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HERBICIDAL ACTIVITY OF PRE AND POST EMERGENT HERBICIDE ON CONTROL *ELEUSINE INDICA* IN AEROBIC RICE SYSTEM

GUNAVATHY SELVARAJH[1], NORHAFIZAH MD ZAIN[1], CHUAH TSE SENG[2] AND ANIDA AMINUDIN[1]

[1]*Faculty of Agro- Based Industry, University Malaysia Kelantan, 17600, Jeli, Kelantan.*
[2]*School of Food Science and Technology, University Malaysia Terengganu, 21030, Kuala Terengganu, Terengganu*
Corresponding author's email: norhafizah.mz@umk.edu.my

Abstract

Aerobic rice system can be an alternate way to cultivate rice in less water conditions. However, weeds are a major constrain in aerobic rice field which decline its success. Weeds are being controlled by herbicides in aerobic rice but not all herbicides are effective in controlling various types of weeds. In this study, two pre-emergent (pretilachor and pendimethalin) and two post-emergent (cyhalofop-butyl and bispyribac-sodium) herbicides were evaluated for effective control of the bioassay species, *Eleusine indica*. It was found that pendimethalin at a higher application rate of 1.0 kg ai ha^{-1} strongly inhibit the emergence and shoot growth of *E. indica* by >75% with negligible effect on the rice growth with stimulation on the leaf greenness. Conversely, pretilachor, cyhalofop-butyl and bispyribac-sodium gave moderate inhibition (55-60% inhibition) on weed emergence and shoot growth at higher application rates of 0.44, 0.1 and 0.035 kg ai ha^{-1}, respectively. Significant inhibitory effects on rice root growth were noticed at highest application rates of pretilachor, cyhalofop-butyl and bispyribac-sodium (40-50% inhibition) across the growth stage of rice seedlings. Great reduction in shoot height, shoot fresh weight, and greenness of rice plant also was evident at 0 DAS across herbicides rates. However, with increasing growth stages, the rice plant became less susceptible to the applied treatments. The finding suggested that pendimethalin at 1.0 kg ai ha^{-1} was the most suitable application rate for inhibiting *E. indica* without injuring the rice seedlings.

Key words: *Eleusine indica*; Herbicides; Aerobic rice.

Introduction

Aerobic rice cultivation is becoming trendy among rice growers in Asia. In the present scenario, direct seeded rice technique (DSR) under aerobic condition is adopted by many growers due to attractive option of less water and labor usage. DSR technique is practiced widely instead of conventional flooded transplanting in order to overcome water and labor shortage (Jabran *et al.*, 2012). In many instances, the DSR method faces failure due to high weed infestation. The weed infestation found to be higher in DSR due to its favourable soil conditions. It has been reported that 90 weed species competing with rice under aerobic system, which causes reduction in grain yield by 23-100% (Chauhan *et al.*, 2015). The weed composition in DSR is highly variable and categorised into three major groups which are grasses, sedges and broad leaved. Mahajan *et al.*, (2014) found that the infestation of grasses in dry direct seeded rice in India is the highest followed by sedges and broadleaved weed respectively. Dominant weed infested DSR varies, and *Eleusine indica* is listed as one of main weed species (Chauhan & Opẽna, 2012).

Since DSR is more prone to the invasion of weed and weed competition, a reliable affordable approach and timely weed management vital to make DSR acceptable commercially (Khaliq & Matloob, 2012). The quick and easy viable method presently available is using herbicides. There are various options available such as manual weeding, tillage practices and biological control, but it is costly and time consuming. The most prevalent option is herbicide usage, where pre and post emergent efficacy in suppressing weed in aerobic rice is widely established (Anwar *et al.*, 2012; Jabran *et al.*, 2012; Singh *et al.*, 2016). Dixon & Clay (2004) reported that pre-emergence activity of herbicides was greater, give extremely good control and even completely kill many of weed species starting from low doses. Similarly, post-emergence herbicide, although applied at later stage had shown significant weed suppression in rice field (Ismail *et al.*, 2013). Studies reported that herbicides could induce phytotoxicity to rice seedlings in aerobic condition. Recently, Chauhan & Johnson (2011) reported herbicide induce phytotoxicity in DSR rice. Many of herbicides are applied at advanced stage of growth in transplanted rice, but in DSR, herbicides were applied 0-3 DAS or 15-25DAS (Khaliq & Matloob, 2012). It is imperative that timing of application can be a determinant factor of herbicide efficacy in suppressing weed.

