over control (Figs. 2–5). Root length, dry weight and number of lateral roots were reduced by different herbicides to the tune of 24–100, 43–100 and 24–100%, respectively over control. Maximum inhibition was observed for pretilachlor. Pendimethalin and butachlor provided complete control of jungle rice firstly by inhibiting its germination that was followed by seedling mortality. Marked reduction in shoot length (35–100%) and weight (39–100%) over control were also recorded. Leaf score was reduced to a significant extent (84–100%). Among pre-emergence herbicides, acetochlor and pretilachlor inhibited shoot growth of this weed to greatest extent. Bispyribac sodium was the most effective post emergence herbicide. Herbicides also caused significant seedling mortality of jungle rice and the highest value (100%) was observed for butachlor and pendimethalin followed by pretilachlor (87%) and ethoxysulfuron ethyl

Fig. 1: Influence of different pre emergence herbicides on germination (%) of test species. Vertical bars above mean denote standard error of four replicates

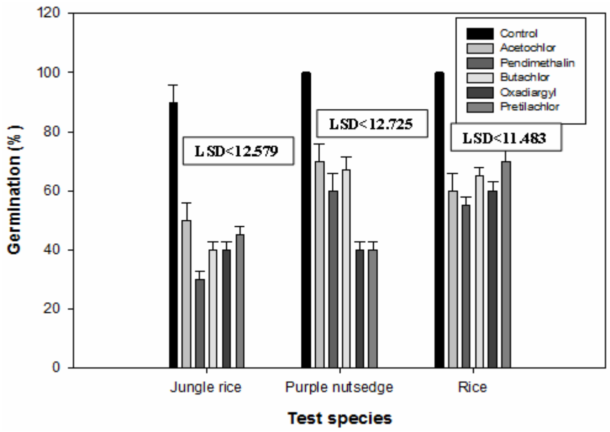
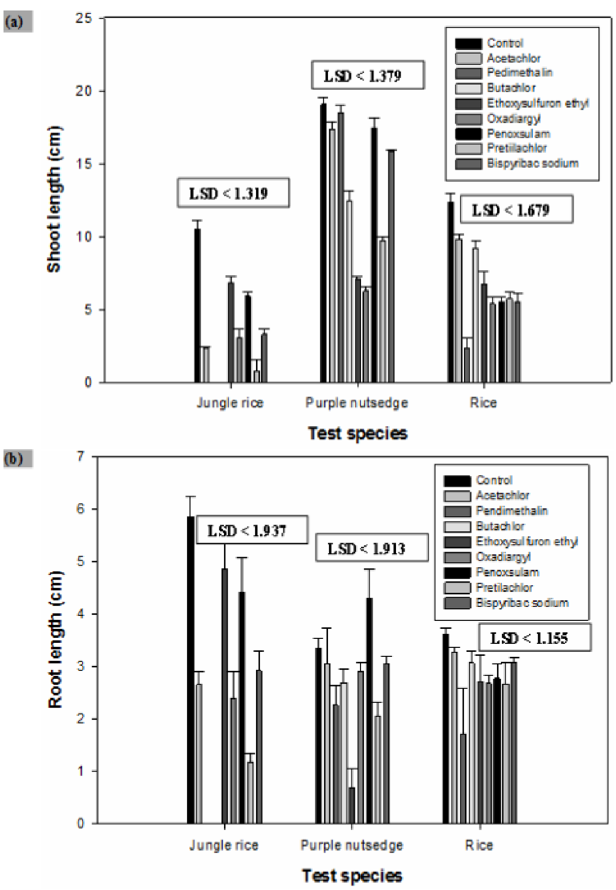


