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Effect of N fertilizer on the emergence and seedling growth of *Eleusine indica* in aerobic rice systems

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Abstract

Weed has become one of the most serious problems in aerobic rice systems. Weed management in aerobic rice system needs a manipulation of optimum N fertilizer application to control the weed and simultaneously enhance aerobic rice growth performance. In this study, three types of N sources (urea, ammonium sulphate and ammonium nitrate) with concentration starting from (0, 60, 120, 180 and 240 kg ha^{-1}) were evaluated for effective control of the bioassay species, *Eleusine indica*, while observing the rice injury level. The data on weed (emergence and shoot fresh weight), while rice growth (root length, shoot fresh weight, shoot height and leaf greenness) was collected in this study. It was found that urea at a higher application rate of 240 kg ha^{-1} strongly inhibit the emergence and shoot growth of *E. indica* by >78%. Conversely, ammonium sulphate and ammonium nitrate gave moderate inhibition (58-65% inhibition) on weed emergence and shoot growth at higher application rates of 240 kg ha^{-1}, respectively. Significant stimulation effect on rice root growth, shoot height, shoot fresh weight and leaf greenness was noticed at a low application rate of 60 kg ha^{-1} urea, while ammonium sulphate and ammonium nitrate only exerts its stimulation effect starting at 120 kg ha^{-1}. Stimulation effect on the rice growth was likely negligible at 0 and 4 DAS across urea, ammonium sulphate and ammonium nitrate application rates. However, the rice plants growth increases with increasing growth stage. The finding suggest that application of urea at 240 kg ha^{-1} starting from 8 DAS of rice growth stage was the most efficient application rate in inhibiting *E. indica* with the stimulation effect on the growth of rice seedlings.

Keywords: *Eleusine indica*; Nitrogen fertiliser; aerobic rice.
Abbreviations: N_nitrogen, DAS_days after sowing.

Introduction

Aerobic rice system is increasingly become popular among farmers. Traditionally, rice is grown in flooded soil or can be simplified as a biggest user of water, whilst direct seeding in aerobic rice system requires less water (Liu et al., 2015). Aerobic rice is high yielding rice grown in non-puddle, aerobic soils with less water (Bouman et al., 2002). However, this system faces higher weed infestation which suppresses the growth performance of rice crop. Weed incursion is most prominent in aerobic rice system compared to flooded conventional cultivation method and has been proven to be the major constraint faced by farmers in managing aerobic rice (Chauhan et al., 2015). However, it is been recognized that rice yield in aerobic conditions to be improved by 27–300% through implementation of suitable weed control methods (Hussain et al., 2008; Mishra and Singh, 2007). Various means can be used to overcome this problem including mechanical, chemical and biological control strategies. However, no one pays attention to the fertilizer application. Nitrogen fertilizer enables plants to absorb balanced nutrients, which promote photosynthesis and growth, and nitrogen also important for synthesis of plant protein, chlorophyll and nucleic acid (Dalga, 2016). Nitrogen fertilizer is crucial for the development of aerobic rice plant since it is one of major nutrient source for the plant. Nori et al. (2008) has used five nitrogen rates (0, 120, 160, 200 and 240 kg N ha^{-1}) and found that the rice growth performance is increasing with the increment in application rate. The nitrogen application (0, 120, 180 and 240 kg ha^{-1}) in rice cultivation field has shown that application starting from 180 kg ha^{-1} found to increase the quality and yield of rice plant, where the plant height and overall performance is better (Anil et al., 2014). However, over dosage of different N fertilizer sources and improper timing may lead to the injury of rice seedlings due to different composition or percentage of constituents. Fageria and Baligar (2005) had stated that appropriate sources, adequate rates, efficient methods and application timing are crucial for sustainable crop production. Further, the researcher specify that, sources of nitrogen and application timing influences N uptake efficiency in crop plants, where important consideration should be given on selection of N fertilizer sources to avoid wastage and toxic accumulation. Hence, aerobic rice fertilizer needs and response towards various N sources still not clearly found to maximize its absorption in certain growth stages and observe the degree of inhibition or stimulation in growth performance. N fertilizer plays an important role in competitive balance of weeds and rice plant. Fertilizer application at correct timings and doses are crucial to improve the competitive ability of crop plants against weeds (Awan et al., 2015). Addition of fertilizer may reduces crop yield, if it increases weed growth because some weeds capable to capture nutrients more faster than the crops (Kristensen et al., 2008) and in some cases crop can be more efficient and quick in taking up fertilizers than weeds (Dhima and Eleftherohorinos, 2001). Weeds are capable of absorbing nutrients faster and in relatively bigger amounts than crop plants (Blackshaw, 2005), and in the presence of a high weed population density, fertilizer application may stimulate weed growth greatly over the crop growth (Mahajan et al., 2009). In contrast, previous study found that the addition of N fertilizer reduces the weed emergence in aerobic rice system where the soil N level was altered (Norhafizah et al, 2017). In addition, Singh et al. (2014) has stated that increased nitrogen rate more than 180 kg ha^{-1} decreases the weed density of grasses, sedges and broadleaf. It is evident that, N fertilizer has the potential to suppress weed if proper dosage and sources used. So, manipulation by determining correct N source is necessary to overcome this problem. Hence, various N fertilizer sources efficacy is evaluated against weed while enhance the growth performance of aerobic rice.