Besides many of presently available herbicides may not be useful to suppress weed in DSR, hence herbicide efficacy on weeds in DSR needs to be re-evaluated based on herbicide phytotoxicity on weed emergence and aerobic rice (Khaliq & Matloob, 2012). However, several herbicides were recommended for weed management in DSR (Akbar *et al.*, 2011). For instance this researcher also found out that application of pendimethalin, pretilachor and butachlor suppress weed in DSR by 70% with paddy showed improvement 7-19%. Similarly, Jabran *et al.*, (2012) reported that in DSR field, bispyribac-sodium, penoxulam and pendimethalin improves the yield and control the weeds effectively by >50%. Application of herbicide such as pendimethalin suppressed the growth of weeds and reduced the weed density by >60% (Nadeem *et al.*, 2006). Hence, different types of herbicide possess different capability in suppressing weeds by not inducing injury to rice seedlings. Therefore the present study conducted to evaluate the pre and post emergent herbicidal activity on controlling *E. indica* and to determine the herbicide phytotoxicity level on aerobic rice seedling.

Materials and Methods

Plant materials and chemicals: Aerobic rice seed were obtained from Malaysian Agricultural Research and Development Institute (MARDI), UPM Serdang, while bioassay species, *Eleusine indica* seed collection was done in rice fields at Pasir Mas, Kelantan, Malaysia (6.07704° N, 102.2384° E), and subjected to propagation in glasshouse. Commercial herbicides were purchased from Agricultural Chemicals (M) Sdn. Bhd. (ACM), Penang, Malaysia.

Soil bioassay: Pre-emergent and post-emergent activity of herbicides were evaluated by using AERON 1 and *E. indica* as the bioassay species. Moist sandy loam soil was filled into plastic pot (8cm diameter by 9cm height) with holes at the bottom. Dry rice seed following aerobic rice seeding rate 150 kg ha^{-1} was buried in soil evenly at depth of 1cm for each pot through direct seeding method. Prior to sowing, the soil was prepared with organic fertilizer at 1500kg ha^{-1} and first NPK fertilizer with rate 150:60:60 were applied during 7 days after seed germination (Othman *et al.,* 2014). The pots were placed in a 80- by 60- by 5-cm tray and water was applied from the bottom of pots for good growth of rice seedlings. The trays were immediately placed in a glasshouse and maintained at relative humidity 75–80% and temperature 25-30^{0}C, with 12-h photoperiod. The herbicides were prepared in three application rates; pretilachlor (0.11, 0.22, and 0.44 kg ai ha^{-1}), pendimethalin (0.25, 0.50, and 1.00 kg ai ha^{-1}), cyhalofop-butyl (0.025, 0.05 and 1.0 kg ai ha^{-1}) and bispyribac-sodium (0.0088, 0.0175 and 0.035 kg ai ha^{-1}). Pre-emergent herbicide (pretilachor and pendimethalin) was applied 3 days after sowing of *E. indica* seed, while post-emergent herbicide (cyhalofop-butyl and bispyribac-sodium) was applied 20 days after sowing of *E. indica*. The rice seedlings at the growth stages of 0, 4, 8, and 12 days were sown with 30 seeds of *E. indica* on the soil surface. Herbicides with different rates were applied onto the soil surface with a micropipette. Non-treated rice plant and *E. indica* seeds were used as control treatments.

Data collection: Number of *E. indica* seedlings emergence was counted and recorded after 30 days of herbicides treatment. Shoot fresh weight of rice seedlings and *E. indica* was determined by harvesting and weighing aboveground living tissues remaining for each seedling. Meanwhile the rice seedlings root length, shoot height and leaf greenness was measured and the data were subjected to percentages expression of the respective controls.

