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INFLUENCE OF SHADING LEVELS ON GROWTH, YIELD AND ANDROGRAPHOLIDE CONTENT OF KALMEGH

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Abstract

We studied the growth, yield and andrographolide content of three local kalmegh (*Andrographis paniculata* (Burm. F.) Nees) responses to varying shade levels under green house conditions, from March to July 2018 in Thailand. Our experiment was laid out in a split plot design with three replications. Three local kalmegh varieties (Prachinburi, Nakhon Prathom and Saraburi) and four shading levels (0%, 25%, 50% and 75% shading) were applied to the test plots. Stem height, stem, leaf and root dry weight and leaf number per plant, seed and leaf dry weight yield and andrographolide content were recorded. The Prachinburi variety, followed by Nakhon Prathom and Saraburi, showed the strongest growth under all shade conditions. Different shading levels strongly influenced growth and kalmegh yield. The highest growth, stem, leaf, root, total dry weight, seed and leaf dry weight yield and andrographolide content were registered at 25% shading levels followed by 0%, 50% and 75% shading levels. We concluded that 25% shading and the local Prachinburi variety kalmegh should be recommended.

Key words**:** Growth, Yield, Andrographolide, Kalmegh, *Andrographis paniculata*, Shading.

Introduction

Andrographis paniculata (Burm. F.) Nees commonly known as kalmegh belonging to family Acanthaceae, is an important medicinal plant, widely used in India and south-east Asian countries, such as Thailand and Indonesia, for treatment of malaria, liver disorder, hypertension, bowel complaints, fever, snake bite, common cold and variety of other ailments (Parasher *et al.*, 2011; Valdiani *et al.*, 2012)

Light is the main environmental factor which determines crop development: all plants are sensitive to it. Light plays an important role in chlorophyll synthesis, enzyme activation, and photosynthesis, thus governing plant growth and development. Excessive light intensity inhibits photosynthesis as it destroys photosynthetic pigments (Kumar *et al.*, 2012). Under natural conditions, kalmegh may be found in both shaded and wide open areas. Purwanto *et al.*, (2011) reported that the shading level influenced growth and yield of kalmegh. Shading at 25% led to the best growth characteristics and yield, while the highest andrographolide content was observed at 50% shading levels. In contrast, Gundadon *et al.*, (2015) found that kalmegh grown under full sunlight did not differ from those under 50% shade in height but produced more branches and leaves as on the main plant stem. Rosli *et al.*, (2018) compared two shade levels, 0%, and 40%, and reported that 40% shaded plants grew taller with greater total leaf area, specific leaf area ratio and net assimilation rate than sun-grown plants. During the growing period, growth and crop yield was closely related to the amount of solar energy received. However, this may be a lack of information on growth and physiology related to different light intensities of kalmegh (Saravanan *et al.*, 2008). Therefore, the aim of this experiment is to evaluate the kalmegh in growth and yield response to different shading levels. So, we evaluated kalmegh growth and yield response to different shading levels.

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Materials and Methods

Plants were grown in the glass house of the Department of Plant Production Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand (13°44'33"N, 100°46'51"E) during March to July 2018.

Experimental design: The experiment was arranged in a split plot design with three replications. The main plot treatments use three local kalmegh varieties (Prachinburi, Nakhon Prathom, and Saraburi) while sub plot treatments used four different shading levels (0%, 25%, 50%, and 75% shading).

For placing the shading material, four 2 m high posts were erected above the treatments and then different shading material was stretched between these posts so that it was hanging on the sides, but not touching the ground to ensure ventilation. Three local kalmegh cultivar such as Prachinburi, Nakhon Prathom, and Saraburi were planted in main-plots. Black perforated plastic matting with 25, 50 and 75% transparency level was used as shade material. For 0% shading, the plant was fully exposed to sunlight.

