Regular Article
ISSN 1226-8267
Journal of Forest Science
Vol. 28, No. 4, pp. 212-219, November, 2012
http://dx.doi.org/10.7747/JFS.2012.28.4.212

Effects of Organic Manure on Seedling Growth and Nodulation Capabilities of Five Popular Leguminous Agroforestry Tree Components of Bangladesh

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Abstract

An experiment was set to understand the seedlings growth and nodulation capabilities of five potentially important leguminous agroforestry tree species of Bangladesh in response to application of organic fertilizer. Study was carried out in nursery bed on the seedlings of *Acacia mangium* Willd., *Acacia hybrid, Acacia auriculiformis* A. Cunn. *ex* Benth, *Albizia lebbeck* (L.) Benth. and *Leucaena leucocephala* (Lam.) de Wit. after sowing their seeds in polybags filled with different medium. Farmyard manure or decomposed cow dung was applied as the source of organic fertilizer with other potting media. The growth and nodulation performance of seedlings of the selected species were measured three moths after the first seed was emerged. The different soil: organic manure ratios' used during the experiment were, 1:1, 2:1 and 3:1. The effects of organic fertilizer on growth and nodulation were compared to that of seedlings grown in control. The results revealed a positive correlation between seedling growth and the different doses of organic fertilization (i.e. seedling growth was enhanced significantly with different treatments containing higher proportion of organic fertilizer). In few cases a negative effect was observed after applying higher levels of organic manure. It was also apparent that the nodulation in terms of nodule number and size was inhibited significantly by the application of organic fertilizer.

Key Words: cow dung, leguminous species, growth performance, nodulation, inhibition.

Introduction

Low soil fertility is one of the greatest biophysical constraints to production of agroforestry crops across the world (Ajayi 2007). This phenomenon is sometimes attributed by the scarcity of chemical or inorganic fertilizer in most of the developing countries including Bangladesh. In such countries, like Bangladesh, the domestic production of inorganic fertilizer is still fewer than the country's actual fertilizer requirements which often causes worth of a lot of governments' money and effort to subsidized these for the convenience of small rural farm owners and tree growers. Organic fertilizers/manure like, cow dung on the other hand are readily available in rural areas of the country since

Received: February 29, 2012. Revised: March 1, 2012. Accepted: March 14, 2012.

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most of the rural farmers rearing cattle for fattening, production of milk, and for ploughing their agricultural fields. Moreover, the uses of such kind of fertilizers are now increasingly recognized and preferred by the farmers throughout the country since the uses of these products are believed to eco-friendly, without any toxic compounds and cheaply available (FAO 2002; Datta and Kar 2006).

A number of studies have although so far been conducted to assess the effect of inorganic or chemical fertilizers on growth and nodulation capabilities of seedlings of some selected agroforestry components in both nursery conditions and natural stands (Prasad and Ram 1986; Fakir et al. 1988; Hossain et al. 1996; Aryal et al. 2000; Bhuiyan et al. 2000; Hossain et al. 2001; Hossain 2003; Uddin et al. 2007; Uddin et al. 2008; Uddin et al. 2009 for example) but unfortunately little is known about the responses of seedlings in respect to application of organic fertilizer. A previous study of Paul and Hossain (1996) attempted to make a comparative evaluation of effect of both commercial fertilizers and organic manure on a popular agroforestry tree component. Our paper therefore tries to reports the results of a nursery experiment conducted to understand the seedling growth and nodulation capabilities of five potentially important agroforestry tree species; namely, *Acacia mangium* Willd., *Acacia hybrid*, *Acacia auriculiformis* A. Cunn. *ex* Benth, *Albizia lebbeck* (L.) Benth. and *Leucaena leucocephala* (Lam.) de Wit. in response to application of decomposed cow dung as organic manure. These species are usually fast growing, medium to large sized and evergreen (*Acacia* spp.) to deciduous (*A. lebbeck* and *L. leucocephala*) in nature (Das and Alam 2001). These species were chosen for the study since they have been used as a common agroforestry tree component in country's rural and sub-urban farms, homesteads and in road side plantations for years and usually preferred by farmers to produce quality timber, fuel, fodder as well as to provide several environmental services (Alam et al. 1997). We believe this study is an initial step for understanding the value of organic manures on seedling growth and their possible adoption in country's conventional agroforestry practice.