Statistical analysis: Experiment was arranged in a completely randomized design (CRD) with five replications. All data were subjected to two-way ANOVA analysis and excluded non-treated control data. The Tukey HSD was used to compare the mean among the treatments. Differences were regarded as significant when the p-values were less than 0.05 ($p<0.05$). In addition, all the percentage of weed data were fitted to a logistic regression model (Kuk *et al.,* 2002) (Sigma Plot 2006 version 10.0, Systat Software, Inc., 225 W Washington St., Suite 425, Chicago, IL 60606) to obtain the concentration that causes 50% inhibition on weed emergence and shoot fresh weight as follow:

$$Y = d / \left(1 + [x / x_0]^b\right)$$

where Y = percentage of weed emergence/shoot fresh weight, d = the coefficients corresponding to the upper asymptotes, x = pendimethalin/ pretilachlor/ chyhalofop-butyl/ bispyribac-sodium, x0 = pendimethalin/ pretilachlor/ chyhalofop-butyl/ bispyribac-sodium rate that required to inhibit the weed emergence/shoot fresh weight by 50% relative to untreated seeds, and b = the slope of the line. Regression analyses were conducted, and ED_{50} (rate that gives 50% inhibition) were calculated from the regression equations.

Results and Discussion

Effect of herbicides on *E. indica*: Experimental results revealed that the herbicide treatments had effects on *E. indica* weed. The data of weed emergence and shoot fresh weight were pooled for a single analysis since there was no significant difference in phytotoxic effect of herbicides on weed sown at different rice plant growth stages (data not shown) (Fig. 1). The highest pendimethalin application rate (1.0 kg ai ha^{-1}), has recorded a great inhibition on weed emergence and shoot fresh weight with >75 % inhibition (Fig. 1). In contrast, pretilachor, cyhalofop-butyl and bispyribac-sodium gave moderate inhibition (55-60% inhibition) on weed emergence and shoot growth at higher application rates of 0.44, 0.1 and 0.035 kg ai ha^{-1}, respectively. It was noticed that pendimethalin was proven highly phytotoxic to *E. indica* where the weed emergence and shoot fresh weight was reduced by 50% at a concentration less than half from the highest application rate (ED_{50}=0.327 kg ai ha^{-1}) when compared to other herbicides (Table 1). Lower weed emergence and shoot fresh weight indicates the superiority of pendimethalin in controlling *E. indica*, which in turn can be simplified as pendimethalin exerts high toxicity to the bioassay species, where severe injury observable.

Treatment using pendimethalin creates high toxicity environment to the problematic weed *E. indica*. It was noticed that at an application rate of 0.5 and 1.0 kg ai ha^{-1} pendimethalin, the shoot of weed become discolored and/or drying up which eventually lead to dying of the weed. This finding is in line with study conducted by Daniel *et al.* (2012) where pendimethalin has been found to reduce weed density and dry weight of various grasses. Additionally, pendimethalin at (1.0 kg ai ha^{-1}) has attained 90.3% weed control efficiency over various grasses including *E. indica* in aerobic rice field (Kumar *et al.,* 2015). Pendimethalin at 1.0 kg ai ha^{-1} gave a 72% reduction in grassy weed density in rice field which promoted the rice yield (Singh *et al.,* 2007). Singh *et al.* (2016) reported that in both different study location, pendimethalin had recorded lower grassy weed density averaged 10-16 and it also suppressed the broadleaved weeds. Thus the effectiveness of pendimethalin in controlling weed flora is well established. Pretilachor, cyhalofop-butyl and bispyribac-sodium does not provide a good control for *E. indica* which indicates it might not be a suitable control for grassy weed. A study conducted by Anwar *et al.* (2012) has revealed that pretilachor controls broad leaf weeds better than grasses or sedge at earlier stage while cyhalofop-butyl and bispyribac-sodium weed control is better at later stage.