Plant material and transplanting: The seeds of three local kalmegh varieties were obtained from Phichit Agricultural Research and Development Center, Phichit Province, Thailand. It was the place which was the largest collecting germplasm, local and hybrid of kalmegh varieties. One seed was sown (10 mm deep) in small plastic bags and then 30-day old seedlings, 150 mm high, were transplanted to plastic plots 300 mm in diameter and 300 mm of deep soil, approximately 30 kg. The soil was Bangkok series and clay in texture (Land development department, 2019) with pH 6.10, ie slightly acidic. One seedling was transplanted into each pot. The pot was irrigated everyday after transplanting until harvest to keep the soil moist. For fertilizer, the plant was given a base dose, 35 kg ha^{-1} of Nitrogen fertilizer was applied as urea (46% of N) at 300 kg (N) ha^{-1} of which 50% was applied as a basal dose and 50% at the 30 DAT. P [150 kg (P_2O_5) ha^{-1}] and K [150 kg (K_2O) ha^{-1}] were applied as ordinary superphosphate (12% of P_2O_5 and 12% of S) and potassium sulfate (50% of K_2O). All of P and K fertilizers were applied at one day before transplanting. Weeds were removed manually at 15, 30 and 60 DAT to minimize weed competition. The kalmegh plants were sampled at 120 DAT to measure their physical characteristics (stem length per plant, stem, leaf, root dry weight per plant, total dry weight, seed, and leaf dry weight yield). Dry weight per plant was measured by drying the plants at 80 °C for 48 h. Plant height was recorded by measuring the length of the plant from the soil surface to the top of the plant. The number of branches per plant was counted each plant and the average number of branches per plant was calculated. Seed and leaf dry weight yield per pot were noted and seed and leaf dry weight yield per square meter were calculated. At 120 DAT, total andrographolide was measured by HPLC following the method of Saxena *et al.,* (2000) and Rajani *et al.,* (2000).

Statistical analysis: The experiment was arranged in a split plot in a randomized complete block design with three replications following Snedecor and Cochran (1980). Analysis of variance using the SPSS for Windows 14.0 software package and means compared by the Least Significant Difference (LSD) test at P=0.05 level of significance.

Results and Discussion

Growth characteristics

Plant height

Plant height (cm) differed significantly differed among the kalmegh varieties at harvest (Table 1). Maximum plant height was recorded from Prachinburi variety: it was 17% greater than the Nakhon Prathom and 37% greater than the Saraburi variety. There was significant ($P<0.05$) difference in plant height between plants grown under the different shading levels. Plants grown under 75% shading were the highest and were 11% higher compared to 50% shading and 26% higher than 25% shading, while plants were grown under full sun (0% shading) were the lowest. These results agree with those of Saravanan *et al.,* (2008). Plant height varied significantly with light intensity. It was reduced up to 32% under full light (0% shading) compared to 25% light. The plants were shorter with reduced internodal length and more compact under full sun. Similarly, Boardman (1977) and Purwanto (2011) also noted that plants grown in the shade tended to be taller with a long stem segment composed of thin-walled cells, larger intercellular spaces and fewer transport tissue and binding tissue. This can be attributed to the activity of auxin. In the other words, kalmegh plants grew well with shading levels of 25 to 75%.

Number of branches

The degree of branching or number of branches per plant varied significantly among the varieties at harvest (Table 1). The Prachinburi variety showed higher branching than the others. Branching was affected by shading level. The highest branching degree was observed in plants under 25% shading. The lowest branching was observed with 75% shading.

Stem dry weight

Significant variation was found in stem dry weight. The Prachinburi variety recorded the highest stem dry weight followed by Nakhon Prathom and then Saraburi varieties. Shade also affected stem dry weight, with the highest stem dry weight obtained at 25% shading and it decreased by 19% at 0% shading, by 27% at 50% and by 30% at 75% shading.

Leaf dry weight and number of leaves per plant

Significant variation was found in leaf dry weight and number of leaves per plant at harvest (Table 1). Again, the Prachinburi variety showed the highest and Saraburi the lowest. Shading levels led to significant ($P<0.05$) differences also, with the highest values at 25% shading, followed by 0% and 50% shading with the lowest at 75% shading. Higher numbers of leaves per plant and leaf dry weight observed at harvest was attributed to increased growth (number of branches per plant) which generated more leaves. Similar finding was reported by Ashok *et al.,* (2002) and Sanwal *et al.,* (2016). The production of plants with more number of branches per plant by the formation of more lateral buds. The results are in conformity with the finding of Kumar *et al.,* (2009) and Parashar *et al.,* (2011) in kalmegh. In contrast to Saravanan *et al.,* (2008) reported that total leaf area per plant was the highest under full light conditions followed 25% shading. Lowest leaf area was recorded in plants grown under 70% shading.

