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Response of *Eleusine indica* to herbicides and N fertilizer in dry seeded rice

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Weed has become one of the most serious problems in dry seeded rice (DSR). To increase the competitiveness with weeds and achieve the optimum growth of rice, weed management in DSR needs an integration of herbicides and optimum nitrogen (N) fertilizer rates. In this study, the bioassay species were treated with a series of pre-emergence (pretilachlor and pendimethalin) and early post-emergence (propanil+thiobencarb) herbicide concentrations and N fertilizer. It was found that pretilachlor at an application rate of 0.25 and 0.50 kg ai ha^{-1} almost completely inhibit the emergence and shoot growth of *E. indica* by 100% inhibition, at 50, 100 and 150 kg ha^{-1} N, respectively, but it showed severe injury symptom to the root of rice seedlings. Meanwhile, pendimethalin and N fertilizer at moderate application rate gave a strong reduction of weed emergence (>50% inhibition) and shoot fresh weight (>85% inhibition), with negligible effect on the rice seedlings growth. Significant inhibitory effects on weed emergence (60% inhibition) and shoot growth (90% inhibition) also were noticed at the highest application rate of propanil + thiobencarb (3.6 kg ai ha^{-1}) and N fertilizer (150 kg ha^{-1} N), with stimulation effects on the growth of rice seedlings. This promising effect showed combination of propanil + thiobencarb and N fertilizer (3.60 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N) was the most efficient treatment for *E. indica* control in dry seeded rice.

Key words: *Eleusine indica*, herbicides, N fertilizer, dry seeded rice.

INTRODUCTION

The dry seeding (DSR) technique of rice cultivation is becoming popular to farmers and growers in many parts of Asia. DSR is primarily practiced as a response to the labour shortage, and is currently practiced in Malaysia, Thailand, Vietnam, the Philippines, and Sri Lanka (Nirmala et al., 2016). However, weeds become the major problems to the sustainability of DSR where it had caused high yield losses in rice production (Ahmed et al., 2015). It was found that estimated losses in rice yields from weeds are up to 90% in DSR systems (Mahajan et al., 2014). The major weeds in direct seeded rice were grasses, followed by broad leaved weeds and sedges

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(Singh et al., 2016). A wider range of weeds, especially those of grasses, are found infesting direct-seeded fields with *Eleusine indica* being among dominant species.

Weed infestation in DSR depends on several factors such as weed seed bank, tillage system, cultivar used, fertilizer, and water management (Mahajan and Chauhan, 2013). Under such circumstances, weed management in DSR is very crucial. Various means can be used to reduce weed infestations, which include mechanical, chemical, and biological control. However, the use of herbicide is considered the best tool to manage weeds in rice field since it is a very effective, reliable and inexpensive method compared to hand weeding (Rashid et al., 2012). Although using herbicides is an economical weed control method, reliance on herbicides alone is not sustainable in achieving season-long weed-free crop (Chauhan and Opena, 2013). It was found that the application of pre and post-emergence herbicides was not enough to achieve optimum weed control in DSR (Chauhan et al., 2015). Therefore, an alternative weed control method is needed. One of the alternative strategies might be through banded application of herbicide and N fertilizer to increase the competitive ability of crops over the weeds.

Nitrogen (N) plays a significant role in crop-weed competition (Unruh, 2013). Previous studies found that the addition of N fertilizer reduces the competitive advantage of the crop while in other cases enhances the growth of weeds (Tosti et al., 2016). The response of crop and weed to N level depends on several factors such as species and density of the crop or weed (Mick Assani, 2016). Studies also reported that a N fertilizer application is able to break weed seed dormancy and influence weed structure and densities (Nichols et al., 2015). According to a study conducted by Nader et al. (2012), N has been used as carrier for enhancing herbicide efficacy, however, N function was not fully understood and was thought to enhance herbicide absorption by the target species. Therefore, this study was conducted to assess the herbicidal activity of pre and early post-emergence herbicides with N fertilizer on *E. indica* and to determine tolerance levels of local rice variety to herbicides and N application.