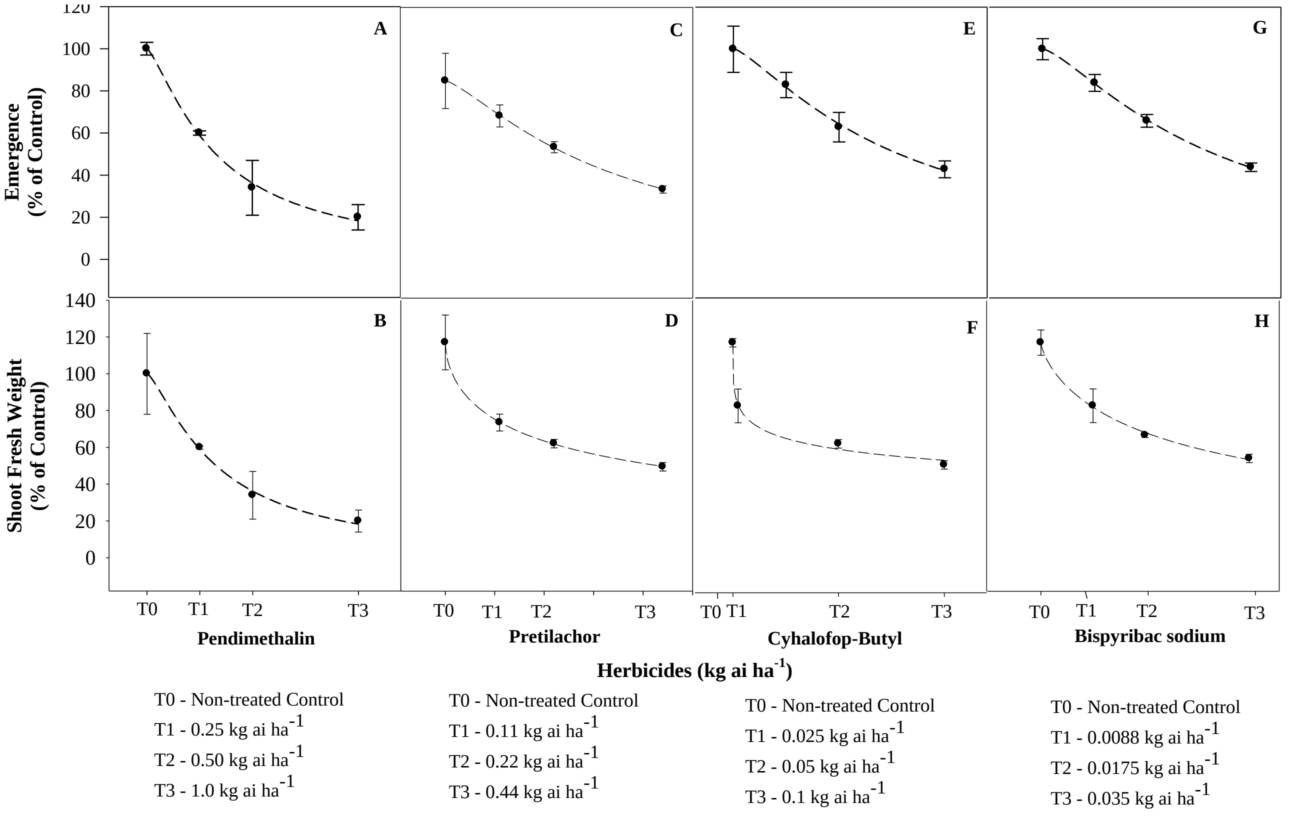

Fig. 1. Effects of herbicide application on weed emergence (A, C, E, G) and shoot fresh weight (B, D, F, H) of *Eleusine indica*. Vertical bar represents standard deviation (SD) of the mean.

Table 1. ED_{50} inhibition rate on weed emergence and shoot fresh weight 30 d after application

ED_{50}	**Emergence**	**Shoot fresh weight**
	ED_{50} Inhibition Rate (kg ai ha^{-1})	
Treatment		
Pendimethalin	0.327	0.327
Pretilachor	0.336	0.246
Cyhalofop-butyl	0.079	0.046
Bispyribac-sodium	0.029	0.026

Effect of herbicides on aerobic rice plant: The injury level of rice seedlings toward herbicides was determined in this study. Since no rate-by-growth-stage herbicide interaction observed, data were pooled over and main effects are presented (Table 2, 3, 4, 5).