Results and discussion

Effect of N fertiliser on weed growth

Fig 1 shows the effect of N fertiliser treatment on *E. indica* bioassay species. Since there was no significant difference in phytotoxic effect of N fertiliser to *E. indica* sown at different growth stage of rice seedlings, data were pooled for analysis. The results indicated that urea at 240 kg ha^{-1} had strongest inhibitory effect on weed emergence and shoot fresh weight of *E. indica* with >78%. By contrast, ammonium sulphate and ammonium nitrate gave moderate inhibitory effects on weed emergence and shoot growth with 58% and 65% inhibition, respectively at similar application rate. Moreover, *E. indica* was found to be very sensitive to urea fertiliser because of low ED_{50} value ranging from 116 to 120 kg ha^{-1}. The inhibitory effect of urea was markedly stronger than that of ammonium sulphate and ammonium nitrate fertiliser on the seed germination and shoot fresh weight of *E. indica* (Fig 1).

Weed control in aerobic rice is a major problem due to poor availability of proper control method (Jabran and Chauhan, 2015). N fertiliser optimisation is crucial to deter the weed growth by not depending solely on herbicides. Soil N level may alter the weed community structure which specifies the failure of herbicide alone to control the respective weeds (Cathcart et al., 2004). Norhafizah et al. (2017) stated that application of N fertiliser at an application rate of 50, 100 and 150 kg ha^{-1} influences the soil environment and improve herbicide efficacy to suppress the emergence and shoot fresh weight of *E. indica*. Irrespective of fertiliser, the present study found that the emergence and shoot fresh weight of *E. indica* decreases with increasing N rate. This finding is supported by earlier work of Abouziena et al. (2007), who investigated the effect of nitrogen rates and weed control treatments on maize yield and associated weeds in sandy soils. It is found that more inhibition was obtained at a higher N rate where the dry weights of Nalta jute and barnyardgrass decreased with increasing N application. However, Khan et al. (2012) stated that the weed density and dry weed biomass was greater at the highest urea application rate 160 kg ha^{-1}. Similarly, Mahajan and Timsina (2011) have documented that N fertiliser in form of urea increases the mean dry weight of grass, sedges and broadleaved weeds with application rate ranging from 120-180 kg ha-1. In this recent study, the relative phytotoxicity of urea on seed germination and growth of *E. indica* was markedly seen at the highest application rate of 240 kg ha^{-1}. According to Singh et al. (2015), addition of urea at 120 kg ha^{-1} attributed to the lower weed density and dry weight of grassy and non-grassy weed flora. This greater weed inhibition might be due to the influence of soil acidity created by the fertiliser. A study conducted by Fageria et al. (2011) reported that increasing rate of N fertiliser from 0-400 mg kg^{-1} of soil increase the soil acidity. Later on, Dalga (2016) has detected higher concentration of fertilizer in soil reduces broadleaved and grass weed seed germination due to osmotic stress or salt toxicity. In connection to this, the increasing of urea rate in the present study might influence the N environment which might increases the soil acidity that is lethal for *E. indica* weed. These results suggest the efficacy of urea in suppress *E. indica* which remain as one of main problematic weed in aerobic rice field.

Effect of N fertiliser on aerobic rice

Aerobic rice successful growth depends on its fertilization strategy, hence the growth performance of aerobic rice towards the N fertiliser application was evaluated on this study. Since no interaction was observed on N fertiliser rate-by-growth-stage, data were pooled over and main effects are presented (Table 1, 2, 3). Significant stimulation effects on rice root growth, shoot height, shoot fresh weight and leaf greenness was noticed at a low application rate of 60 kg ha^{-1} urea with value ranging from 116-127% of the non-treated control. Conversely, ammonium sulphate and ammonium nitrate only exerts its stimulation effect starting at 120 kg ha^{-1} with 105-126% of the non-treated control. Stimulation effect on the rice growth was likely negligible or less affected at 0 and 4 DAS across urea, ammonium sulphate and ammonium nitrate application rates where the rice seedlings growth exhibited at least 94% of non-treated control (Table 1, 2, 3). However, the rice plants growth increases with increasing of growth stages.

