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Allelopathic Effects of Invasive *Acacia mangium* on Germination and Growth of Local Paddy Varieties

N.A.N. Ismail and F. Metali
Environmental and Life Sciences Group, Faculty of Science,
Universiti Brunei Darussalam, Jalan Tungku Link, BE1410, Brunei Darussalam

Abstract: Laboratory and plant house experiments were conducted to study the allelopathic effects of *Acacia mangium* (Fabaceae), an invasive plant species in Brunei Darussalam, on germination and growth of two local paddy varieties. Germination, relative growth rates and/or biomass allocations of paddy Laila and Pusu were determined using a series of aqueous leaf extract concentrations and aqueous soil extract concentrations from *A. mangium* plantation and heath forest. Laila appears to be the most sensitive target species as its germination and relative growth rates based on elongations lengths (RERs) were affected by all the three types of extracts. Mean percentages germination of seeds treated with *A. mangium* leaf and *A. mangium* and heath forest soil extracts significantly decreased in Laila and Pusu as extract concentration increased to 10/12%. Mean RERs of seeds treated with 12% of *A. mangium* leaf extract were significantly slower in both Laila and Pusu as compared to the control. Both types of soil extracts significantly decreased RERs in Laila but not Pusu. At 5% of leaf extract concentration, Laila seedlings allocated a higher proportion of dry mass to roots but a lower proportion of dry mass to shoots (or a higher root-to-shoot ratio) than in other treatments but this differential allocation did not translate into greater final total dry biomass or faster growth rates. *Acacia mangium* negatively affected germination and growth of paddy. It is suggested that careful planning needs to be undertaken before using invasive species in any integrated land use systems.

Key words: Allelopathy, *Acacia mangium*, paddy, germination, relative growth rate, biomass allocation

INTRODUCTION

Genus *Acacia* (Fabaceae) is regarded as one of the most successful alien species that has invaded many areas worldwide (Henderson, 2007; Richardson and Rejmanek, 2011), including tropical countries in South East Asia. *Acacia auriculiformis* was first introduced to Brunei Darussalam in the 1950s to solve the problems of soil erosion occurring along the Tutong-Muara highway highway levee (Osunkoya *et al*., 2005). Then, *A. mangium* was also planted to accomplish the long-term objective of making Brunei self-sustainable in timber and furniture industries. In the late 1980s and early 1990s, *A. mangium* was used for revegetation and rehabilitation work with the aim to increase the productivity of secondary forest and highly degraded heath forest in Brunei (Mr Alex Cheng, Brunei Plantation Unit, pers. comm.).

The setback of planting *Acacia* in Brunei is that there have been evidences showing that the species have spread quite extensively and rapidly into the open, disturbed and degraded areas and forests over the years and they have outgrown many native plants in particular heath forest species, such as *Melastoma malabathricum*, *Ploairium alternifolium*, *Commersonia bartramia* and *Gymnostoma nobili* (Osunkoya *et al*., 2005). This invasion may result in the loss of biodiversity and disruption of structure and dynamics of the natural ecosystems (Osunkoya *et al*., 2005; Peh, 2010; Hussain *et al*., 2011a). They have also trespassed the fruit and coffee farms in Tutong district in Brunei, where they were initially planted at the edge of the farms in order to provide shade to the fruit trees (Osunkoya *et al*., 2005), also reported by K. Omar Ali, Agronomist in Department of Agriculture and Agrifood, pers. comm.

Acacia mangium is an evergreen tree, which may grow up to 15-20 m in height (Coode *et al*., 1996). *Acacia mangium* has larger phyllodes, which are the flattened leafy petioles than that of *A. auriculiformis* (Lakshmi and Gopakumar, 2009). *Acacia mangium* has creamy-white flowers and coiled pods, which contain fruits and seeds (Lakshmi and Gopakumar, 2009).

The term 'Allelopathy' was first introduced in 1937 by an Australian plant physiologist, Hans Molisch (Bhowmik and Inderjit, 2003). Allelopathy in plants is a

Corresponding Author: F. Metali, Environmental and Life Sciences Group, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, BE1410, Brunei Darussalam Tel: +6732463001

biological process by which plants release allelochemicals that affect germination, growth and reproduction of other plants (Olofsdotter, 1998). These allelochemicals may be released to the environment by different plant parts, such as leaves, phyllodes, flowers, bark and twigs, through volatilization and leaching as well as from the soil through the decomposition of plant residue and root exudation (Chou, 1983; Rice, 1984; Oyun, 2006; Das *et al*., 2012). The release of allelochemicals by plants is regulated by environmental conditions, such as soil moisture content, temperature, light intensity, nutrients and other microorganisms, such as fungi and viruses (El-Khawas and Shehata, 2005).

Plants possessing allelopathic potential are able to inhibit or stimulate the germination or growth of another species (Crafts and Robbins, 1962; Whittaker, 1970; Harborne, 1977; Rice, 1984) or may also impose no effect on the target species (Reigose *et al*., 1999). Many reports have shown that *Acacia* have a phytotoxic effect on the agricultural crops, which may decrease crop yields (Korner and Nicklisch, 2002; Leu *et al*., 2002). *Acacia auriculiformis* have shown to have hindered germination and growth of herbaceous plants, *Callistephus chinensis* (aster) and *Chrysanthemum coronarium* (chrysanthemum), if the *A. auriculiformis* leaf extracts were soaked long enough (Barman *et al*., 1997), thus suggesting that prolong exposure to the allelochemicals would inhibit the germination and growth of certain species.

Kamal *et al*. (1997) have reported that the growth of wheat was highly inhibited by the leaf leachates of *A. auriculiformis* and *A. nilotica*. The lower *A. nilotica* leaf extract concentrations (0.25 and 0.5%) have promoted the growth of shoots and roots in peas, while as the concentration increases, there was a decrease in the shoot and root length and fresh and dry weight of shoot and root (Al-Wakeel *et al*., 2007). Three native tree species in India, namely *Ficus infectoria* (white fig), *Emblica officinalis* (Indian gooseberry) and *Acacia leucophloea* and a crop plant, *Cicer arietinum* (Indian chickpea), were proposed to be used in agroforestry, however all the three species have showed a phytotoxic effect on the germination and growth of the Indian chickpea in the laboratory setting (Siddiqui *et al*., 2009).