Root length of aerobic rice: Highest application rate of pretilachlor had an adverse impact on the rice root length, followed by cyhalofop-butyl, bispyribac-sodium and pendimethalin (Table 2, 3, 4, 5). Averaged across pretilachlor, cyhalofop-butyl and bispyribac-sodium rate, rice root growth at 0 DAS were 76-77% of the non-treated control. However, the root injury of rice seedlings was likely negligible when treated with pendimethalin at this growth stage (almost 90% of the non-treated control). Early at 4 DAS, the root length of rice seedlings were less likely affected by pretilachlor, cyhalofop-butyl and bispyribac-sodium treatment, where the rice seedlings growth exhibited at least 80% of non-treated control.

Shoot height and shoot fresh weight of aerobic rice: It was found that the rice seedlings growth start to reduce with the increasing of herbicide rates (Table 2, 3, 4, 5). Strong reduction was noticed at highest application rate of bispyribac-sodium followed by cyhalofop-butyl, pretilachlor and pendimethalin (10-40% inhibition). Averaged across pendimethalin rate, a small reduction on shoot height and shoot fresh weight was noticed at 0 DAS (90% of the non-treated control). However, at 12 DAS, the rice seedlings development was increased similar to control (almost 100% of the non-treated control). Conversely, the growth of rice seedlings that treated with cyhalofop-butyl and bispyribac-sodium treatment exhibited 82-90% of the non-treated control at the same growth stage.

Leaf greenness of aerobic rice: A decrease in leaf greenness of the rice seedlings under herbicides stress was observed (Table 2, 3, 4, 5). There was a significant reduction in leaf greenness of the rice seedlings subjected to pretilachlor, cyhalofop-butyl and bispyribac-sodium at different application rates. The greenness was significantly reduced by 20-30% at the highest application rate of pretilachlor, cyhalofop-butyl and bispyribac-sodium herbicide. It was interesting to note that, a slight stimulation of 7-10% was observed in the leaf greenness of the rice seedlings that were treated with pendimethalin. Similarly, averaged across herbicides rate, a lesser increase on leaf greenness was also noticed as the rice growth stage increased. However, a great reduction on leaf greenness was found when the rice seedlings were treated with pretilachlor at 0 DAS, but starting at 4 DAS, the leaf greenness was likely similar to control. On the other hand, the increase in leaf greenness was not too pronounced after the rice seedlings were treated with cyhalofop-butyl and bispyribac-sodium in between 0-12 DAS.

Both pre-emergent and post emergent herbicides affected the performance of aerobic rice shoot height, shoot fresh weight, root length and greenness content. However, unlike the other three herbicides, pendimethalin has stimulated the aerobic rice greenness instead of reduction. Maximum reduction was observed in pretilachor, cyhalofop- butyl and bispyribac-sodium. The crop injury level is not more than 30% for all herbicides. The aerobic rice phytotoxicity was lesser in aerobic soil conditions. This finding was in line with Chauhan & Johnson (2011), where direct seeded rice exhibit higher phytotoxicity in saturated soil rather than aerobic soil. Other than this factor, herbicide application timing also greatly influences the crop injury level. The crop injury is severe at 0 and 4 DAS while later application on 8 and 12 DAS shows lesser injury. This recent results was in line with a research conducted by Dilipkumar *et al.,* (2012) where the rice seedlings were more tolerant to treatments of pretilachor and sunflower extract as they grew older. Similarly Besancon *et al.*, (2016) also found that the timing of herbicide application in early development of crop stages is not suitable. In contrast, Khaliq & Matloob (2012) reported that all pre and post emergence herbicide induced crop injury irrespective of their application timing. Besides, they also found that the increased concentration of pendimethalin at 1.137 kg ai ha^{-1} more than the recommended rate gave negative implication on rice in the form of yellowing and chlorosis of leaf, reduced root and shoot length. In connection to this, results from our study shown that there was slight detectable impact on the aerobic rice growth when treated with pendimethalin. It was noticed no visible injury, was observed like chlorosis, stunting, wilting or necrosis in aerobic rice plants, even at the highest application rate (1.0 kg ai ha^{-1}), suggesting that this application rate was efficient in controlling *E. indica* without injuring the rice seedlings.

Table 2. Effects of pendimethalin rates and growth stage at application on rice root length, shoot height, shoot fresh weight and greenness content 30 d after application.

