Research Note

Synergistic effect of urea and pendimethalin on *Eleusine indica* suppression and rice growth performance in an aerobic rice system

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***Eleusine indica* is one of the most problematic weeds in aerobic rice systems in many countries. A pot study was conducted to evaluate the phytotoxic effect of urea fertiliser and pendimethalin on *E. indica*, while observing aerobic rice tolerance level and agronomic performance with the application rate. Four treatments were evaluated which were control (urea only without pendimethalin), and three rates of pendimethalin (0.25, 0.50, and 1 kg ai ha^{-1}) in combination with optimum urea fertiliser (240 kg ha^{-1}). Results demonstrated that banded application of urea and pendimethalin gave positive response in suppressing *E. indica* over control. A 0.50 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea and 1 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea gave significant suppression on the emergence and seedlings growth of *E. indica* with 60 - 90% inhibition relative to the control. The aerobic rice growth performance was highly stimulated and the grain yield production was higher for treatments of both 0.50 kg ai ha^{-1} and 1 kg ai ha^{-1} pendimethalin. These results imply that a lower application rate of 0.50 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea can be adopted to control *E. indica* effectively while enhancing the growth performance of aerobic rice plants since it is cost saving and reliable.**

Keywords: *Eleusine indica*, aerobic rice, herbicide, pendimethalin, toxicity, urea

The aerobic rice system has been widely adopted by around 50% of rice growers all over the world because it consumes less water, use minimal labour, and reduces wages rate due to adoption of the dry direct seeded rice method (Pandey and Velasco 2005). The dry direct seeded method is widely adopted because it requires 35-57% less water compared to the transplanting method (Farooq et al. 2011). Direct seeding might be a strategic solution to save water, but sowing rice seeds into dry soil exposes the crop to many problems. One of the most prevalent problems is heavy weed infestation that impedes the success of the aerobic rice system (Juraimi et al. 2013). Mahajan et al. (2014) found that dry direct seeded rice is most heavily infested by grasses with sedges and broad-leaved weeds respectively being the next highest infestations. Among the most dominant and problematic weed present in aerobic rice systems in Malaysia is *Eleusine indica* (Sunyob et al. 2012; Kumar et al. 2015; Mahajan et al. 2010). *E. indica* does not only infested rice fields, but it also has been found in many other places such as orchards and plantations. Mohamed et al. (2008) reported that *E. indica* was predominantly found in farms, orchards, and young palm oil nurseries and that *E. indica* causes loss of food quality and quantity in farms and plantations.

However, *E. indica* control has been hindered due to the development of resistance towards many herbicides. *E. indica* has developed resistance to glufosinate-ammonium (Jalaludin et al. 2010), paraquat (Buker et al. 2002), pendimethalin, prodiamine, and trifluralin (Nyporko et al. 2002) and metribuzin (Brosnan et al. 2008). Farmers tend to increase the dosage of herbicides applied if the first attempt to kill the weeds failed. Repeated usages of similar chemicals cause the weeds to develop resistance (Juraimi et al. 2013). Herbicides induce toxicity for both soil and rice plants (Chauhan and Johnson 2011). Over dosage of

0041–3216/2023/010059–067

herbicides application causes rice plant stunting, slower growth and necrosis on main culm and leaves of rice (Ahmed and Chauhan 2015). Herbicide usage can be reduced and replaced with hand or manual weeding, but it is laborious and impractical for large scale production. Madhukumar et al. (2013) stated that traditional weed management is not a practical choice to be used in rice fields since it is time consuming, costly, and tedious. Instead, an integration method of herbicide and N fertiliser can be adopted because it is economical, provides early weed control and is less time consuming.

Nitrogen fertiliser plays an important role in the competitive balance of rice and weeds. Fertiliser timing and dosage manipulation create an environment that allows crops to absorb more nutrients than the weeds (Kristensen et al. 2008). Selvarajh et al. (2017) found that N fertiliser (urea at 240 kg ha^{-1}) showed a detrimental effect on the growth of *E. indica* weed, while it enhanced the growth performance of aerobic rice. Norhafizah et al. (2017) stated that banded application of herbicides and N fertilisers reduces weed growth, while imposing less injury to the aerobic rice system. Pendimethalin has been proven to control grassy weeds effectively in rice fields (Bhurer et al. 2013; Selvarajh et al. 2018), though in a previous study it was mentioned that *E. Indica* developed resistance to pendimethalin (Nyporko et al. 2002). The effects of herbicides and N rates have been studied separately, but there is less research on the combined effects of these factors on weeds and rice. Very limited information is available on how *E. Indica* responds to high N application doses co-applied with different herbicides concentrations. A better understanding of the competitiveness and response of *E. Indica* towards pendimethalin and urea is needed for the development of appropriate herbicide and fertilizer management strategies which can reduce weed infestation, help farmers to manage weed sustainably with minimal cost, and thus contribute to the increase in national rice yield production. Therefore, this study was carried out to determine the efficacy of urea and pendimethalin in suppressing *E. Indica*, while stimulating aerobic rice growth performance.