The main aim of this study is to investigate the allelopathic effects of *Acacia mangium* on germination and growth of two local paddy varieties. The encroachment of *Acacia mangium* to many disturbed forests in Brunei Darussalam as well as its potential ability to inhibit the growth of certain native tree species and agricultural plant species have been quite distressing, so, the present study attempts to determine the effects of allelochemicals from *Acacia mangium* (test species) on crops in laboratory and plant house settings.

We are interested in answering the following questions:

- Does the aqueous leaf extract of *Acacia mangium* and aqueous soil extract of *Acacia mangium* plantation affect the percentage germination and relative elongation rates of selected local paddy varieties?
- Does the aqueous leaf extract of *Acacia mangium* increase the Relative Growth Rate (RGR) and affect the biomass allocations of selected local paddy varieties?

MATERIALS AND METHODS

Target species: *Oryza sativa* L. (Poaceae) or commonly known as paddy is a monocot grass, which is able to grow all-year round and may grow to a height of 2 m (Duke, 1983). Ensuring optimum growth of rice is imperative as rice is a staple food for most Asians (Olofsdotter, 1998). Paddy Laila, a new hybrid rice variety developed by the International Rice Research Institute in the Philippines, was introduced in 2009 by the Department of Agriculture, Brunei Darussalam (Ministry of Industries and Primary Resources Brunei) to meet the local demand for rice, so the people will be self-dependent on the country's own resources (K. Omar Ali, Agronomist in Department of Agriculture and Agrifood, pers. comm.). Paddy Pusu is a hill paddy variety native to Borneo and it has been traditionally planted by Bruneian rice farmers. The seeds of paddy Laila and Pusu used in this study were supplied by the Department of Agriculture, Ministry of Industry and Primary Resources, Negara Brunei Darussalam.

Sampling of leaves and soils: Leaves of *Acacia mangium* were collected in September-October 2013. The term leaf or leaves will be used to represent phyllode or phyllodes. The leaves were washed with distilled water and air-dried in an air-conditioned room for a week before they were grinded into fine powders. Soil cores were collected in December 2013-January 2014 from *Acacia mangium* plantation site (04.59305°N, 114.515.80°E) and primary heath forest (04.60006°N, 114.51611°E) at the Andulau Forest Reserve, respectively. The soils from each site were bulked and passed through sieve to remove any debris.

Preparation of aqueous leaf and soil extracts: Dried powdered leaves of *A. mangium* were used to prepare a series of aqueous extract concentrations (0.5, 1.0, 2.5, 5.0, 7.5, 10.0 and 12.0%) following the methods described by Hussain *et al*. (2011a). The leaves were soaked in distilled water and left for 24 h in the laboratory before the solution mixtures were vacuum-filtered. Fresh soil samples were used to prepare the aqueous soil extracts of the following concentrations; 1, 5 and 10% using the techniques described by Conway *et al*. (2002). Soils were soaked in distilled water and left for 36 h in the refrigerator before the solution mixtures were vacuum-filtered. The filtrates were refrigerated at 4°C until needed. Distilled water was used as the control for this experiment.

Seed germination and elongation experiments: An experimental design of 4 replicates of 25 seeds for each paddy variety (n = 2 paddy varieties) were used in this study. In each treatment, 5 mL of test solution was added to all the respective petri dishes at initial stage, followed by 3 mL of the same test solution for every two days. The petri dishes were placed in the plant house (25-33°C) and seed germination was scored every two days until the end of the experiment (20 days). Radicle emergence of at least 1 mm was used as the criterion for seed germination. When the seeds germinated, the initial radicle length and shoot length were measured as well as their final radicle and shoot lengths on day 14 after germination, which were used in the calculation of the relative growth rates. Relative growth rates (RGR, mm mm^{-1} day^{-1}) based on elongation lengths over a period of time were calculated by following equation (Hunt, 1982):

$$RGR = (\log_e EL_2 - \log_e EL_1)/(t_2 - t_1)$$

where, EL_2 and EL_1 are final and initial mean elongation lengths (mm), respectively and t_2-t_1 was 14 days.

Growth and biomass allocation of paddy Laila in response to *A. mangium* aqueous leaf extract: Only paddy Laila was used in this investigation because of insufficient supplies of seeds. Germination was carried out in the laboratory (20-21°C). The seedlings (n = 50) of 6-7 cm in height were chosen at random and transferred to plastic pots (base diameter = 11 cm, height = 13 cm), which were filled with black loamy soils (about 900 g) that have been sieved and autoclaved. All pots were kept in the plant house (25-33°C) and left to acclimatise for one week. Ten seedlings (n = 10) with mean height of 16-17 cm were selected at random for the initial harvest in February 2014 to provide estimates of initial size.

Only *A. mangium* leaf extracts with the concentrations of 1.0, 5.0 and 10.0% were used for the seedling growth experiment because these concentrations showed a negative effect on germination and relative elongation lengths of paddy Laila. Following the method of Orr *et al*. (2005), the seedlings (n = 10 seedlings per treatment, including distilled water for control) were hand-watered with 5 mL of leaf extracts or distilled water every morning for a period of three weeks while in the final 10 days, 10 mL of extracts or distilled water were added to the seedlings for every 2 days. A final harvest of all surviving seedlings was conducted after 30 days. At both harvests, the lengths of the shoot and root were determined. Each seedling was separated into shoot and root fractions and each fraction was oven-dried at 60°C for 7 days (until the dry mass was constant).