Materials and Methods

Study condition

The whole experiment was carried out in nursery polybags under exposure of a typical tropical climate. May and January being the hottest and coldest month in the area when the crucial temperature reaches up to 32.6°C and 14.1°C respectively (Ahmed 1990). Most of the rainfall in the area took place between early June to late September (Table 1). Geographically the area is formed by hills of low to medium high with slope ranges from gentle to steep and soils of color yellowish red to yellowish brown (Osman et al. 1992; Huda et al. 2006).

The experiment species

The potential agroforestry tree species selected for the study were: *A. auriculiformis*, *A. mangium*, *A. hybrid*, *L. leucocephala* and *A. lebbeck*. Seedlings were raised in the nursery using healthy and disease free seeds of corresponding species collected from a local seed bank.

Table 1. Background information of the study place

Parameters	Data	Source
GPS coordinates		Huda et al. 2006
Latitudes	22°27′30″ and 22°29′0″North	
Longitudes	91°46′30″ and 91°47′45″East	
Mean monthly temperature		Ahmed 1990
Maximum	29.75°C	
Minimum	21.14°C	
Average annual rainfall	2,500-3,000 mm	Ahmed 1990
Soil		
pH	5.5	Badruddin et al. 1989
Texture	Loam to sandy clay loam	Aryal et al. 1999

The potting media

Different potting media was used as treatments and control and for filling the polybags of size 6 inch x 4 inch. The soils were obtained from a depth of 4-10 cm of degraded hill forest land and collected 3-4 weeks before filling the polybags. The sand used was coarse textured and collected from stream bed. Cow dung was collected locally and kept under shed for four weeks before use at normal room temperature (i.e. 25°C to 29°C) to allow them to decompose and air-dry. All potting media (i.e. soils, sands and cow dung) were sieved (< 3 mm) before filling the polybags to remove stones, roots and other inert debris. The different soil-cow dung ratios' applied during the experiment were 1:1, 2:1 and 3:1 respectively.

Experimental design and treatment combinations

A randomized complete block design with ten replicates of each treatment was followed. Each species was subjected to five different treatments and there were altogether 250 polybags. The treatments were,

T_0-pure sand only
T_1-soil only
T_2-soil: cow dung (1:1)
T_3-soil: cow dung (2:1)
T_4-soil: cow dung (3:1)

Care, maintenance and precautions

The polybags were kept under nursery shade to protect strong sunlight and heavy rainfall. Proper care maintenance and precaution were confirmed during whole study period. Watering was done everyday morning and weeding was done in every third day.

Data collection and analysis

Plants were harvested three months after first seed was emerged. The variables measured were; shoot and root length, collar diameter, root diameter, leaf number, nodule number and nodule size.

The data sets were statistically analyzed by using SPSS (version 11). They were subjected to a one-way analysis of variance (ANOVA) test to understand the significant differences between treatment means which were then compared with the control means using Duncan's multiple range test (Bliss 1967). Relative ratios (e.g., shoot length, root length, collar diameter, nodule number, nodule size and leaf number) were calculated by using the following formula as per suggested by Rho and Kil (1986):

$$R=(T/Tc)\text{x}100 \quad (1)$$

where, R is the relative ratio, T mean data of tested plant and Tc the mean data of control.

Results

Shoot length (cm)

It was found that shoot lengths were varied significantly with different treatments as well as within the selected species. Table 2 represents the average shoot lengths (cm) of the treated seedlings. A trend of increases in shoot length with increasing proportion of cow dung in the soil (T_2-T_4) was observed, except in case of *L. leucocephala*, where it was not statistically significant. The highest shoot length (35.20 cm) was recorded for *A. lebbeck* at T_4 treatment whereas the lowest shoot length (14.25 cm) was observed in *A. hybrid* at T_2 treatment followed by 14.55 cm at T_0 treatment for the same species and 14.85 cm in *A. auriculiformis* at T_0 treatment. The highest relative elongation ratio (RER) of shoot (199.6%) was found in *A. hybrid* at T_4 treatment while the lowest (69.6%) was observed in *A. lebbeck* at T_4 treatment (Fig. 1).