MATERIALS AND METHODS

Plant materials

Seeds of the bioassay species, *Eleusine indica* were collected from rice fields of Pasir Mas, Kelantan, Malaysia (6.07704°N, 102.2384°E), and propagated in a glasshouse; meanwhile local rice seeds (MR263) were provided by Kelantan Agricultural Development Authority (KADA), Kelantan, Malaysia.

Chemicals

Commercial herbicides were purchased from Agricultural Chemicals (M) Sdn. Bhd. (ACM), Penang, Malaysia, while the N fertilizer was purchased from Golden Rengat Enterprise, Temerloh Pahang.

Soil bioassay

Herbicidal activity of pre and early post-emergence herbicides with N fertilizer was carried out by using the MR263 local rice variety and *E. indica* as the bioassay species. Moist silty loam soil was filled into a plastic pot (8 cm diameter by 9 cm height) with holes at the bottom. Through the direct seeding method, the rice seed was covered with soil immediately after sowing (Zheng et al., 2016). The pots were placed in an 80- by 60- by 5-cm tray and water was applied from the bottom of pots for proper growth of rice seedlings. The trays were immediately placed in a glasshouse and maintained at relative humidity of 75 to 80% and a temperature range of 25 to 30°C, with a 12-h photoperiod. The herbicides and N fertilizer were prepared in three application rates; pretilachlor (0.125, 0.25 and 0.50 kg ai ha^{-1}), pendimethalin (0.25, 0.50 and 1.00 kg ai ha^{-1}) propanil + thiobencarb (0.9, 1.8 and 3.6 kg ai ha^{-1}) and N fertilizer (50, 100 and 150 kg ha^{-1}). N fertilizer at each application rate was applied onto the soil surface with different growth stage of rice seedlings in the pots to provide different level of N in the soil. Then, 30 seeds of *E. indica* were sown on the soil surface when the rice seedlings were at the growth stages of 0, 10 and 15 days after being sowed in the soil. After one day, 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.

Observations

The number of emerged *E. indica* seedlings was counted and recorded after 30 days of treatment. Meanwhile, the shoot fresh weight of *E. indica* and rice seedlings was determined by harvesting and weighing aboveground living tissues remaining for each seedling. Root lengths and shoot heights of rice seedlings were then measured, and the data were expressed as percentages of the respective controls.