Biomass allocations of seedlings were determined using dry mass ratios (dry mass of plant part divided by total plant dry mass) for shoot (shoot mass ratio, SMR) and root (root mass ratio, RMR) as well as root-to-shoot mass ratio per seedling from the final harvest only (Hunt, 1982). The total dry mass of shoot and root was also calculated for each seedling from the final harvest only. Relative Growth Rate (RGR) in terms of height and biomass were calculated following Hunt (1982):

$$RGR = (\log_e W_2 - \log_e W_1)/(t_2 - t_1)$$

where, W_2 and W_1 are final and initial total height (cm) for RGR_{height} and final and initial total seedling dry masses (g) for $RGR_{biomass}$ and t_2-t_1 was 30 days. The calculations of RGR values were based on height and biomass of seedlings harvested in March 2014.

Statistical analysis: The mean final germination percentage±standard error of mean (SE) was calculated for each leaf extract or soil extract concentration per paddy variety. Final percentage germination was relatively expressed to initial seed numbers used in the experiment. Prior to analysis, germination percentages and biomass allocations were arcsine-square transformed, while relative growth rates were $\log_{10}$-transformed if residuals were not normally distributed after fitting models to the untransformed data. The germination percentages between treatments and within each paddy variety were analysed using a one-way analysis of variance (ANOVA) and Tukey's honest significant difference (Tukey's HSD) tests. However, for convenience in interpretation, untransformed data appear in all tables and figures. All statistical analyses were conducted using R version 3.0.3 (R Development Core Team, 2014).

RESULTS

Effects of *Acacia mangium* leaf extract: The mean final percentages germination were significantly different among *A. mangium* leaf extract concentrations for paddy Laila ($F_{7,\ 24} = 3.17$, $p<0.05$; Fig. 1a) and paddy Pusu ($F_{7,\ 24} = 4.50$, $p<0.01$, Fig. 1b). The mean percentages germination of paddy Laila and paddy Pusu were low at 7.5% (94±2%) and 12% (93±1%) *A. mangium* leaf extract concentrations, respectively (Fig. 1a and b). The mean relative growth rates based on elongation lengths were significantly different among *A. mangium* leaf extract concentrations for the two paddy varieties; paddy Laila ($F_{7,\ 72} = 27.11$, $p<0.001$; Fig. 2a) and paddy Pusu ($F_{7,72} = 6.03$, $p<0.001$, Fig. 2b). There seems to be a general decreasing trend in the mean RERs of paddy Laila and paddy Pusu as the different concentrations of *A. mangium* leaf extract increase (Fig. 2a and b). The mean RERs of paddy Laila and Pusu were significantly greater by 41 and 15% in the control than at 2.5 and 12% *A. mangium* leaf extract concentrations, respectively (Fig. 2a and b).

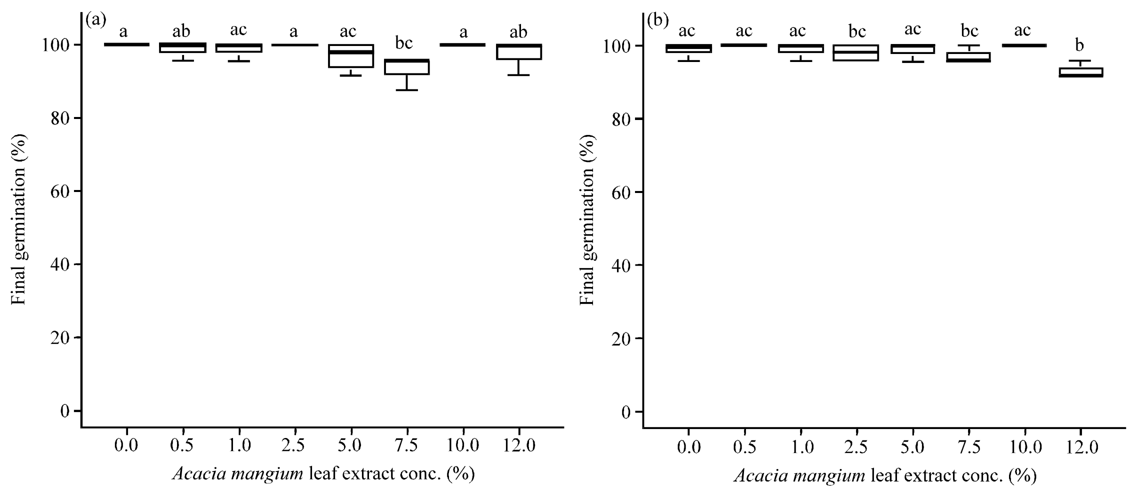

Fig. 1(a-b): Boxplots of final germination percentages of: (a) Paddy Laila, (b) Paddy Pusu in different aqueous concentrations (0% or control, 0.5, 1.0, 2.5, 5.0, 7.5, 10.0 and 12.0%) of *Acacia mangium* leaf extracts. Each treatment involved four replicates of 25 seeds and the experiment was conducted over 20 days. Different letters within a panel indicate significant differences between the species mean values at 5% significance level