Root dry weight

Root dry weight also varied with variety at harvest (Table 1). This followed the trend observed for the other parameters with Prachinburi variety showing the highest root dry weight and the Saraburi variety showing the lowest.

Pod dry weight

Pod dry weight differed among three varieties (Table 2): the trend was the same as for the other varieties with Prachinburi showing the maximum. Pod dry weight was also attributed to increased growth (number of branches per plant) and was consistent with reports by Kumar *et al.,* (2009); Parashar *et al.,* (2011).

Total dry weight

The trend for total dry weight followed the same pattern, agreeing with the work of Araki *et al.,* (2014). However, Omar *et al.,* (2016) found that shoot fresh weight and shoot dry weight disclosed increased with the increase in shade level or lowered total sun exposure, i.e. the minimum shoot dry weight was obtained under full sunlight. Singh *et al.,* (2011) also reported that growth and dry matter yield decreased with lower sun exposure. This might be due to the fall of lower leaves, which became yellow and dry due to shading. Saravanan *et al.,* (2008) also concluded that the total herbage and andrographolide content were the highest under full light conditions and that kalmegh is suitable for open cultivation. Purwanto *et al.,* (2011) confirmed this. However, significant differences in total biomass were not observed for plants grown under 70 and 50% light levels.

Plant yield and andrographolide content

Seed dry weight yield

Maximum seed dry weight (g m^{-2}) was found in Prachinburi variety and was 27% greater than Nakhon Prathom and 48% greater than Saraburi. The same trend with shading level was observed.

Leaf dry weight yield

Leaf dry weight yield (g m^{-2}) was again highest in the Prachinburi variety and 15% lower for Nakhon Prathom and 30% larger than Saraburi with the same trend for shading level.

Andrographolide content

The Prachinburi variety produced more andrographolide content than Nakhon Prathom and Saraburi see Table 3. Andrographolide content decreased significantly with increasing shading levels. The highest andrographolide content (2.9%) was recorded at the 25% shading level. It was reduced by 60% at the 75% shading level. Saravanan (2008) reported that the role of light in biosynthesis of andrographolide and the metabolism control was not yet understood. Shade indirectly plays a role, by altering the basic processes, like photosynthesis and respiration, and thereby changing the flux of metabolites and reducing power generated through the light reaction which may in turn, modify synthesis and accumulation of andrographolide. Similar findings were reported by Purwanto *et al.,* (2011), who found that the kalmegh plant needed shading in the 25% to 50% range for optimum growth and andrographolide production. Rosli *et al.,* (2018) reported that andrographolide content of shaded plants was slightly higher than sun-grown plants. Liphan and Detpiratmongkol (2017) reported a maximum andrographolide content at 20% shading level and a minimum at the 80% shading level.

Significant variations were found in respect of growth and yield among the local kalmegh cultivars. As three local kalmegh cultivars (Nakhon Prathom, Prachinburi and Saraburi) cultivars had significant in nine characteristics with each other. Prachinburi had the tallest plants (300 mm), number of branches per plant (34.6) and highest weights per plant: stem (53.9 g), leaf (25.9), root dry weight (14.2 g), pod dry weight (5.1 g), total dry weight (95.4 g), seed (48.3 g) and leaf dry weight yield (365 g m^{-2}) followed by the Nakhon Prathom and Saraburi

Table 1: Plant height, branches number plant^{-1}, stem dry weight, leaf dry weight and number of leaves plant^{-1} of three local kalmegh varieties at harvest (120 days after planting) affected by different shading levels.