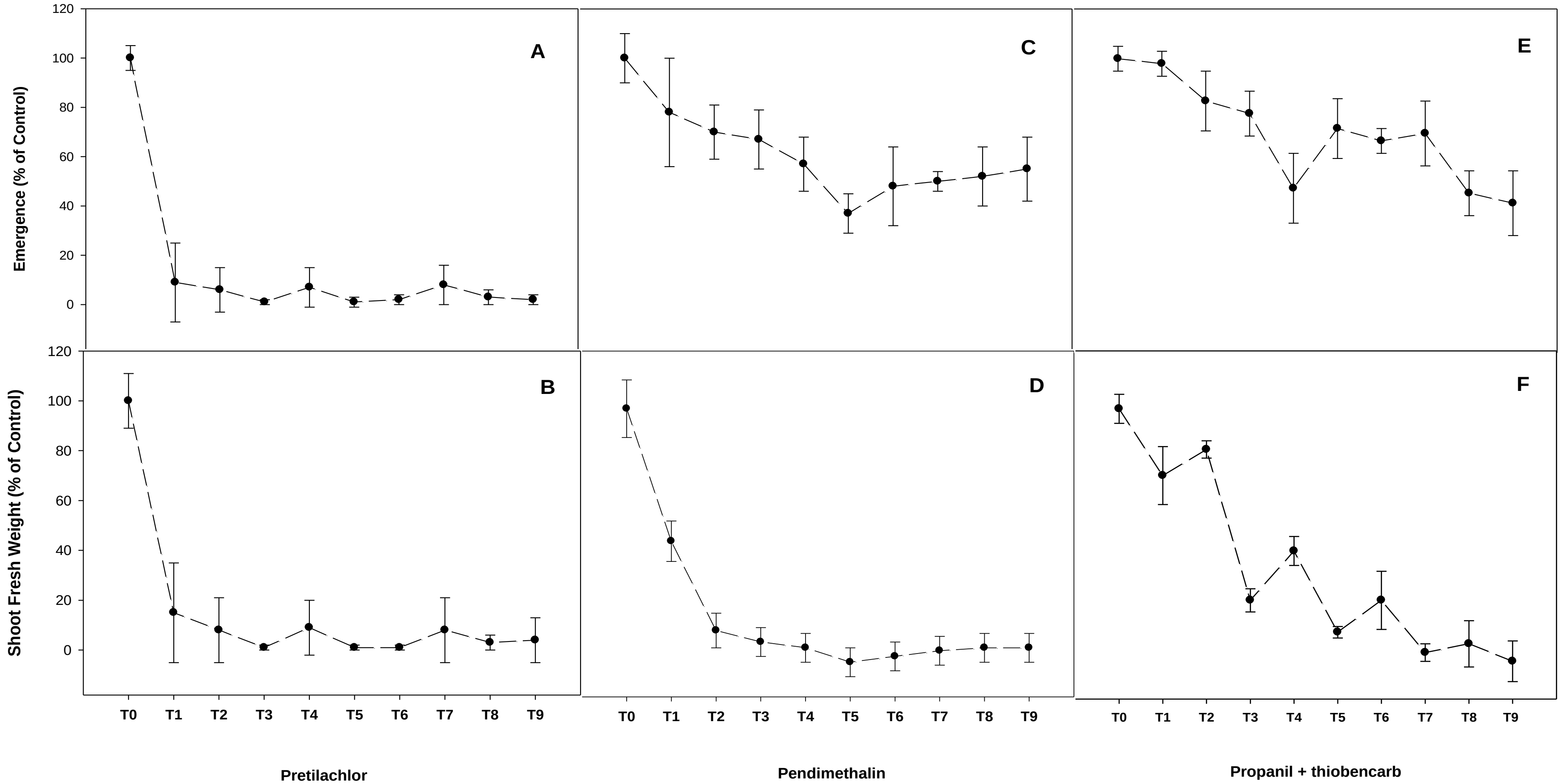
Statistical analysis

Each experiment was arranged in a completely randomized design (CRD) with five replications. Each experiment was repeated twice. All data were subjected to two-way ANOVA 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 ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of herbicides and N fertilizer on weed

The effects of commercial herbicides and N fertilizer treatment on *E. indica* seeds are presented in Figure 1. Since there was no significant difference in phytotoxic effects of commercial herbicides and N fertilizer to *E. indica* sown at different growth stages of rice plants, all data were pooled for analysis. It was found that pretilachlor at an application rate of 0.25 and 0.50 kg ai ha^{-1} almost completely inhibited the emergence and shoot growth of *E. indica* by 100% inhibition, at 50, 100 and 150 kg ha^{-1} N, respectively (Figure 1A and B). These



T0 - Control
T1 – 0.125 kg ai ha^{-1} pretilachlor + 50 kg ha^{-1} N
T2 – 0.25 kg ai ha^{-1} pretilachlor + 50 kg ha^{-1} N
T3 - 0.50 kg ai ha^{-1} pretilachlor + 50 kg ha^{-1} N
T4 – 0.125 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N
T5 – 0.25 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N
T6 – 0.50 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N
T7 – 0.125 kg ai ha^{-1} pretilachlor + 150 kg ha^{-1} N
T8 – 0.25 kg ai ha^{-1} pretilachlor + 150 kg ha^{-1} N
T9 – 0.50 kg ai ha^{-1} pretilachlor + 150 kg ha^{-1} N