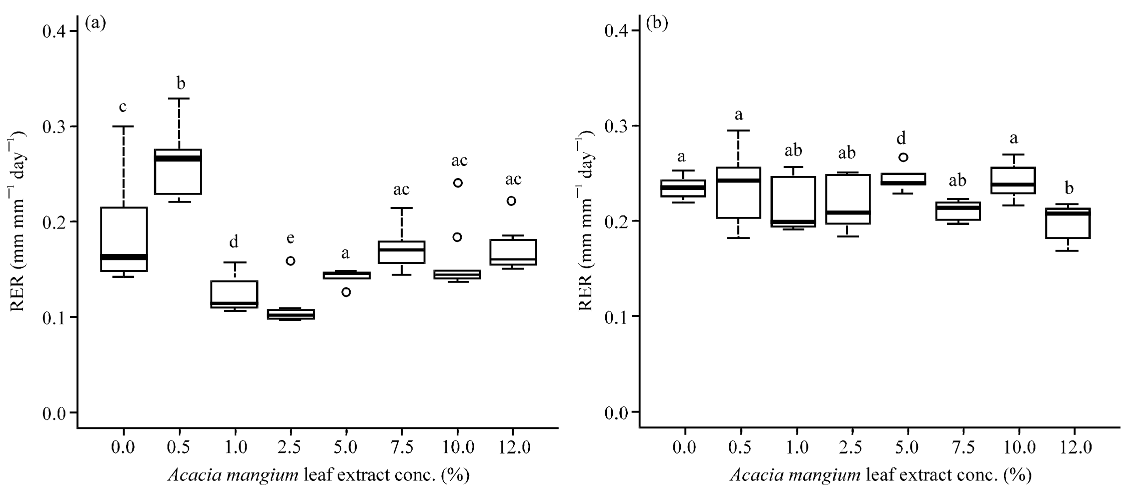

Fig. 2(a-b): Boxplots of relative growth rate based on elongation lengths (RER, mm mm^{-1} day^{-1}) of: (a) Paddy Laila and (b) Paddy Pusu in different aqueous concentrations (0% or control, 0.5, 1.0, 2.5, 5.0, 7.5, 10.0 and 12.0%) of *Acacia mangium* leaf extracts. Each treatment involved 10 seedlings (n = 10) and the experiment was conducted over 14 days. Different letters within a panel indicate significant differences between the species mean values at 5% significance level

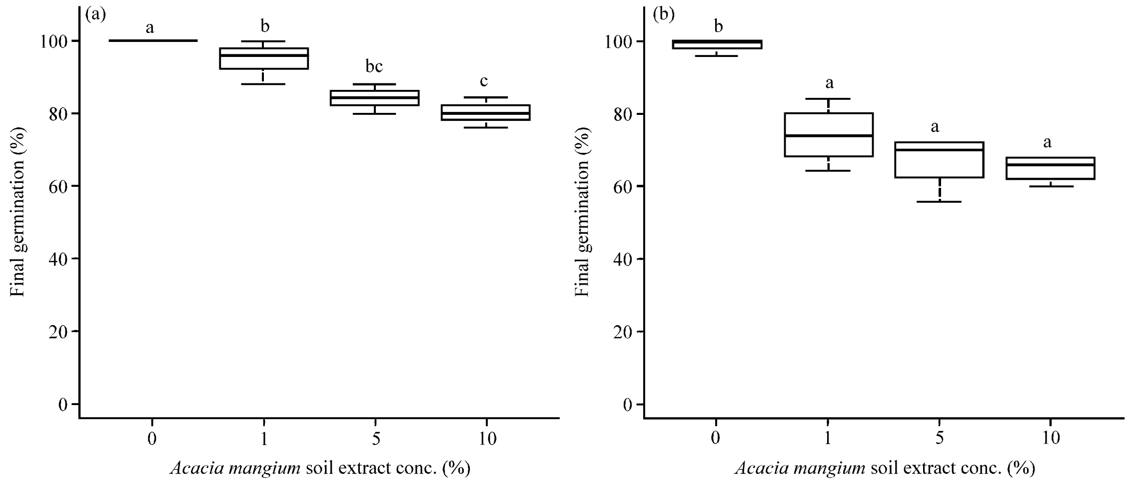

Fig. 3(a-b): Boxplots of final germination percentages of: (a) Paddy Laila and (b) Paddy Pusu in different concentrations (0% or control, 1.0, 5.0 and 10.0%) of aqueous extracts of soils from *Acacia mangium* plantation. Each treatment involved four replicates of 25 seeds and the experiment was conducted over 20 days. Different letters within a panel indicate significant differences between the species mean values at 5% significance level

Effects of *Acacia mangium* soil extract: The mean final percentages germination were significantly different among *A. mangium* soil extract concentrations for paddy Laila ($F_{3, 12}$ = 29.18, p<0.001; Fig. 3a) and paddy Pusu ($F_{3, 12}$ = 46.11, p<0.001, Fig. 3b). Generally, there was a decreasing trend of mean percentages germination of Paddy Laila (from 100% at 0 to 80±2% at 10%) and Paddy Pusu (from 99±1% at 0 to 65±2% at 10%) as *Acacia* soil extract concentration increases (Fig. 3a and b). The mean percentages germination for paddy Laila and Pusu seeds started to decrease at 1% (Fig. 3a and b). The mean percentages germination of paddy Laila and paddy Pusu at 1% (Laila: 95±3% and Pusu: 74±4%), 5% (Laila: 84±2% and Pusu: 67±4%) and 10% (Laila: 80±2% and Pusu: 65±2%) were significantly lower than the control (0%; Laila: 100% and Pusu: 99±1%) treatment (Fig. 3a and b).

The mean relative growth rates based on elongation lengths (RER) were significantly different among *A. mangium* soil extract concentrations for paddy Laila ($F_{3, 36}$ = 11.55, p<0.001; Fig. 4a) but not for paddy Pusu (p>0.05, Fig. 4b). The aqueous extract of *A. mangium* soil extract seem to have a stimulating effect on the RER of paddy Laila as the mean RER at 1% (0.29±0.01 mm mm^{-1} day^{-1}), 5% (0.27±0.01 mm mm^{-1} day^{-1}) and 10% (0.26±0.01 mm mm^{-1} day^{-1}) were higher than control (0.18±0.02 mm mm^{-1} day^{-1}) (Fig. 4a).

Effects of primary heath forest soil extract: The mean final percentages germination were significantly different among the primary heath forest soil extract concentrations for paddy Laila ($F_{3, 12}$ = 5.47, p<0.05; Fig. 5a) and paddy Pusu ($F_{3, 12}$ = 41.62, p<0.001; Fig. 5b). Generally, there was a decreasing trend of mean percentages germination of Paddy Laila (from 100% at 0 to 89±3% at 10%) and Paddy Pusu (from 99±1% at 0 to 71±3% at 10%) as primary heath soil extract concentration increases (Fig. 5a and b). The mean percentages germination for paddy Pusu and paddy Laila seeds started to decrease at 1% (Fig. 5a and b). The mean percentages germination of paddy Pusu at 1% (82±1%), 5% (73±3%) and 10% (71±3%) were significantly lower than the control (0%; 99±1%) treatment (Fig. 5b). As for paddy Laila, the mean percentages germination at 5% (91±4%) and 10% (89±3%) were significantly lower than the control (100%, Fig. 5a).