Materials and methods

Plant materials

Aerobic rice seeds were provided by Malaysian Agricultural Research and Development Institute (MARDI), University Putra Malaysia Serdang, Selangor, Malaysia, while *E. indica* seeds were collected from rice fields at Pasir Mas, Kelantan, Malaysia (6.07° N, 102.24° E). The *E. indica* seeds were scarified using sand paper to remove the seed coat. The naked seeds were soaked in 0.2% potassium nitrate solution for 24 hours to break the seed dormancy. The viability of *E. indica* seeds was tested to ensure the seeds had germination percentage higher than 90%. The seeds were then rinsed with distilled water before being used in the subsequent experiments.

Soil analysis

Soil analysis was conducted before urea and pendimethalin application in order to know the soil environment. Top soil collected from Agro Technopark, University Malaysia Kelantan, Jeli Campus, Malaysia (5.75 °N, 101.87 °E) was air-dried, finely ground, and sieved to pass a 2-mm screen. The soil textures were determined by using a textural triangle (Anderson and Ingram 1993). Soil pH was measured by using a digital pH meter (HI 3220, HANNA Instruments, Inc., 584 Park East Drive, Woonsocket, RI 02895) and Electrical Conductivity (EC) was measured by using an EC meter (Anderson and Ingram, 1993). Soil organic carbon was determined based on the Walkley-Black chromic acid wet oxidation method (McLeod 1973). The content of N, P, K, Ca, Mg, B, Fe, Zn, Mn, and Cu was determined using inductively coupled plasma (ICP) and elemental analysis.

The soil physico-chemical properties are presented in Table 1. The soil sample was categorised as sandy-clay-loam (24% clay, 6% silt, and 70% sand). Sandy soil is considered as well drained soil that has good aeration which is conducive for healthy root growth, thus leads to a healthy crop. The soil pH of this soil was acidic, and this might be due to the higher Fe in the soil. This was in agreement with Selvarajh et al. (2020a, b), who stated that higher Fe and Al cause increment in soil acidity. The amounts of N, P, K, Ca, and Mg were lower in the soil. It had been reported that acidic soil is the causal factors for lesser nutrients availability in soil, and impedes plant nutrient uptake (Selvarajh and Ch'ng 2022).

Table 1: Selected soil physico-chemical analysis

Properties	Results
Soil texture	Sandy clay loam
Clay (%)	24
Silt (%)	6
Sand (%)	70
Organic carbon (%)	0.55
pH	4.88
Available N (%)	0.05
Available P (ppm)	9
Available K (meq 100 g^{-1})	0.09
Available Ca (meq 100 g^{-1})	0.61
Available Mg (meq 100 g^{-1})	0.11
Available B (ppm)	1
Available Fe (ppm)	86.65
Available Zn (ppm)	1.1

Soil experiment

A total of 40 kg of sandy-clay-loam (24% clay, 6% silt and 70% sand; pH 4.88) was filled in a plastic pot (59 cm diameter × 51 cm height). Using the direct seedling method dry rice seed was buried in the soil evenly at a depth of 1 cm with a distance of 5 cm between the seeds, so that their density would correspond to an aerobic rice seeding rate of 150 kg ha^{-1} (Muda Agricultural Development Authority 2014). Prior to sowing, the soil was prepared with Plantmate Organic Fertiliser at the rate of 1500 kg ha^{-1} (Othman et al. 2014). The pots were arranged in an open space field-like condition where its temperature was between 25 ^{0}C to 34 ^{0}C. The experiment was conducted in triplicate, where each treatment was evaluated in three pots with a total of 12 pots for four treatments. The experiments were arranged in completely randomised design. The treatments evaluated are listed in Table 2.