Treatments	Plant height (cm)	Branches number plant^{-1}	Stem DW (g plant^{-1})	Leaf DW (g plant^{-1})	No. ofleaves plant^{-1}
Plant varieties (A)					
Prachinburi	30.44	34.56	53.87	25.86	192.40
Nakhon Pathom	25.18	28.05	46.96	22.36	158.73
Saraburi	19.28	23.42	39.27	19.14	116.50
Shading levels (B)					
0 %	19.47	31.32	55.21	24.27	185.95
25 %	22.76	36.82	65.00	27.48	243.16
50 %	27.08	25.48	39.75	20.73	125.89
75 %	30.56	21.08	27.85	17.31	68.51
LSD (0.05) (A)	3.63	3.32	6.66	2.99	26.71
LSD (0.05) (B)	2.84	4.20	6.02	2.40	21.38
LSD (0.05) (AxB)	ns	ns	ns	ns	ns
C.V. (A) (%)	12.84	10.22	12.59	11.78	15.12
C.V. (B) (%)	11.52	14.78	13.20	10.79	13.85

ns = No significant at the 0.05 probability level; DW = dry weight.

Table 2: Root, pod and total dry weight of three local kalmegh varieties at harvest (120 days after planting) affected by different shading levels.

Treatments	Root DW (g plant^{-1})	Pod DW (g plant^{-1})	Total DW (g plant^{-1})
Plant varieties (A)			
Prachinburi	14.21	5.06	95.36
Nakhon Pathom	11.59	4.43	81.13
Saraburi	9.17	2.97	66.38
Shading levels (B)			
0 %	11.58	4.08	84.72
25 %	14.51	5.09	95.10
50 %	10.70	3.95	73.22
75 %	9.68	3.50	70.78
LSD (0.05) (A)	1.42	0.60	9.32
LSD (0.05) (B)	1.16	0.57	8.35
LSD (0.05) (AxB)	ns	ns	ns
C.V. (A) (%)	10.77	12.80	16.31
C.V. (B) (%)	10.07	13.76	14.42

ns = No significant at the 0.05 probability level; DW = dry weight.

varieties. These results confirm other work (Detpiratmongkol *et al.* 2016; Detpiratmongkol *et al.*, 2017; Liphan and Detpiratmongkol 2017). Sandeep *et al.*, (2009) also reported that genotypes have a significantly different effect on plant height, stem, and leaf dry matter, total dry matter and dry matter yield.

Plant growth is affected by many environmental factors, such as insolation, temperature, soil, fertilizer and so forth. Significant differences in growth, yields, and

Table 3: Seed and leaf dry weight yield and andrographolide content in the leaf of three local kalmegh varieties at harvest (120 days after planting) affected by different shading levels.

Treatments	Root DW (g plant^{-1})	Pod DW (g plant^{-1})	Total DW (g plant^{-1})
Cultivars (A)			
Prachinburi	48.25	365.27	2.78
Nakhon Prathom	35.34	310.76	2.41
Saraburi	25.02	255.22	1.81
Shading levels (B)			
0%	39.74	374.43	2.62
25%	48.67	449.47	2.93
50%	34.48	297.40	2.60
75%	21.92	120.37	1.18
LSD (0.05) (A)	5.96	53.88	0.03
LSD (0.05) (B)	5.64	81.81	0.32
LSD (0.05) (AxB)	ns	ns	ns
C.V. (A) (%)	14.52	15.31	12.22
C.V. (B) (%)	15.74	26.61	13.76

ns = No significant at the 0.05 probability level; DWY = dry weight yield.

andrographolide content were observed with shading levels. The maximum seed dry weight yield and total dry weight were obtained for the crop under 25% shade followed by the full sun (0% shade) and 50% shade levels. The lowest was recorded at 75% shade levels. We concluded that the optimum growth of kalmegh required limited shading for better growth. However, shade at more than 50% would decrease plant growth due to the high reduction of photosynthesis. As shown in Tables 1 and 2, the stem, leaf, and root dry weight and total dry weight and yield were the highest under 25% shading, followed 0% and 50% shading levels and lowest growth characters were recorded under 75% shading levels. This previous work of Liphan and Detpiratmongkol (2017) who were found that shading affected on growth and yield of kalmegh.

Conclusion

Our study clearly indicated that the highest plant height, stem leaf and root dry weight, total dry weight, seed, and leaf dry weight yield were obtained by the Prachinburi variety, followed by Nakhon Prathom and Saraburi. For different shading levels; shading had significant difference for all the growth characters studies. The maximum of stem, leaf and root dry weight, total dry weight and dry weight yield were obtained with 25% shading levels and the minimum was achieved with 75% shading level. Therefore, the use of the Prachinburi variety and plants grown under 25% shading levels were recommended.

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