T0 - Control
T1 – 0.25 kg ai ha^{-1} pendimethalin + 50 kg ha^{-1} N
T2 – 0.50 kg ai ha^{-1} pendimethalin + 50 kg ha^{-1} N
T3 – 1.00 kg ai ha^{-1} pendimethalin + 50 kg ha^{-1} N
T4 – 0.25 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N
T5 – 0.50 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N
T6 – 1.00 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N
T7 – 0.25 kg ai ha^{-1} pendimethalin + 150 kg ha^{-1} N
T8 – 0.50 kg ai ha^{-1} pendimethalin + 150 kg ha^{-1} N
T9 – 1.00 kg ai ha^{-1} pendimethalin + 150 kg ha^{-1} N

T0 - Control
T1 – 0.90 kg ai ha^{-1} propanil + thiobencarb + 50 kg ha^{-1} N
T2 – 1.80 kg ai ha^{-1} propanil + thiobencarb + 50 kg ha^{-1} N
T3 – 3.60 kg ai ha^{-1} propanil + thiobencarb + 50 kg ha^{-1} N
T4 – 0.90 kg ai ha^{-1} propanil + thiobencarb + 100 kg ha^{-1} N
T5 – 1.80 kg ai ha^{-1} propanil + thiobencarb + 100 kg ha^{-1} N
T6 – 3.60 kg ai ha^{-1} propanil + thiobencarb + 100 kg ha^{-1} N
T7 – 0.90 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N
T8 – 1.80 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N
T9 – 3.60 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N

Figure 1. Effects of commercial herbicides and N fertilizer application on weed emergence (A, C, E) and shoot fresh weight (B, D, F) of *Eleusine indica*. Vertical bar represents standard deviation (SD) of the mean.

Table 1. Effects of pretilachlor + N fertilizer rates and growth stage at application on rice root length, shoot height and shoot fresh weight 30 day after application.

Main effect	Root length	Shoot height	Shoot fresh weight
	(% of Control)		
Treatment			
T1 - 0.125 kg ai ha^{-1} pretilachlor + 50 kg ha^{-1} N	95^{cd}	84^{b}	92^{cd}
T2 - 0.25 kg ai ha^{-1} pretilachlor + 50 kg ha^{-1} N	100^{de}	80^{ab}	66^{a}
T3 - 0.50 kg ai ha^{-1} pretilachlor + 50 kg ha^{-1} N	85^{b}	74^{ab}	76^{ab}
T4 - 0.125 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N	94^{cd}	80^{ab}	73^{ab}
T5 - 0.25 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N	74^{a}	86^{b}	94^{cd}
T6 - 0.50 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N	94^{cd}	77^{ab}	78^{ab}
T7 - 0.125 kg ai ha^{-1} pretilachlor + 150 kg ha^{-1} N	88^{cd}	81^{ab}	74^{ab}
T8 - 0.25 kg ai ha^{-1} pretilachlor + 150 kg ha^{-1} N	101^{e}	71^{a}	77^{ab}
T9 - 0.50 kg ai ha^{-1} pretilachlor + 150 kg ha^{-1} N	75^{ab}	85^{b}	83^{b}
Application timing (DAS)*			
0	83^{a}	74^{a}	73^{a}
5	97^{b}	83^{b}	85^{b}
10	97^{b}	93^{c}	88^{b}
Non-treated control (T0)	100	100	100

* The rice seeds were treated with pretilachlor + N fertilizer at 0, 5 or 10 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.

treatments were highly toxic to the bioassay species, where the seedling injury was severe. Conversely, pendimethalin and N fertilizer at a moderate application rate gave >50% inhibition on the emergence of *E. indica* (Figure 1C). Strong reduction in shoot fresh weight of weeds also was noticed at this application rate with >85% inhibition (Figure 1D).