The split NPK fertiliser in the form of urea, Christmas Island Rock Phosphate (CIRP), and Muriate of Potash (MOP), was applied on the soil surface 12 days after rice seed germination. CIRP and MOP (60 kg ha^{-1}) were applied once, and the urea was applied in four splits using a micropipette. The highest urea-N, 90 kg ha^{-1} was applied at the first application 12 days after seed germination. The other applications of urea were at tillering, panicle initiation and heading stage with the recommended rate of 50 kg ha^{-1} for aerobic rice (Othman et al. 2014). A total of 100 seeds of *E. indica* were sown on the soil surface in the pots after the first application of urea fertiliser. After 1 day of *E. indica* seed sowing, different rates of pendimethalin were applied on the soil surface with a micropipette. The number of emerged, shoot length, radicle length and shoot fresh weight of *E. indica* seedlings were counted and recorded at the time of aerobic rice plant harvesting at 97 days after sowing. The rice plants were harvested upon maturity, where the grain yield reached 14% moisture content. The root length, shoot height, shoot fresh weight, leaf greenness, tiller number, panicle length, panicle number, and grain yield of aerobic rice were recorded at the time of harvesting.

Table 2: List of treatments evaluated in field-like conditions

Treatments	Description
T0	240 kg ha^{-1} urea + 60 kg ha^{-1} CIRP + 60 kg ha^{-1} MOP
T1	0.25 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea + 60 kg ha^{-1} CIRP + 60 kg ha^{-1} MOP
T2	0.50 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea+ 60 kg ha^{-1} CIRP + 60 kg ha^{-1} MOP
T3	1.00 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea+ 60 kg ha^{-1} CIRP + 60 kg ha^{-1} MOP

CIRP: Christmas Island Rock Phosphate. MOP: Muriate of Potash

Statistical analysis

All data were subjected to one-way ANOVA analysis. The data were expressed as percentages of the control. Tukey's HSD test was used to separate the means among the treatments. Differences were regarded as significant when $P \leq 0.05$.

Results and discussion

Effect of pendimethalin and urea on *E. indica* growth

The phytotoxic activity of urea and pendimethalin on *E. indica* in soil is shown in Figure 1. Urea (240 kg ha^{-1}) and pendimethalin (0.25, 0.50, and 1.00 kg ai ha^{-1}) displayed promising results in suppressing the bioassay species and exhibited varied degrees of *E. indica* inhibition. The most significant treatments that gave > 60% inhibition were T2 (0.50 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea) and T3 (1.00 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea). However, there was no significant difference among these two treatments on the suppression of *E. indica*. The emergence of *E. indica* was reduced by 76% and 88%, respectively after the treatment with T2 and T3 relative to the control (Figure 1A). This result is similar to that of weed shoot fresh weight, where the strongest reduction was observed in T3 (93%) followed by T2 (84%) over the control (Figure 1B). This indicates that the increased dosage of pendimethalin gave higher mortality of *E. indica*. The growth of treated *E. indica* at T2 and T3 was retarded with symptoms of leaf yellowing, wilting and necrosis starting from 3 days after treatment which eventually led to plant death after 2 weeks. However, the moderate application of pendimethalin at rate of 0.50 kg ai ha^{-1} (T2) showed greater inhibition as well. At T1, the inhibition of weed emergence and shoot fresh weight were 56 and 65%, respectively. This showed that the co-application of urea and pendimethalin reduces the emergence of *E. indica* in the soil. In addition, the shoot and root growth of the *E. indica* were retarded due to the urea and pendimethalin toxicity. Inhibition of root and shoot were higher in T3 (> 70%) followed by T2 (> 60%) as compared to T1 and T0 (Figures 1C and 1D).