The mean relative growth rates based on elongation lengths (RER) were significantly different among primary heath forest soil extract concentrations for paddy Laila ($F_{3, 36}$ = 5.40, p<0.01; Fig. 6a) but not for paddy Pusu ($F_{3, 36}$ = 0.974, p>0.05; Fig. 6b). Generally, there was an increasing trend in the mean RER of paddy Laila (from 0.18±0.02 mm mm^{-1} day^{-1} at 0% to 0.25±0.01 mm mm^{-1} day^{-1} at 10%) as the concentration of the primary heath forest soil extract increases (Fig. 6a).

Growth and biomass allocation of paddy laila: The mean shoot mass ratios ($F_{3, 12}$ = 5.47, p<0.05; Fig. 7c), root mass ratios ($F_{3, 12}$ = 41.62, p<0.001; Fig. 7d) and root-to-shoot ratios ($F_{3, 24}$ = 7.11, p<0.01, Fig. 7e) of paddy Laila

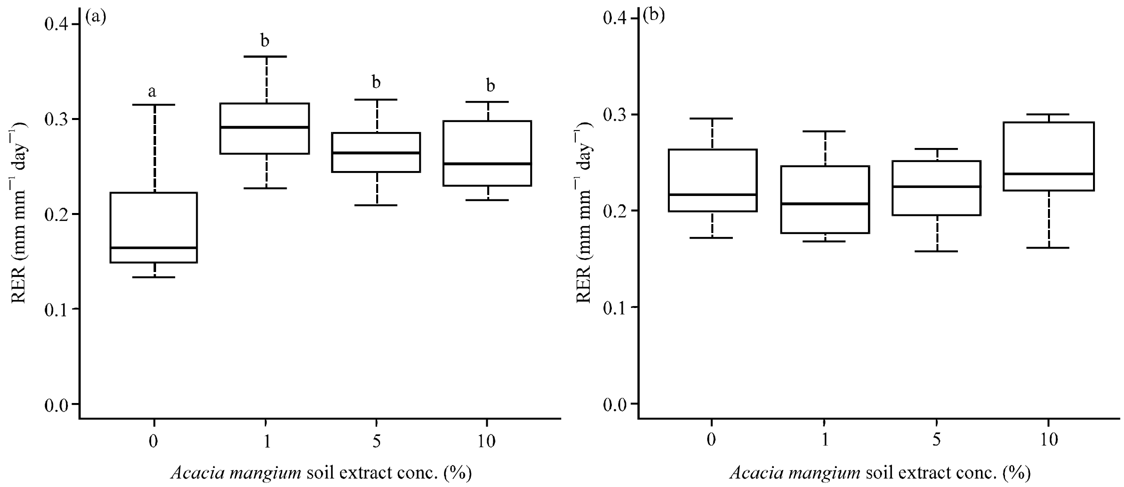

Fig. 4(a-b): Boxplots of relative growth rate based on elongation lengths (RER, mm mm^{-1} day^{-1}) of: (a) Paddy Laila and (b) Paddy Pusu in different aqueous concentrations (0% or control, 1.0, 5.0 and 10.0%) of *Acacia mangium* soil extracts. Each treatment involved 10 seedlings (n = 10) and the experiment was conducted over 14 days. Different letters within a panel indicate significant differences between the species mean values at 5% significance level

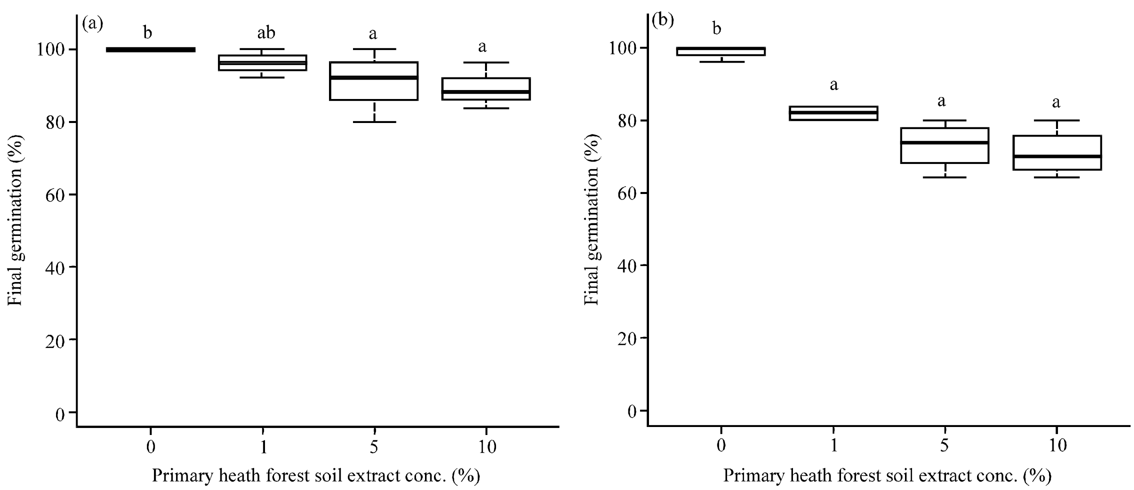

Fig. 5(a-b): Boxplots of final germination percentages of: (a) Paddy Laila and (b) Paddy Pusu in different concentrations (0% or control, 1.0, 5.0 and 10.0%) of aqueous extracts of soils from the primary heath forest. Each treatment involved four replicates of 25 seeds and the experiment was conducted over 20 days. Different letters within a panel indicate significant differences between the species mean values at 5% significance level

seedlings were significantly different across aqueous extracts concentrations of *A. mangium* leaves. Generally, there was an increasing trend of biomass allocations to roots (from 0.23 at 0% to 0.32 at 5%) but a decreasing trend of biomass allocation to shoots (from 0.77 at 0% to 0.68 at 5%) of paddy Laila seedlings as leaf extract concentrations increase to 5% (Fig. 7c and d). After 5%, there was a decline trend of biomass allocations to roots but not shoots in the Laila seedlings (Fig. 7c and d). At 5%, plants allocated a higher proportion of dry mass to roots and lower proportions of dry mass to shoots (or a higher root-to-shoot ratio) than in the 0, 1 and 10% treatments (Fig. 7c, d and e), but this did not translate into greater final total dry biomass or faster growth rates (Fig. 7a, b and f). Relative growth rates ($RGR_{biomass}$ and RGR_{height}) and total final dry biomass of paddy Laila were not significantly affected by the *A. mangium* leaf extracts ($p>0.05$; Fig. 7a, b and f).