Early post-emergence herbicide, propanil + thiobencarb was found to have the greatest inhibitory effects on seedling emergence of *E. indica* at the highest application rates of 3.6 kg ai ha^{-1} propanil + thiobencarb and 150 kg ha^{-1} N, with 60% inhibition (Figure 1E). It was noticed that this treatment also was highly toxic to the bioassay species, where it reduced the weed shoot fresh weight by > 90% with symptoms of leaf wilting and discoloration (Figure 1F). Similar symptoms were also found at moderate to low concentration where the seedling injury was severe. By contrast, untreated seedlings for all herbicide types remained healthy 30 days after treatment.

Weed control is a major problem in direct-seeded rice (DSR) because the weed seeds germinate simultaneously with the rice seeds (Kumar, 2017). According to Singh et al. (2014), the density of grasses, sedges and broad leaf weeds in direct seeded rice increased with increased nitrogen rates from 120 to 150 kg ha^{-1}, but a further increase in nitrogen rate (180 kg N ha^{-1}) leads to a decrease in weed density. Later on, Awan et al. (2014) found that added N favoured rice biomass production more than it did the weed where weed biomass increased by 82 to 160%, whereas rice biomass increased by 92 to 229%, with the application of 50 to 150 kg N ha^{-1}. Meanwhile, Morshed et al. (2015) reported that *Scirpus maritimus* L. in Boro rice showed the maximum visual abundance (58%) after treated with N up to 202.4 kg ha^{-1} under selected pre-emergence and post-emergence herbicides; suggesting that availability of nitrogen in soil favoured the luxuriant growth of weeds. In this study, it was noticed that the emergence and shoot fresh weight of *E. indica* showed a different response along with herbicides and N rates (Figure 1). Therefore, manipulating the N environment could be effective in enhance herbicide efficacy.

Effect of herbicides and N fertilizer on rice

The degree of susceptibility of rice seedlings toward herbicides and N fertilizer was evaluated in this study. Since herbicides and N fertilizer rate-by-growth-stage interaction was observed, data were pooled over and main effects are presented (Tables 1 to 3). Moderate application rates of pretilachlor and N fertilizer (0.25 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N) had an adverse impact on the rice root length (Table 1). Averaged across pretilachlor and N fertilizer rates, rice seedlings growth at 0 DAS were 70 to 80% of the non-treated control. It was observed that starting at 5 DAS, the growth of rice seedlings were less affected by the pretilachlor and N fertilizer treatment, where the rice seedlings growth exhibited at least 80% of non-treated control. In contrast, the injury of rice seedlings was likely negligible when

Table 2. Effects of pendimethalin + N fertilizer rates and growth stage at application on rice root length, shoot height and shoot fresh weight 30 day after application.

Main effect	Root length	Shoot height	Shoot fresh weight
		(% of Control)	
Treatment			
T1 - 0.25 kg ai ha^{-1} pendimethalin + 50 kg ha^{-1} N	76^{ab}	90^{d}	72^{ab}
T2 - 0.50 kg ai ha^{-1} pendimethalin + 50 kg ha^{-1} N	78^{ab}	84^{c}	78^{abc}
T3 - 1.00 kg ai ha^{-1} pendimethalin + 50 kg ha^{-1} N	71^{a}	78^{a}	69^{a}
T4 - 0.25 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N	90^{bcd}	86^{c}	83^{cde}
T5 - 0.50 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N	95^{d}	97^{d}	94^{e}
T6 - 1.00 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N	80^{ab}	80^{ab}	75^{abc}
T7 - 0.25 kg ai ha^{-1} pendimethalin + 150 kg ha^{-1} N	95^{d}	90^{d}	89^{de}
T8 - 0.50 kg ai ha^{-1} pendimethalin + 150 kg ha^{-1} N	94^{cd}	82^{bc}	85^{cde}
T9 - 1.00 kg ai ha^{-1} pendimethalin + 150 kg ha^{-1} N	88^{bcd}	78^{a}	80^{bcd}
Application timing (DAS)*			
0	81^{a}	79^{a}	79^{a}
5	84^{a}	89^{b}	79^{a}
10	90^{b}	93^{b}	89^{b}
Non-treated control (T0)	100	100	100

* The rice seeds were treated with pendimethalin + N fertilizer at 0, 5 or 10 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 propanil+thiobencarb + N fertilizer rates and growth stage at application on rice root length, shoot height and shoot fresh weight 30 day after application.