Table 2. Shoot lengths (cm) of selected agroforestry tree seedlings grown in polybags treated with different media in nursery conditions

Treatments	Species				
	A. mangium	*A. hybrid*	*A. auriculiformis*	*A. lebbeck*	*L. leucocephala*
T_0	17.19b*	14.55b	14.85b	20.76c	27.25ab
T_1	17.80b	14.25b	16.35b	25.90b	23.48b
T_2	30.80a	26.05a	20.05a	33.85a	23.75b
T_3	26.70a	29.05a	22.50a	33.45a	27.85ab
T_4	30.32a	28.10a	21.55a	35.20a	34.95a

*Values in the columns followed by the same letter(s) are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT).

Root length (cm)

The mean root lengths of the selected tree seedlings are shown in Table 3. Similar to shoot length there observed an increasing trend of root lengths (cm) with increasing proportion of cow dung in the soil, except in case of *A. auriculiformis* and *L. leucocephala* where the effect was found inhibitory. In case of *A. auriculiformis*, the increment of root lengths in different treatments was not statistically significant. The highest root length (37.20 cm) was observed in *L. leucocephala* at T_1 treatment followed by 33.50 cm at T_4 treatment in *A. auriculiformis*. The lowest data (11.30 cm) was recorded for *L. leucocephala* at T_3 treatment. Both the maximum (156.3%) and minimum (47.5%) relative elongation ratio (RER) of root was found in case of *L. leucocephala* respectively at T_1 and T_3 treatment (Fig. 2).

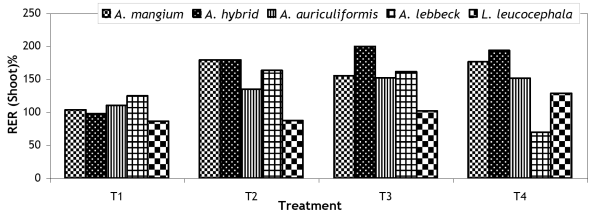

Fig. 1. Relative elongation ratio (RER) of shoot of selected agroforestry tree seedlings at different treatments (*where*, T_0-pure sand only; T_1-only soil; T_2-soil: cow dung at 1:1 ratio; T_3-soil: cow dung at 2:1 ratio; T_4-soil: cow dung at 3:1 ratio).

Collar diameter (mm)

Table 4 represents the collar diameter of selected leguminous tree seedlings at different treatments. The application of cow dung (treatment T_2-T_4) had significant effect on the increment of collar diameter and the maximum increment (3.52 mm) was observed in case of *L. leucocephala* at T_2 treatment followed by 3.46 mm at T_4 treatment for the same species. The lowest response in diameter growth was found at T_1 treatment in case of *A. hybrid*. The maximum relative elongation ratio (RER) of collar diameter (156.4%) was found in *A. hybrid* at T_2 treatment whilst the lowest (93.1%) was in *A. mangium* at T_1 treatment (Fig. 3).

Root diameter (cm)

Cow dung applications (T_2-T_4) barely accelerated the increment of root diameter of the selected agroforestry tree seedlings except in case of *A. mangium*. In most cases the

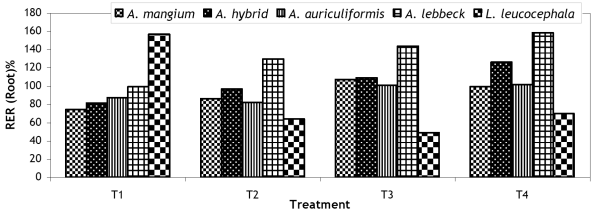

Fig. 2. Relative elongation ratio of root of selected agroforestry tree seedlings at different treatments (*where*, T_1-only soil; T_2-soil: cow dung at 1:1 ratio; T_3-soil: cow dung at 2:1 ratio; T_4-soil: cow dung at 3:1 ratio).