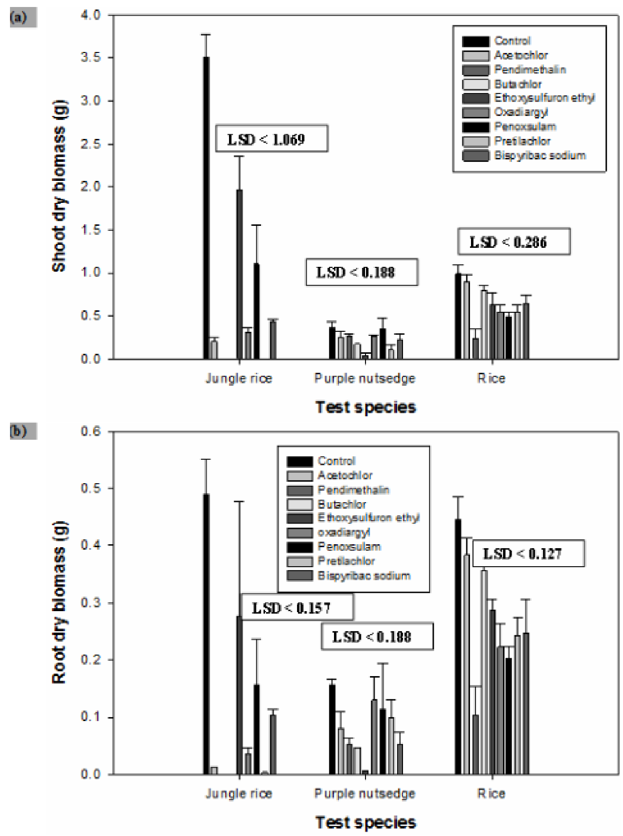
Fig. 2: Influence of different pre and post emergence herbicides on (a) shoot length and (b) root length of test species. Vertical bars above mean denote standard error of four replicates



(67%).

Purple nutsedge: Oxadiargyl and pretilachlor suppressed sprouting of tubers of purple nutsedge by 60% over control (Fig. 1). Pre- or post-emergence herbicides significantly

Fig. 3: Influence of different pre and post emergence herbicides on (a) shoot and (b) root dry biomass of test species. Vertical bars above mean denote standard error of four replicates



suppressed seedling growth and caused mortality of purple nutsedge (Figs. 2–5). Acetochlor, pendimethalin, butachlor, penoxsulam and bispyribac sodium did not reduce seedling growth of purple nutsedge to a considerable extent. Nonetheless, ethoxysulfuron ethyl and oxadiargyl effectively reduced its seedling growth. Ethoxysulfuron ethyl recorded maximum inhibition of root length (79%), shoot (88%) and root (96%) dry biomass and leaf and root score (62%) indicating its activity on both aerial and below ground parts. Although effective in retarding aerial growth of purple nutsedge, oxadiargyl was less effective against below-ground growth (Figs. 1b & 2b). Highest seedling mortality (70%) of purple nutsedge was recorded with ethoxysulfuron ethyl and was followed by pretilachlor and oxadiargyl that were however, at par ($P \le 0.05$). Although showing its effectiveness against root and shoot dry biomass, bispyribac sodium recorded minimum mortality (17%).

Rice: Pre-emergence application of herbicide had negative bearing on rice germination as well (Fig. 1). Pendimethalin decreased germination of rice by 45% over control. Pretilachlor recorded least (30%) inhibition of rice germination. Irrespective of their application timing, all herbicides also had suppressive influence on seedling growth of rice (Figs. 2–5) by reducing root length and dry weight by 9–49 and 15–77% over control. Suppression in shoot length and dry weight ranged from 18–69 and 9–76%, while leaf score was dropped by 18–36% over control. Pendimethalin exhibited its negative implications in the form of yellowing and chlorosis of leaves, reduced root and shoot length and biomass accumulation in these parts. It reduced rice seedling shoot length, dry weight and leaf score by 69, 76 and 36%, respectively over control. Minimum lateral root spread was also observed for this herbicide. It also accounted for 36% seedling mortality in rice. Application of acetochlor (pre-emergence), and ethoxsulfuron ethyl and bispyribac sodium (post emergence) was less phytotoxic to rice seedlings. Minimum seedling mortality (≈4%) was noticed for penoxsulam that was followed by oxadarigyl, bispyribac sodium and ethoxysulfuron ethyl indicating their relatively lower phytotoxicity to young rice seedlings (Fig. 5).