This finding is in line with Anil et al. (2014) who indicate that N fertilisation and proper timing is crucial for good quality of rice crop. In improving agricultural production, N plays a vital role in supplying nutrients to the rice plant despite of losses due to late absorption or excessive application (Liu et al., 2014). In connection to this, the timing of fertiliser application remains in concern. In this study, it is clear that N fertiliser application appeared to give a better growth performance of aerobic rice with increasing of rice growth stages, irrespective of N rates (Table 1, 2, 3). Significance on stimulation effect of rice seedlings towards urea and ammonium nitrate were noticed starting at 8 DAS and above, but ammonium sulphate starting to trigger the stimulation effect only at 12 DAS. These results are in agreement with previous findings documented by Qiao-gang et al. (2013) where nitrogen absorption in rice plant increased with increment in growth stage. Interestingly, urea had showed promising effect on suppress the emergence and growth of *E. indica* (Fig 1) while enhance the growth of rice plants compared to ammonium nitrate and ammonium sulphate. According to Xiang et al. (2013), the determinant factor for the growth of aerobic rice is N content. Urea is used widely for agricultural production where it possess high N content (46%), whilst ammonium nitrate and ammonium sulphate N content is 33.5% and 21%, respectively. Thus, our result suggests that urea is the best contributor of N source for rice plant, while suppressing weed.

Table 1. Effects of urea fertilizer rates and growth stage at application on rice root length, shoot height, shoot fresh weight and leaf greenness value 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 - 60 kg ha^{-1} Urea	116 [b]	111 [b]	127 [b]	120 [b]
T2 - 120 kg ha^{-1} Urea	123 [bc]	114 [bc]	137 [bc]	124 [b]
T3 - 180 kg ha^{-1} Urea	127 [bc]	119 [cd]	145 [cd]	129 [bc]
T4 - 240 kg ha^{-1} Urea	131 [c]	121 [d]	152 [d]	136 [c]
Application timing (DAS)*				
0	110 [a]	106 [a]	110 [a]	115 [a]
4	115 [ab]	109 [a]	114 [a]	117 [a]
8	124 [c]	116 [b]	149 [b]	123 [b]
12	127 [c]	119 [b]	152 [b]	128 [b]

* The rice seeds were treated with N fertilizer 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.

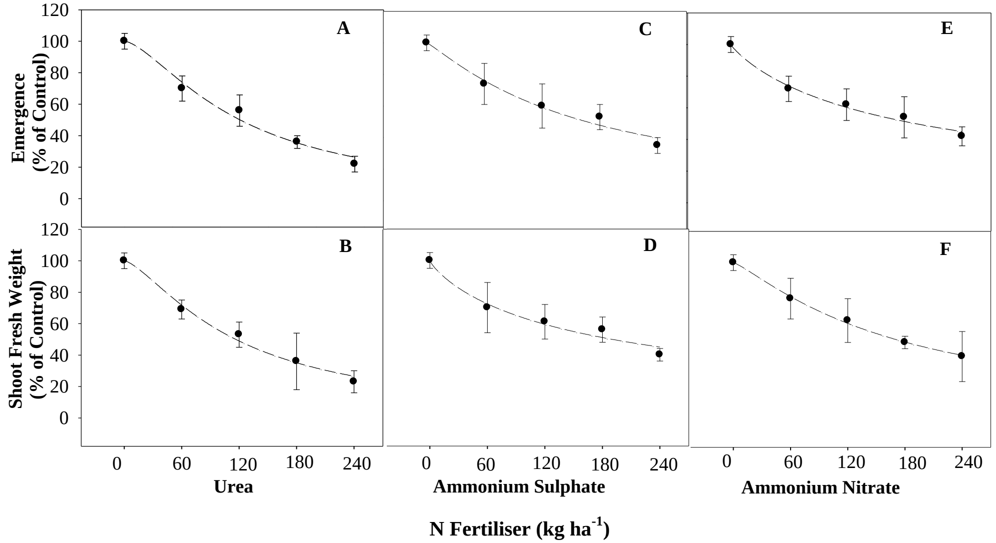

Fig 1. Effects of N fertilizer application on weed emergence (A, C, E) and shoot fresh weight (B, D, F) of *Eleusine indica*. Vertical bar represents standard deviation (SD) of the mean.