Table 3. Root lengths (cm) of selected agroforestry tree seedlings grown in polybags treated with different media under nursery conditions

Treatments	Species				
	A. mangium	*A. hybrid*	*A. auriculiformis*	*A. lebbeck*	*L. leucocephala*
T0	24.90ab	20.05ab*	33.10a	18.87b	23.80b
T1	18.40b	16.25b	28.80a	18.63b	37.20a
T2	21.40ab	19.30ab	27.05a	24.38ab	16.60bc
T3	26.64a	21.80ab	33.20a	27.04a	11.30c
T4	24.68ab	25.20a	33.50a	29.77a	16.60bc

*Values in the columns followed by the same letter(s) are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT).

Table 4. Collar dia (mm) of selected agroforestry tree seedlings grown in polybags treated with different media under nursery conditions

Treatments	Species				
	A. mangium	*A. hybrid*	*A. auriculiformis*	*A. lebbeck*	*L. leucocephala*
T_0	1.75b*	1.56b	1.65d	2.92b	2.90a
T_1	1.63b	1.50b	1.89cd	2.77b	3.03a
T_2	2.67a	2.44a	2.10bc	3.37a	3.52a
T_3	2.60a	2.32a	2.26b	3.09ab	3.22a
T_4	2.64a	2.22a	2.65a	3.05ab	3.46a

*Values in the columns followed by the same letter(s) are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT).

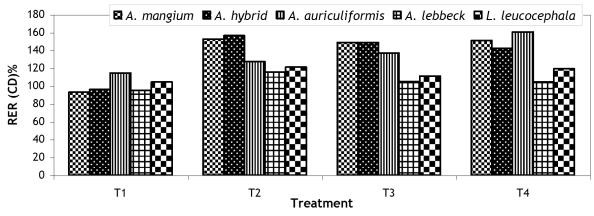

Fig. 3. Relative elongation ratio of collar diameter of selected agroforestry tree seedlings at different treatments (*where*, T_1-only soil; T_2-soil: cow dung at 1:1 ratio; T_3-soil: cow dung at 2:1 ratio; T_4-soil: cow dung at 3:1 ratio).

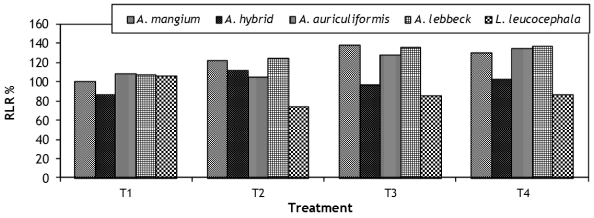

Fig. 4. Relative leaf ratio (RLR) of selected agroforestry tree seedlings at different treatments (*where*, T_1-only soil; T_2-soil: cow dung at 1:1 ratio; T_3-soil: cow dung at 2:1 ratio; T_4-soil: cow dung at 3:1 ratio).

Table 5. Root dia (cm) of selected agroforestry tree seedlings grown in polybags treated with different media under nursery conditions

Treatments	Species				
	A. mangium	*A. hybrid*	*A. auriculiformis*	*A. lebbeck*	*L. leucocephala*
T_0	2.65c	2.95a*	4.33a	4.04a	3.41a
T_1	2.02c	2.45a	3.63ab	3.97a	2.65a
T_2	2.80c	3.03a	2.73c	3.92a	2.81a
T_3	5.64a	2.85a	3.50b	2.64b	3.28a
T_4	4.47b	2.95a	3.88ab	3.21ab	3.00a

*Values in the columns followed by the same letter(s) are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT).

Table 6. Leaf number of selected agroforestry tree seedlings grown in polybags treated with different media under nursery conditions

Treatments	Species				
	A. mangium	*A. hybrid*	*A. auriculiformis*	*A. lebbeck*	*L. leucocephala*
T_0	8.30b	7.90ab*	7.70b	7.70b	9.40ab
T_1	8.30b	6.80b	8.30b	8.20b	9.90a
T_2	10.10ab	8.80a	8.00b	9.50a	6.95b
T_3	11.40a	7.60ab	9.85a	10.40a	8.00ab
T_4	10.80a	8.10ab	10.35a	10.50a	8.10ab

*Values in the columns followed by the same letter(s) are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT).

highest increment was recorded at T_0 treatment (Table 5). The maximum increment (5.64 cm) was found at T_3 treatment in *A. mangium*, where the minimum growth (2.02 cm) was found for the same species at T_1 treatment.