Main effect	Root length	Shoot height	Shoot fresh weight	Leaf greenness
	(% of Control)			
Treatment				
T0 - Non-treated control	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}
T1 - 0.25 kg ai ha^{-1} pendimethalin	99 ^{a}	95 ^{ab}	96 ^{ab}	110 ^{ab}
T2 - 0.50 kg ai ha^{-1} pendimethalin	89 ^{b}	93 ^{b}	91 ^{b}	108 ^{ab}
T3 - 1.0 kg ai ha^{-1} pendimethalin	83 ^{b}	92 ^{b}	90 ^{b}	107 ^{b}
Application timing (DAS)*				
0	88 ^{a}	91 ^{a}	89 ^{a}	103 ^{a}
4	91 ^{ab}	96 ^{ab}	91 ^{a}	109 ^{a}
8	96 ^{bc}	97 ^{ab}	96 ^{ab}	107 ^{a}
12	101 ^{c}	99 ^{b}	100 ^{b}	104 ^{a}

* Herbicide treatment done to rice seeds at 0, 4, 8 or 12 days after sowing (DAS). Main effect mean within the same column followed by the same letter has no significant difference at p<0.05 after determined by a Tukey test.

Table 3. Effects of pretilachor rates and growth stage at application on rice root length, shoot height, shoot fresh weight and greenness content 30 d after application.

Main effect	Root length	Shoot height	Shoot fresh weight	Leaf greenness
		(% of Control)		
Treatment				
T0 - Non-treated control	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}
T1 - 0.11 kg ai ha^{-1} pretilachor	83 ^{b}	90 ^{b}	88 ^{b}	98 ^{a}
T2 - 0.22 kg ai ha^{-1} pretilachor	68 ^{c}	83 ^{c}	78 ^{c}	92 ^{b}
T3 - 0.44 kg ai ha^{-1} pretilachor	51 ^{d}	75 ^{d}	73 ^{c}	81 ^{c}
Application timing (DAS)*				
0	77 ^{a}	85 ^{a}	87 ^{ab}	77 ^{a}
4	78 ^{a}	88 ^{ab}	85 ^{ab}	98 ^{b}
8	79 ^{a}	90 ^{ab}	82 ^{a}	99 ^{b}
12	79 ^{a}	91 ^{b}	91 ^{b}	99 ^{b}

* Herbicide treatment done to rice seeds at 0, 4, 8 or 12 days after sowing (DAS). Main effect mean within the same column followed by the same letter has no significant difference at p<0.05 after determined by a Tukey test.

Table 4. Effects of cyhalofop-butyl rates and growth stage at application on rice root length, shoot height, shoot fresh weight and greenness content 30 d after application

Main effect	Root length	Shoot height	Shoot fresh weight	Leaf greenness
	(% of Control)			
Treatment				
T0 - Non-treated control	100 [a]	100 [a]	100 [a]	100 [a]
T1 - 0.025 kg ai ha^{-1} cyhalofop-butyl	83 [b]	84 [b]	78 [b]	91 [b]
T2 - 0.05 kg ai ha^{-1} cyhalofop-butyl	73 [c]	74 [c]	68 [c]	82 [c]
T3 - 0.1 kg ai ha^{-1} cyhalofop-butyl	59 [d]	67 [d]	59 [d]	71 [d]
Application timing (DAS)*				
0	76 [a]	78 [a]	73 [a]	85 [a]
4	80 [b]	83 [b]	77[ab]	86 [a]
8	81 [b]	83 [b]	80 [b]	88 [ab]
12	82 [b]	83 [b]	82 [b]	89 [b]

* Herbicide treatment done to rice seeds at 0, 4, 8 or 12 days after sowing (DAS). Main effect mean within the same column followed by the same letter has no significant difference at $p<0.05$ after determined by a Tukey test

Conclusions

Pre-emergent herbicide, pendimethalin has a potent phytotoxicity to control *E. indica* weed, with less injury to aerobic rice seedlings. Pendimethalin has a strong promising herbicidal activity on *E. indica* and might have control on other types of weed in aerobic rice fields. A further study is needed to elucidate the effectiveness of pendimethalin herbicide in controlling other types of weed and can be used as a mixture or sequential application with other ingredients.

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