DISCUSSION

All test species expressed differential response to the application of pre and post emergence herbicides thereby reflecting variable levels of sensitivity and control. This indicated the herbicide-group based susceptibility, and urgency to broaden the spectrum of weed control when weed flora of site has sedges as an integral component. Water "*the best herbicide in rice*" can not be employed for this purpose in dry seeded rice. Hence, herbicide usage seems inevitable to combat weed menace since no other viable alternative is available till date to rescue rice farmers (Rahman *et al*., 2012). Associated with herbicide usage, was reduction in germination and growth of rice as well (Figs. 1–5). Studies clearly demonstrated that irrespective of timing of application, herbicides were detrimental to rice growth and survival too, even under aerobic conditions. In this context, Bond *et al*. (2007) and Chauhan and Johnson (2011) who found that herbicides cause plant stunting and reduction of rice growth. Though more phytotoxic under saturated soil conditions, herbicide application to aerobic soil also restricted rice growth (Chauhan & Johnson, 2011). Weeds affect dry seeded rice when rice stand is also in its infancy. Ideally, herbicide application as pre and early post emergence can help control weeds at these times but unfortunately rice was also prone to their ill effects at early growth stage. Use of herbicides often ensures efficient weed control during critical periods in DSR but any growth reduction arising from their usage at initial growth stage can harm subsequent stand establishment of rice as well. Man and Lim (1986) reported that although different herbicides caused some sort of injury to rice plants, butachlor was least injurious to rice, and plants overcame phytotoxicity within one to three weeks. Since herbicides were phytotoxic to rice, they might influence yield levels under field conditions but this aspect was not evaluated in present work. However,

Fig. 4: Influence of different pre and post emergence herbicides on (a) leaf and (b) root score of test species. Vertical bars above mean denote standard error of four replicates

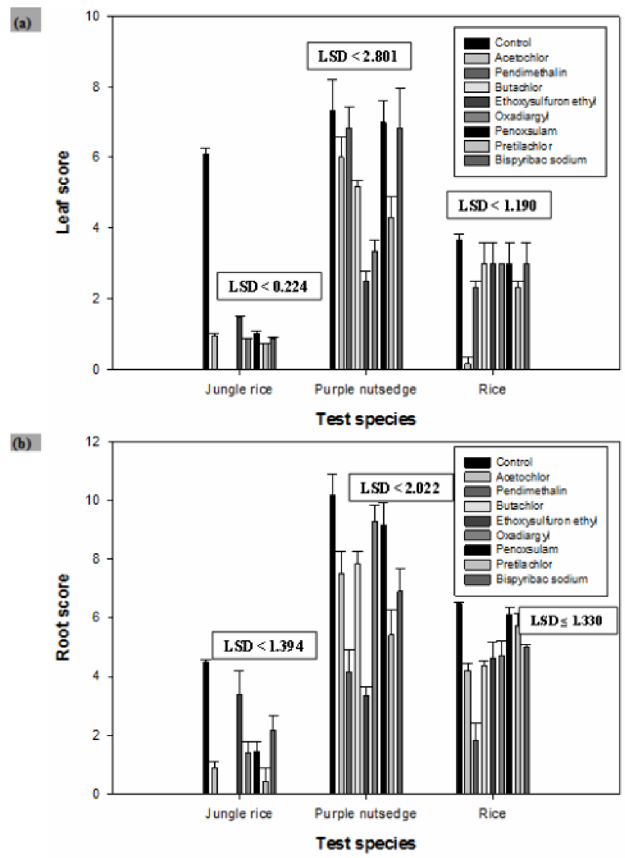
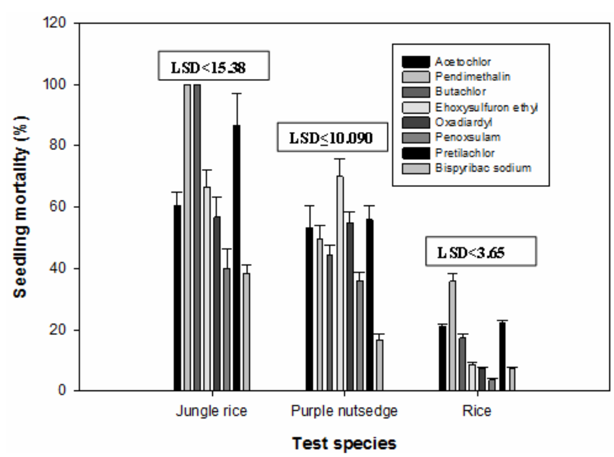


Fig. 5: Influence of different pre and post emergence herbicides on seedling mortality of test species. Vertical bars above mean denote standard error of four replicates



Mahajan *et al*. (2011) stated that rice injury following herbicide treatment (chlorosis & growth inhibition) occurs frequently and is transitory as crop usually recovers in a few weeks and still producing desired grain yields.