Number of leaves

It was apparent that the application of organic manure had significant positive effect on the number of leaves of the selected agroforestry tree seedlings. In most cases the highest numbers of leaves were recorded at T_4 treatment. A tendency of increasing leaf numbers with different treatments was observed. The highest data (11.40) was recorded at T_3 treatment followed by 10.80 at T_4 treatment, both for *A. mangium* (Table 6). The lowest leaf number was observed at T_1 treatment in *A. hybrid*. The highest relative leaf ratio (RLR) was found in case of *A. mangium* (137.5%) at T_3 treatment while the lowest (73.9%) was observed in *L. leucocephala* at T_2 treatment. Fig. 4 shows the calculated RLR of the selected tree seedlings.

Nodule number

Nodulation in terms of nodule number (seedling^{-1}) was found to affect greatly during the period of experiment by treatments containing higher proportion of cow dung. Nodulation performance was appeared resistant to treatments containing cow dung (T_2-T_4), i.e. no or significantly lowest nodules were observed (Table 7). However, in case of *A. lebbeck* it was found relatively less effective. In all cases nodulation was significantly increased at T_0 treatment (pure sand) followed by at T_1 treatment. The highest number of nodule (20.00) was recorded in *A. lebbeck* at T_0 and T_2 treatment, while the lowest was 0.28 in *A. mangium* at T_2 treatment. Maximum relative nodulation ratio (RNR) was found in *A. lebbeck* (100%) at T_2 treatment while the minimum (24.4%) was in *L. leucocephala* at T_1 treatment (Fig. 5).

Nodule size (mm)

The nodule sizes of the selected leguminous tree seed-

Table 7. Nodule number of selected agroforestry tree seedlings grown in polybags treated with different media under nursery conditions

Treatments	Species				
	A. mangium	*A. hybrid*	*A. auriculiformis*	*A. lebbeck*	*L. leucocephala*
T_0	8.30a	10.70a*	7.30a	20.00a	4.10a
T_1	5.40b	4.20b	6.00b	12.50b	1.00b
T_2	0.28c	0.00c	0.00c	20.00a	0.00c
T_3	0.00c	0.00c	0.00c	4.10c	0.00c
T_4	0.00c	0.00c	0.00c	6.00c	0.00c

*Values in the columns followed by the same letter(s) are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT).

Table 8. Nodule size (mm) of selected agroforestry tree seedlings grown in polybags treated with different media under nursery condition

Treatments	Species				
	A. mangium	*A. hybrid*	*A. auriculiformis*	*A. lebbeck*	*L. leucocephala*
T0	2.73a	2.27a*	2.21a	3.36a	2.06a
T1	2.06b	2.35a	1.73b	3.67a	1.37b
T2	1.20c	0.00b	0.00c	2.32b	0.00c
T3	0.00d	0.00b	0.00c	1.65c	0.00c
T4	0.00d	0.00b	0.00c	1.22c	0.00c

*Values in the columns followed by the same letter(s) are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test (DMRT).

lings in response to different media are given in Table 8. It was observed that treatments containing higher proportion of cow dung had significant negative effect on the sizes of the nodules. In case of *A. lebbeck* nodules were present in each treatments and the biggest nodule size (3.67 mm) was found in it at T_1 treatment. The smallest nodule (1.20 mm) was found at T_2 treatment in case of *A. mangium.*

Nodule shape and color

During whole experiment period the nodule shape of the selected agroforestry tree species were found varied considerably. The shape of nodules of species belongs to Mimosoideae family (i.e. *Acacia* spp. and *A. lebbeck*) were found fan or finger shaped, lobed and semi-globose (Table 9). Most of the young nodules of *A. lebbeck* were round, white and smooth-surfaced while older nodules were of various shapes. In case of *L. leucocephala* (Papilionoideae) the nodule shape was found bifurcate, globose and elongated.