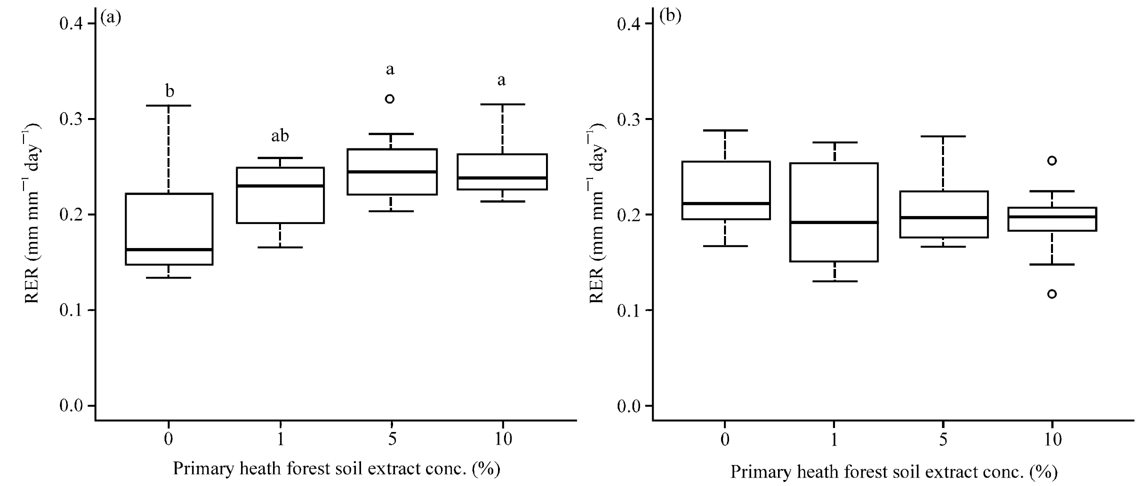

Fig. 6(a-b): Boxplots of relative growth rate based on elongation lengths (RER, mm mm^{-1} day^{-1}) of: (a) Paddy Laila and (b) Paddy Pusu in different aqueous concentrations (0% or control, 1.0, 5.0 and 10.0%) of aqueous extracts of soils from the primary heath forest. Each treatment involved 10 seedlings (n = 10) and the experiment was conducted over 14 days. Different letters within a panel indicate significant differences between the species mean values at 5% significance level