Table 2. Effects of ammonium nitrate fertilizer rates and growth stage at application on rice root length, shoot height, shoot fresh weight and leaf greenness value 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 - 60 kg ha^{-1} Ammonium Nitrate	99 [a]	104 [ab]	98 [a]	108 [b]
T2 - 120 kg ha^{-1} Ammonium Nitrate	105 [ab]	110 [bc]	112 [b]	114 [bc]
T3 - 180 kg ha^{-1} Ammonium Nitrate	116 [ab]	115 [cd]	121 [b]	119 [cd]
T4 - 240 kg ha^{-1} Ammonium Nitrate	121 [b]	119 [d]	125 [b]	124 [d]
Application timing (DAS)*				
0	94 [a]	99 [a]	97 [a]	105 [a]
4	98 [a]	104 [a]	101 [a]	107 [a]
8	118 [b]	115 [b]	119 [b]	116 [b]
12	119 [b]	117 [b]	127 [b]	119 [b]

* The rice seeds were treated with N fertilizer 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 ammonium sulphate fertilizer rates and growth stage at application on rice root length, shoot height, shoot fresh weight and leaf greenness value 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 - 60 kg ha^{-1} Ammonium Sulphate	100 [a]	103 [ab]	104 [ab]	117 [b]
T2 - 120 kg ha^{-1} Ammonium Sulphate	102 [a]	109 [bc]	111 [bc]	126 [c]
T3 - 180 kg ha^{-1} Ammonium Sulphate	110 [ab]	115 [cd]	117 [cd]	129 [cd]
T4 - 240 kg ha^{-1} Ammonium Sulphate	118 [b]	121 [d]	123 [d]	136 [d]
Application timing (DAS)*				
0	100 [a]	103 [a]	105 [a]	118 [a]
4	104 [ab]	105 [a]	109 [ab]	121 [a]
8	107 [ab]	113 [b]	113 [ab]	123 [a]
12	113 [b]	117 [b]	116 [b]	125 [b]

* The rice seeds were treated with N fertilizer 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.

Materials and Methods

Plant materials and chemicals

Aerobic rice seed (AERON 1) were obtained from Malaysian Agricultural Research and Development Institute (MARDI), UPM Serdang, while bioassay species, *Eleusine indica* seed were collected from rice fields at Pasir Mas, Kelantan, Malaysia (6.07704° N, 102.2384° E), and subjected to propagation in glasshouse. Commercial fertilizers were purchased from Agricultural Chemicals (M) Sdn. Bhd. (ACM), Penang, Malaysia.

Soil bioassay

Activity of N fertilizer was evaluated by using AERON 1 aerobic rice variety 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 1 cm for each pot through direct seeding method. Prior to sowing, the soil was prepared with organic fertilizer at 1500 kg ha^{-1} (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 vigorous 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 N fertilizers (urea, ammonium nitrate and ammonium sulphate) were prepared together with P fertilizer (Crystal Island Rock Phosphate) and K fertilizer (Muriate of Potash) in four application rates; (60:60:60, 120:60:60, 180:60:60 and 240:60:60 kg ha^{-1}). Fertilizer was applied when the rice seedlings were at the growth stages of 0, 4, 8, and 12 days after being buried in the soil. 30 seeds of *E. indica* were sown on the soil surface 1 day after fertilizer application. Fertilizer with different rates was applied onto the soil surface with a micropipette. Non-treated rice plant and *E. indica* seeds were used as control treatments.

Observations

After 30 days of herbicides treatment, emerged *E. indica* seedlings was counted and recorded. Shoot fresh weight of *E. indica* and rice seedlings was determined by harvesting and weighing aboveground living tissues remaining for each seedling. Meanwhile the root length, shoot height and leaf greenness of rice seedlings were measured and the data were expressed as percentages 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) (SigmaPlot 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:

$Y = d/ (1+[x/x_o]^{b})$ where Y = percentage of weed emergence/shoot fresh weight, d = the coefficients corresponding to the upper asymptotes, x = urea/ ammonium sulphate/ ammonium nitrate/, x0 = urea/ ammonium sulphate/ ammonium nitrate 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.

Conclusion

In summary, urea has shown promising detrimental effect on *E. indica* weed while simultaneously enhance the growth performance of aerobic rice plant. The efficacy of urea in controlling *E.indica* highlights its potential in controlling other weeds in rice fields. An additional study is needed to elucidate the mechanism of action of urea fertilizer in weed control.

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