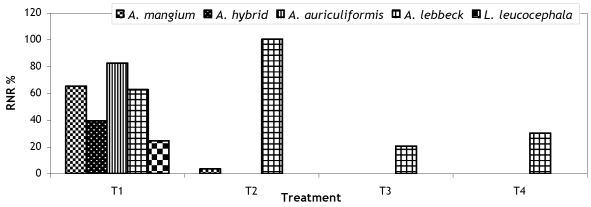

Fig. 5. Relative nodulation ratio (RNR) of selected agroforestry tree seedlings at different treatments (*where*, T_1-only soil; T_2-soil: cow dung at 1:1 ratio; T_3-soil: cow dung at 2:1 ratio; T_4-soil: cow dung at 3:1 ratio).

Table 9. Characteristics of nodules of selected agroforestry tree species in nursery

Species	Nodule morphology	Color and appearance
Acacia spp.	Fan shaped	Brown, rough surfaced
A. lebbeck	Finger-shaped, semi-globose and lobed	White, smooth surfaced
L. leucocephala	Branched, elongated	Brown, rough surfaced

Discussion and Conclusion

Morphological characteristics are the visually determinable attributes of tree and seedlings to understand their performance in response to different fertilizer application and to adopt them in desirable agroforestry systems (Duguma and Tonye 1994). Our study clearly revealed that organic fertilizations yielded better growth and quality of the seedlings of selected agroforestry tree species, i.e. they have beneficial effect on the improvement of growth parameters of seedlings. This result is also supported by the study of Sundralingam 1983; Sanginga et al. 1989; Walker et al. 1993; USEPA 1999; Bhuiyan et al. 2000, who reported the accelerated growth parameters in response to the application of different fertilizer both organic and chemical on species like, *Tectona grandis* and *Casuarina* sp. The beneficial use of organic manure or cow dung for improving growth of leguminous agroforestry tree seedlings are also in accordance with the works of several authors (Das 1984;

FAO 1987; Paul and Hossain 1996 for example).

In some cases it was evident that the higher doses of organic fertilizers have negative effect on seedlings (i.e. reduced growth) which might hamper the seedling growth by initiation of toxic effects. This supported the findings of Van den Driessche (1980), who reviewed both positive and negative effects of application of nursery fertilizers on subsequent seedling growth and survival. Negative effects of fertilization were also reported for Sitka spruce (Benzian et al. 1974) and Lobolly pine (Pharis and Kramer 1964; Schomaker 1969). Similarly, Kadeba (1978) and Hartley (1977) reported that an addition of access fertilizer on *Pinus caribaea* depressed growth and increased mortality of the seedlings.

Fertilizers have also an important effect on biological nitrogen fixation (BNF). Since nodulation is the primary indicator of BNF, we therefore studied these (i.e., nodules number plant^{-1}, nodule size, shape, color etc.) to realize the effects of organic manure on nodulation performance of the selected species. Our study revealed that nodulation in terms of nodule number and size was reduced considerably which indicated a significant negative effect of organic fertilizer on nodulation performance. This is in against the findings of Schröder et al. 2007. The nodule shapes and color were also reported in this study which consistent with the findings of Anegbeh et al. (2003) who reported the characteristics of nodules of four agroforestry tree species in Niger Delta, Nigeria, and Corby (1971) who reported the shape and color of some selected leguminous tree crops.

The present investigation clearly concluded that in the context of sustainable agriculture and agroforestry, the use of leguminous or nitrogen fixing trees (NFT) and the application of organic manure might be an effective biological strategy directed at sustainability. In an agro-ecosystem this means profitable and eco-friendly production without hamper to the environment (Hossain 2003). Our study also suggested that decomposed cow dung or organic manure hold a great potentiality to be an appropriate alternative or substitutes of chemical compound fertilizers which could certainly contribute to resolve present fertilizer dilemma and to enhance productivity. A huge amount of dried and processed cow dung in the country is still used as fuel in domestic cooking in spite of their probable best use in agricultural and forestry sector. A mass awareness creation program is necessary to make people concern about this. Again, although fertilizers showed significant positive (or negative) effects on growth and nodulation (through BNF) of agroforestry trees and seedlings but still little is known about the bio-physical mechanism behind of it mainly due to limited infrastructure and lab facilities in the country. Developments of further facilities are therefore recommended to study and identify this occurrence in detail.

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