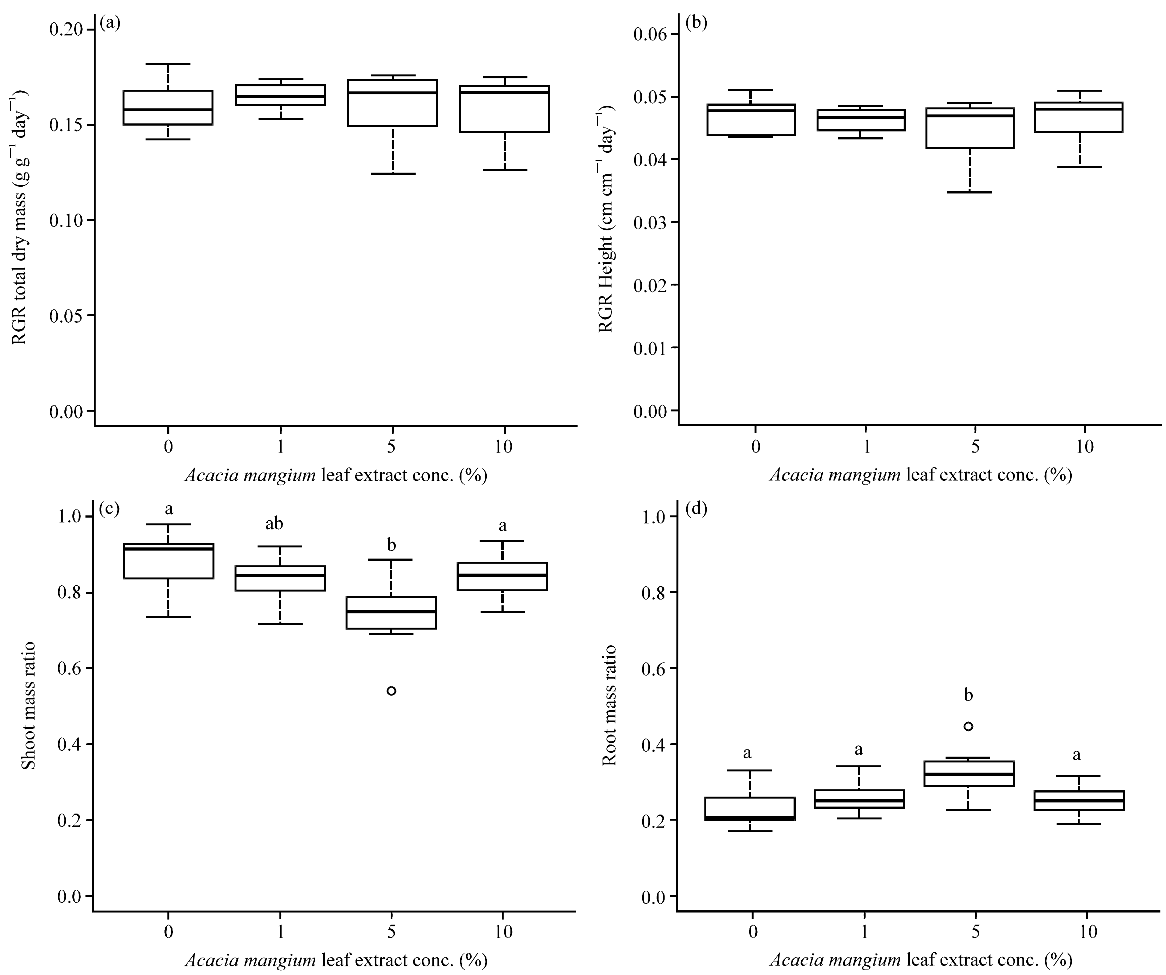

Fig. 7(a-f): Continue

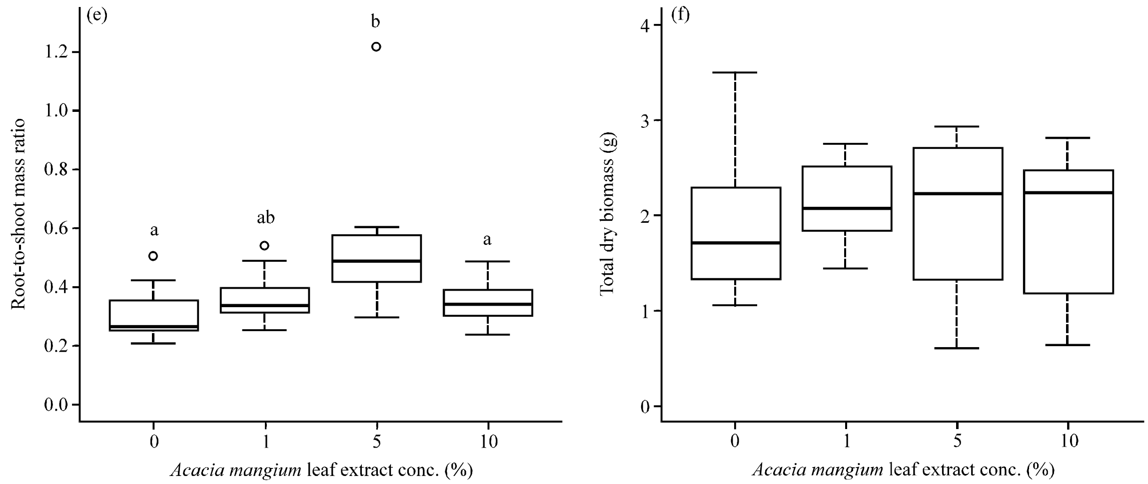

Fig. 7(a-f): Differences in (a) Relative growth rate based on biomass, $RGR_{biomass}$ (g g^{-1} day^{-1}), (b) Relative growth rate based on height, RGR_{height} (cm cm^{-1} day^{-1}), (c) Shoot mass ratio, SMR, (d) Root mass ratio, RMR, (e) Root to shoot mass (root:shoot) and (f) Total final dry biomass (g) of paddy Laila seedlings at different concentrations (0% or control, 1.0, 5.0 and 10.0%) of aqueous *Acacia mangium* leaf extracts. Each treatment involved one replicate of 10 seedlings and the experiment was conducted over 30 days. Different letters within a panel indicate significant differences between the species mean values at 5% significance level

DISCUSSION

Effects of *Acacia mangium* leaf and soil extracts on germination and growth of crops: *A. mangium* and other *Acacia* species have been implicated as allelopathic species because they release allelochemicals that affect the germination and development of other plant species, for example *A. melanoxylon* and *A. dealbata* inhibited the germination and suppressed root growth of weeds, such as *Dactylis glomerata* (orchard grass), *Lolium perenne* (ryegrass) and *Rumex acetosa* (sorrel) and crops, such as lettuce (Hussain *et al*., 2011a). However, none of these studies look into the allelopathic of *A. mangium* in paddy.

The mechanism of allelopathy depends on three factors, namely, the concentration of the test species, the part or organ of the test species at which the extract is obtained and finally, the target species that may respond to the allelochemicals (Hussain *et al*., 2011a). Chou *et al*. (1998) reported that *A. confusa* and some other *Acacia* species have shown phytotoxic effect at an aqueous extract concentration of as low as 0.5%. The phytotoxic present in *A. confusa* are water-soluble (Chou *et al*., 1998), thus suggesting that aqueous extract solutions of plants can be used in the study of allelopathy. The aqueous extracts used to study allelopathic effects of a species may derived from different plant parts, such as roots, stems, flowers and leaves as well as soils and the degree of allelopathic potential may differ for every plant parts (Hussain *et al*., 2011a). Leaf is the most common part of plants, which is used in many allelopathic studies (Conway *et al*., 2002; Kato-Noguchi *et al*., 2011; Baratelli *et al*., 2012), so this is why we use *Acacia* leaves (phyllodes) in our current study.

The present findings reported that *A. mangium* leaves have phytotoxicity effect on the two local paddy varieties in Brunei Darussalam (paddy Laila and paddy Pusu). For example in paddy Laila and paddy Pusu, the mean percentages germination were significantly lower at 7.5 and 12%, respectively, suggesting that the higher the concentration of the extract, the more prominent the degree of germination inhibition is. Similar studies also reported the negative effects of increasing leaf extract concentrations of *Acacia* species on germination and growth of crops (Bora *et al*., 1999; Oyun, 2006; Hussain *et al*., 2011a, b). We also observed a delay in the germination of the first seed, for example in paddy Laila, which showed a delayed by a day as compared to the control treatment. This observation was similar to Rafiqul-Hoque *et al*. (2003), who reported that *A. auriculiformis* leaf leachates delayed the germination process of target plant species. We observed that the *A. mangium* leaf extracts seemed to promote the growth of fungus, thus affecting the germination of the first seed and final germination percentages of target species. The test solutions can be autoclaved, however, autoclaving the aqueous extracts is not advisable as high temperatures may cause an alteration or loss of active allelochemicals (Orr *et al*., 2005).