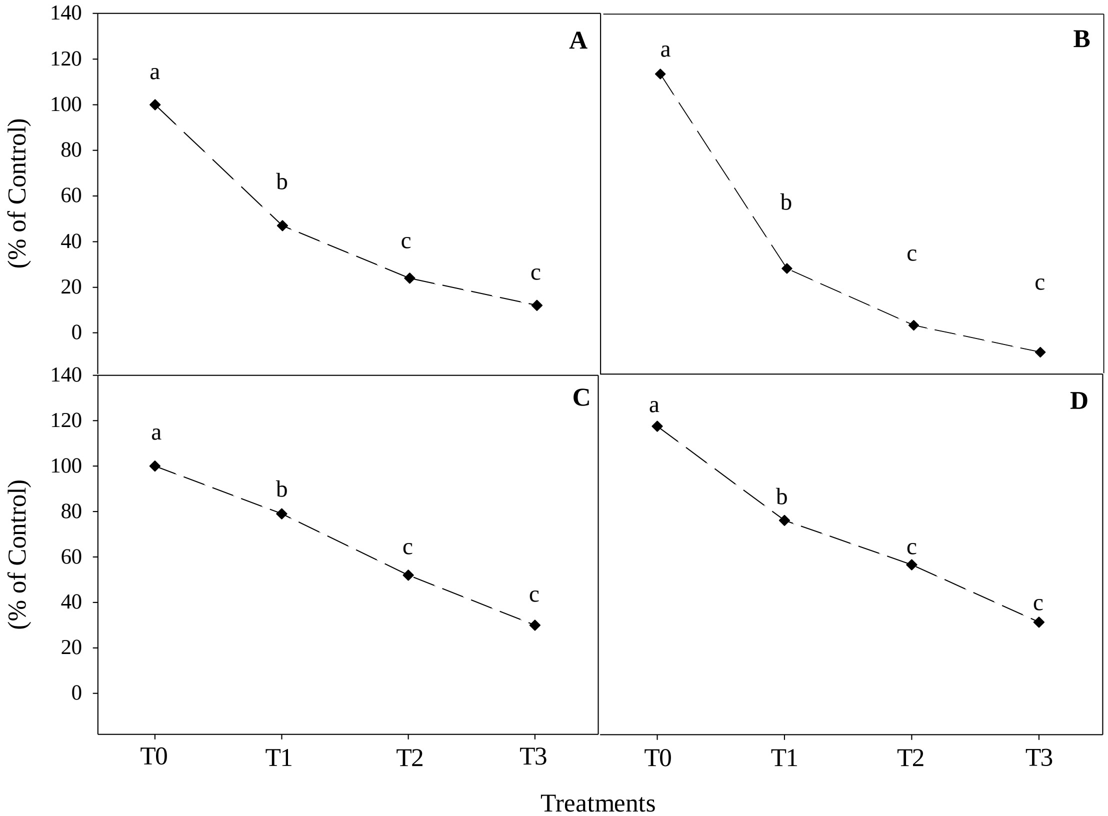

Figure 1: Effects of urea and pendimethalin application on weed emergence (A), shoot fresh weight (B), root length (C) and shoot height (D) of *Eleusine indica*. The treatments evaluated were: T0: non-treated control, T1: 0.25 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea, T2: 0.50 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea, and T3:1.00 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea. Values shown are percentages of the control values. The different letters indicate the significant difference between treatments by Tukey's HSD test at $p \leq 0.05$.

During the period of this study, it was observed that for all application rates of pendimethalin, many seedlings of *E. indica* growth were impeded in the soil compared to seedlings in the control. The *E. indica* seedlings grow healthily in both control and T1 after 3 weeks of treatment. However, at a rate of 0.50 kg ai ha^{-1} (T2) and 1.00 kg ai ha^{-1} (T3), the *E. indica* seedlings that emerged eventually showed damage and deformation due to the inhibition of root growth. This finding is in line with Chuah et al. (2014) who stated that weeds grow slowly in treated soil and finally deformed due to strong root inhibition. The root and shoot are equally affected due to translocation of applied treatments from roots towards shoot, resulting in a comparable degree of inhibition. Cruz-Hipolito et al. (2011) stated that the herbicide translocation from the root of the whole plant causes the weed to exhibit symptoms of chlorosis and necrosis leading to death. In addition, the decrease in shoot growth reduced the survival of *E. indica* seedlings, suggesting that T3 and T2 can be adopted for *E. indica* control since the herbicidal activity is more effective than T1.

Previously, it had been documented that *E. indica* had developed resistance to pendimethalin (Yemets et al. 2003), but in this study, banded application of pendimethalin and urea showed a positive effect. This implies that banded applications create a toxic environment for the weed which further prevents it from establishing in the soil. Urea applied at (240 kg ha^{-1}) was proven to be lethal to *E. indica* (Selvarajh et al. 2017). Hence, when the urea is applied together with pendimethalin, the phytotoxic effect of these treatments on *E. indica* is greater because urea has been said to inhibit photosynthesis and enzyme cholinesterase (Powles and Yu, 2010), while pendimethalin inhibit shoot, root and cell division of plants (Ni et al. 2016).

Effect of pendimethalin and urea on aerobic rice growth

The effects of urea and pendimethalin on an aerobic rice plant are presented in Table 3. Compared to the control growth of the rice was stimulated when pendimethalin was applied which clearly indicates that there was no phytotoxic effect of the applied treatment exerted on the aerobic rice plants. Khaliq and Matloob (2012) stated that the application of pre-emergence herbicide 1 days after sowing (DAS) affects the rice seed germination and shows detrimental effect on the root-shoot elongation under aerobic conditions. Khaliq and Matloob (2012) further elaborated that application, and timing of pre-emergence herbicide in aerobic rice needs to be adjusted to minimise injury to rice crops. Pendimethalin applied at 12 DAS did not show any adverse effect on the growth of the aerobic rice. Pendimethalin at 1 kg ai ha^{-1} exerted significant stimulation effect on the aerobic rice plant growth over control when applied banded with 240 kg ha^{-1} of urea.