Acetochlor, butachlor and pretilachlor (anilides) are mostly active as surface active pre-emergence herbicides interfering with the synthesis of protein, nucleic acid and long-chain fatty acids as their primary mode of action. They are taken up primarily by germinating shoot and secondly through root passing through continuous, and 2 cm or thicker protective layer of herbicide in the soil. Although butachlor is quite effective against grasses in wet-seeded rice, its efficacy is lowered in dry-seeded rice presumably due to low soil moisture of dry seeded rice fields (Bindra *et al*., 2002; Saha *et al*., 2003; Yadav *et al*., 2008). Pendimethalin belongs to dinitroanaline group that prevents germination and arrests root and shoot development of susceptible weeds by acting as mitotic poison (microtubule assembly inhibitor). The observed reduction in rice germination and seedling growth in present studies (Fig. 1) revealed its phytotoxicity, which is also reported elsewhere (Koger *et al*., 2006). Penoxsulam seizures root growth by inhibiting translocation of photosynthates from source (leaves) to roots. Our results regarding penoxsulam effect on rice growth differ from those of Pacanoski and Glatkova (2009) who reported no visual injury symptoms at any application rate. Ethoxysulfuron ethyl (sulfonylurea) and bispyribac sodium (pyrimidinyloxy benzoic chemical family) has the enzyme acetolactate synthase as target site. The inhibition of this enzyme retards the synthesis of branch chain amino acids like valine, leucine and isoleucine, thereby hampering growth due to rapid cessation. All the plants whether susceptible or not posses this enzyme; selectivity, however, arises due to metabolic detoxification because some of the plants convert it into inactive form. Bispribac sodium induces shoot and root injury in rice (Braverman & Jordan, 1996) and up to 65% root injury coupled with reduced length and biomass has been documented (Bond *et al*., 2007). Oxadiargyl (oxadiazole family) mode of action is similar to protoporphyringen oxidase (PPO). Rice sensitivity was lower in dry-seeded than wet-seed rice to oxadiargyl (Gitsopoulos & Froud-Williams, 2004).

Herbicides are phytotoxic by design, and any selectivity achieved depends upon several factors (environment, dose, stage & timing and method of application). Thus, phytotoxicity of the herbicide to crop itself is not uncommon due to the selectivity achieved under field conditions under various conditions. Nevertheless, even if a herbicide molecule is phytotoxic to dry seeded rice, its potential for weed management will still depend on relative benefits compared with other methods of weed control. Present study concludes that acetochlor, butachlor, ethoxysulfuron ethyl and penoxsulam were relatively less harmful to rice and were still effective against weeds. Positivity of weed control associated with these may be considerable and outweigh toxic effects on rice seedling, and possibly on yield, if any. Although less toxic to rice, bispyribac sodium was ineffective against purple nutsedge. Pendimethalin simultaneously diminished germination and seedling growth of rice. Our results suggested that although inevitable in DSR, pre-emergence herbicides exhibited suppressing effects on rice, but their application timing with reference to growth stage and soil moisture regimes need to be adjusted merely because reduction in rice germination owing to their use is hardly compensated. Sensitivity to pendimethalin is related to mesocotyl length that varies among rice cultivars. Possible reason may be the development of coleoptile node about 1.5–2 cm below the treated layer in resistant cultivars with short mesocotyls as against susceptible ones, which elongate and develop coleoptile node at soil surface thus experiencing higher injury. Hence, selection of a suitable rice cultivar coupled with optimized application time can be beneficial in future. Deep seeding (3–4.5 cm) and use of seed coating by protectants may be helpful in securing rice germination against pre-emergence herbicides.

In conclusion, post-emergence herbicide was superior as germination of rice is not effected, but they also caused growth stunting and crop usually recovers after a few weeks. Such negative effects can also be compensated by crop and management practices. Efforts are needed to lessen adverse effects of herbicides on rice through judicious manipulation of factors that regulate their activity in the field. The adjuvants can be explored to enhance efficacy and selectivity of applied herbicides. Moreover, interaction of herbicides with fertilizer and water input also need to be addressed to get better insight into their activity. Cultivars with greater tolerance to different herbicide may also be exxplored. For effective weed control in DSR and to avoid damage to rice, this information should be considered before any herbicide is recommended.

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