In the present findings, the decreasing trend of percentages germination as the concentration of *A. mangium* leaf extract increases also reflect a decreasing trend in the mean RERs of some species. Jadhav and Gayanar (1992) and Kamal *et al*. (1997) also reported that *A. auriculiformis* inhibits the growth of rice and cowpeas. Blum *et al*. (1999) reported that root is the first organ affected in allelopathy by disrupting its uptake of water and ions. This is in agreement with the study by Hussain *et al*. (2011a), who reported similar findings, where the root lengths were significantly affected by the aqueous extract of *A. melanoxylon* flowers. Oyun (2006) also reported that the inhibition of seedling growth is related to the inhibition of nutrient uptake, which affect the growth the plants shoot and root. Tannins, wax, flavonoids and phenolic acids are the potential major allelochemicals that inhibit the seed germination of the crop plants, to some extent (Oyun, 2006). This coincides with the findings of El-Khawas and Shehata (2005), who reported that the same chemicals were responsible in the deleterious effect of *Acacia* species on the germination and growth of other plants.

In the present study, the germination percentages of Paddy Laila and Pusu were negatively affected by both types of aqueous soil extract concentrations. This is not surprising as allelochemicals may also be passed into the soils via the fallen leaf litters (Chou *et al*., 1998). The *Acacia* plantation in Brunei was established since 1998 and it is very likely that the soils may contain allelochemicals that are released by the invasive *A. mangium* trees. The amount of the phytotoxic chemicals present in the soil depends on the density of the fallen leaves, the rate of leaf litter decomposition, the distance of *Acacia* trees from other plants, rainfall, soil type and soil pH (Mann, 1987; Saxena *et al*., 1996; Escudero *et al*., 2000; Nilsson *et al*., 2000).

Our findings showed an increasing trend of RERs as the concentration of soil extract from *Acacia* plantation and primary forests increases. There were no *A. mangium* trees present in the primary heath forest at Andulau Forest Reserve but we can deduced that allelochemicals are also present in soils with *A. mangium* and heath forest species. However, it is not certain as to whether the allelochemicals in both aqueous soil extracts are the same or not until the active compounds in aqueous soil extracts are identified. Being an invasive species, the allelopathy mechanism in *Acacia* helps them to survive and avoid competition with other plants, especially the native species. As for primary forests, there seem to be certain degree of allelopathy happening in this forest type (Chou *et al*., 1998). The species in natural forest may possess allelopathic potential even though it was not as dominant as the *A. mangium* soil extract. This might be due to the plants interacted with the allelochemicals are able to stimulate or inhibit the release of allelochemicals and may also affect the effectiveness of allelopathy in the soil as well as their ability to choose for low or high concentrations of allelochemicals (Inderjit *et al*., 2011).

Based on this study, paddy Laila and paddy Pusu seem to be a good candidates for the study of allelopathic potential of *A. mangium* and other allelopathic species as the germination at control was consistent at 100%, hence the allelopathic effect of *A. mangium* on these species is more convincing.

Effects of *Acacia mangium* leaf extracts on growth and biomass allocation of crops: Biomass allocations (SMR, RMR and shoot-to-root ratio) showed significant results at 5% *A. mangium* leaf extract concentration. The RMR was significantly low at 10% compared to at 5%, suggesting that at 10%, the roots were the most affected tissues by *A. mangium* leaf extract. This coincides with other findings, which reported that root elongation was more sensitive in response to the extracts of allelopathic plants (Meissner *et al*., 1982; Rafiqul-Hoque *et al*., 2003). As a result, plant shoots were affected and they were unable to attain optimum shoot growth due to the disruption in root elongation, thus reducing water and nutrient uptake (Rafiqul-Hoque *et al*., 2003). None of the increases in biomass allocations were translated into faster RGRs or greater final total dry biomass, thus suggesting that the total dry mass and the height were less affected by the allelochemicals present in *A. mangium* leaf extract or the need to extend the duration of seedling growth experiments.

Future study should focus on extending the list of target species to other common crops, fruit trees and native plants in Brunei and lengthening the duration of the seedling experiment because seedlings may take a long time to response to environmental changes. Since all plant parts may be potential sources of allelopathy, it might be useful to investigate the different parts, such as stems, barks and flowers, of the *A. mangium* tree on seed germination, growth and biomass allocations of target species. It is also recommended to use extracts from mixed plant parts as allelopathic activity always work when there are more than one allelochemical (Einhellig, 1995). Finally, it is recommended that the allelopathic studies to be replicated *in-situ* as it would be interesting to observe the natural patterns of seed germination and seedling growth in the field.

Implications of study: *Acacia,* such as *A. auriculiformis*, has been recommended worldwide as a potential agroforestry species, which are planted with shrubs and crops (Petmak and Williams, 1991). However, it is crucial to investigate the allelopathic effects of *Acacia* species on target shrub/crop species that will be used in agroforestry sites (King, 1979). Allelopathic species, such as *A. mangium* and *A. nilotica*, have the potential to be used as natural herbicides because of their abilities in killing weeds (El-Khawas and Shehata, 2005). However, we need to treat this matter with caution because we do not want to introduce invasive plant species as agroforestry candidate and natural biological control.

CONCLUSION

From the present findings, *A. mangium* leaf extract seems to exhibit allelopathic potential on local rice varieties by decreasing germination percentages and relative growth rates in terms of elongation lengths of paddy Laila and paddy Pusu. *Acacia mangium* soil extract also decreased the germination percentages of paddy Laila and paddy Pusu and decreased RERs of paddy Laila, to a certain extent but not paddy pusu. Biomass allocations of paddy Laila seedlings were significantly affected by the *A. mangium* leaf extracts to a certain degree but not their relative growth rates and total final dry biomass.

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