Table 3: Effects of pendimethalin + urea fertiliser application on rice growth

Treatment	Root length	Shoot height	Shoot fresh weight	Leaf greenness	Tiller number	Panicle number	Panicle length	Grain yield
	(% of control)							
T0	100[a]	100[a]	100[a]	100[a]	100[a]	100[a]	100[a]	100[a]
T1	110[b]	107[a]	106[ab]	105[ab]	132[b]	125[b]	107[ab]	108[b]
T2	123[c]	124[b]	119[bc]	113[bc]	133[b]	147[c]	114[bc]	143[c]
T3	128[c]	128[b]	123[c]	123[c]	152[c]	151[c]	126[c]	148[c]

Note: Mean values within column with different letter(s) indicate significant difference between treatments by Tukey's HSD test at $p \leq 0.05$. The treatments evaluated were: T0: non-treated control, T1: 0.25 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea, T2: 0.50 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea, and T3:1 kg ai ha^{-1} pendimethalin + 240 kg ha^{-1} urea. Values shown are percentages of control values.

Overall, the aerobic rice plant in treatments T1, T2, and T3 showed a significant growth in performance over T0. In average across pendimethalin treatments, the root lengths were stimulated by 7 - 28% in T1, T2, and T3 over T0 (control). The longer root length indicates that the plant showed positive response towards the application of treatments with increased pendimethalin rates. Likewise, the rice plant shoots fresh weight and shoots height increased with increasing dosage of pendimethalin. Averaged across three pendimethalin treatments, the shoot height, shoot fresh weight and leaf greenness were stimulated by 7 - 28%, 6 - 23% and 5 - 23%, respectively over the control. It might be due to the efficacy of NPK fertiliser application that marked the counter effects of pendimethalin. Selvarajh et al. (2017), Selvarajh and Ch'ng (2021) stated that fertiliser application induces the growth of rice plant vigorously despite of herbicide application.

In this study, the tillers emerged 30 DAS, panicle initiation was at 45 DAS, heading stage was 60 DAS and the harvest time was 97 DAS. The tiller number increased as the concentration of pendimethalin increased due to less competition with *E. indica* weed. T3 treatment significantly increased tiller numbers production compared to other treatments (Table 3). Rice panicles number and panicle length were significantly higher in treatments T2 and T3 compared to T0. The improvement in rice plant physical growth, contributes to higher grain yield. Grain yield was 48% higher in T3, followed by T2 (43%) over T1 (Control). The grain yield produced by aerobic rice in T3 and T4 were significantly higher than T1 and T2. The increased grain yield in treatments T3 and T4 were in line with the reduction of *E.indica* weeds, which indicates that the rice-weed competition for nutrients is under control. Sunil et al. (2014) mentioned that the increase in yield was due to better control of weeds throughout the crop growth, resulting in better nutrients, moisture and light availability for the crop in which the productive tiller per hill and panicle number production are higher. This finding is supported by Jabran and Chauhan (2015), who concluded that implementation of weed control practices increases the grain yield by 15 - 307% in an aerobic rice system. These results suggest that pendimethalin is influenced by urea to suppress *E. indica* weed effectively without injuring the aerobic rice plants.

Conclusion

Application of urea at 240 kg ha^{-1} influences pendimethalin efficacy in suppressing *E. indica*, while enhancing the performance of aerobic rice. At 240 kg ha^{-1} urea, 0.50 and 1 kg ai ha^{-1} pendimethalin had shown promising result to suppress *E. indica*. A test needs to be undertaken in field conditions for two season of rice cycles to further evaluate the effectiveness of this banded application of pendimethalin and urea fertiliser. Information generated in this study is crucial in developing appropriate strategies to manage *E. indica* without injuring aerobic rice plants and has a potential to benefit farmers in managing the weeds sustainably.

Acknowledgement

The authors would like to thank the Malaysian Ministry of Education and University Malaysia Kelantan Jeli Campus for providing research facilities. The research was funded by Malaysia Fundamental Research Grant Scheme (Grant No: FRGS/1 /2022/WAB04/UMK/02/4) and (Grant code: R/FRGS/A0700/01091A/004/2022/ 01078).

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