Main effect	Root length	Shoot height	Shoot fresh weight
		(% of Control)	
Treatment			
T1 - 0.90 kg ai ha^{-1} propanil + thiobencarb + 50 kg ha^{-1} N	87^{a}	72^{a}	68^{b}
T2 - 1.80 kg ai ha^{-1} propanil + thiobencarb + 50 kg ha^{-1} N	93^{ab}	67^{ab}	47^{a}
T3 - 3.60 kg ai ha^{-1} propanil + thiobencarb + 50 kg ha^{-1} N	94^{ab}	83^{ab}	30^{a}
T4 - 0.90 kg ai ha^{-1} propanil + thiobencarb + 100 kg ha^{-1} N	97^{ab}	111^{cd}	95^{cd}
T5 - 1.80 kg ai ha^{-1} propanil + thiobencarb + 100 kg ha^{-1} N	96^{ab}	109^{cd}	80^{bc}
T6 - 3.60 kg ai ha^{-1} propanil + thiobencarb + 100 kg ha^{-1} N	107^{ab}	109^{cd}	77^{b}
T7 - 0.90 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N	111^{b}	133^{def}	100^{d}
T8 - 1.80 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N	112^{b}	138^{ef}	111^{d}
T9 - 3.60 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N	182^{c}	147^{f}	146^{e}
Application timing (DAS)*			
0	89^{a}	97^{a}	85^{a}
10	110^{b}	106^{a}	98^{b}
15	127^{b}	136^{b}	104^{b}
Non-treated control (T0)	100	100	100

*The rice seeds were treated with propanil+thiobecarb + N fertilizer at 0, 5 or 10 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.

treated with moderate application rate of pendimethalin and N fertilizer (0.50 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N), and it also found that the growth of rice seedlings started to increase at 10 DAS (Table 2). It was interesting to note that the rice seedlings growth was greatly stimulated at the highest application rate of propanil + thiobencarb and N fertilizer (3.60 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N). Averaged across propanil + thiobencarb and N fertilizer rate, a small reduction on rice root length, shoot height, and shoot fresh weight was noticed at 0 DAS (85 to 95% of the non-treated control). It was observed that starting at 10 DAS, the growth of rice seedlings were stimulated by propanil + thiobencarb with N treatment, where both root length and shoot height exhibited 106 to 110% of non-treated control (Table 3).

According to Knezevic et al. (2013) the timing of application was important in determining the effectiveness of a particular herbicide. This finding was in line with our study where weed control with early application of pretilachlor and pendimethalin at 0 and 5 DAS was toxic to rice crop. However, late application of pre and early post-emergence herbicides at 10 and 15 DAS respectively, was good for crop growth. Under DSR, highest application rates of propanil + thiobencarb and N fertilizer could reduce the emergence and shoot fresh weight of *E. indica* by 60 to 90%, respectively (Figure 1E and F). Interestingly, the rice root and seedling growth were highly stimulated at this application rate (Table 3). Thus, the study results suggest that this combination rate was efficient in control *E. indica* without injuring the rice seedlings. The period between 10 and 15 DAS was found suitable for effective weed control for this application rate with respect to toxicity to rice plants.

Conclusions

Early post-emergence herbicide mixture of propanil + thiobencarb (3.60 kg ai ha^{-1}) possesses promising herbicidal activity for control of *E. indica* along with N application (150 kg ha^{-1} N), thus highlighting its potential for controlling many weed species in rice fields. However, some additional experiments in the field should be undertaken to study the synergistic or antagonistic effect of herbicide and N fertilizer on rice-weed competition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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