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RESPONSE OF KALMEGH (*ANDROGRAPHIS PANICULATA* (BURM. F.) NEES) TO SHADING AT DIFFERENT GROWTH STAGES

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Abstract

Kalmegh (*Andrographis paniculata* (Burm. F.) Nees) is a very useful medicinal herb widely used for treatments of various human diseases and symptoms: fever, cold, laryngitis, diarrhea and inflammation. However, its enhanced growth and yield as a response to shading at different growth stages have not been studied yet. Therefore, in this study, an experiment was undertaken, from January to April 2018, to investigate the effects of shading at different growth stages on the growth, yield and quality of kalmegh. The design of this pot experiment was split plot with three replications; three local kalmegh cultivars—Prachinburi, Phichit 4-4 and Phisanulok 5-4—were the main plots. The four sub-plots were 20% shading at 3 stages of growth *viz*: 30, 60 and 90 days after planting (DAP) till harvest and no shading which was the control. Several agronomic characteristics of kalmegh such as plant height, stem, dry weight of leaf and root, total dry weight and leaf dry weight yield were recorded. The results indicated that Prachinbuti cultivar gave the tallest plant and stem heights as well as the highest dry weight of leaf and root, total dry weight and leaf dry weight yield, followed by Phisanulok 5-4 and Phichit 4-4. Shading at different stages of growth clearly affected the growth and yield of kalmegh. No shading resulted in the least growth and yield while shading at 90 DAP promoted some growth and yield that was higher than no shading but lower than shading at 30 and 60 DAP. The maximum values of growth and yield were observed under 20% shading at 30 DAP.

Key words **:** Yield, Andrographolide, Kalmegh, Shading.

Introduction

Medicinal plants are essential for human beings who utilize them for basic preventive and curative health care. Kalmegh (*Andrographis paniculata* (Burm. F.) Nees) is an important herb for treatments of fever, diarrhea, dysentery, cough, sore throat, bronchitis, arthralgia, haematometra, hypertension and snake bite (Madav *et al.,* 1995; Matsuda *et al.,* 1994). Kalmegh that is continuously harvested from trees in the forest may become depleted (Yusron and Januwati, 2004). However, it is widely cultivated in India, Sri Lanka, Malaya Peninsula, China and Thailand, but a proper cultivation method is required. The need for kalmegh plant as a raw material for traditional medicine continues to increase, but the supply of kalmegh harvested from farms is still unpredictable (Purwanto *et al.,* 2011). Environmental conditions such as shading strongly affect its growth (Kosma *et al.,* 2013). Solar radiation with a proper intensity, quality and duration of exposure is an important factor for its growth. When the received light intensity is low, the amount of light received by the surface area of each leaf within a specified period of time will be low as well. Lack of light leads to metabolism disorder and decreases in photosynthesis and carbohydrate synthesis rates. Conversely, if the light intensity is too high, the plant may suffer from high temperature stress and drought stress (Parwanto *et al.,* 2011). Therefore, the objective of this study was to determine the effects of 20% shading at different growth stages on the growth, yield and quality of harvested kalmegh. This study was also meant to identify the optimum period for shading, in terms of days after planting till harvest, and inform farmers so as to enhance their kalmegh production.

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Materials and Methods

A field experiment was conducted at an experimental plot in the area of the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand, during January to April, 2018. Seeds of kalmegh (Prachinburi, Phisanulok 5-4 and Phichit 4-4

varieties) were obtained from the Phichit Agricultural Research and Development Center, Pichit Province, Thailand. Three seeds were dropped into a 1-cm deep hole in the soil in plastic pots (30 cm in diameter). The pots were arranged in the experimental plot according to a split plot randomized complete block design with 3 replications. The main plot had all three varieties of kalmegh placed in it with 20% shading. It was divided into 4 sub-plots where each received 20% shading only during one of the 4 different growth stages of kalmegh. The sub-plot treatments included the following: (1) shading for 30 days after planting (DAP) till harvest, (2) shading for 60 DAP till harvest; (3) shading for 90 DAP till harvest and (4) without shading. When the seeds grew to 15 DAP, one plant from each pot was selected and the others were pulled off from the pot. At 30 DAP, 3 replicates of each of the 3 varieties for a total of 9 pots altogether were placed in each of the four sub-plots. According to a number of previous studies, plant growth, shoot dry weight and leaf dry weight yield were the highest under 20% shading condition (Liphan and Detpiratmongkol, 2017). Therefore, in this study, the shade treatments were imposed in the field with black artificial shade nets that provided 20% shading and the no shade control provided 100% of the sun light without any shading. To keep the soil moist, all of the pots received daily irrigation of water equivalent to 5 mm of rainfall till harvest. All of the pots were weeded manually for three rounds at 15, 30 and 60 DAP.

At 120 DAP, all of the plants were washed off the dirt clinging on them with water, pulled off the pots and their heights were measured with a meter scale. Next, they were separated into fresh leaves, stems and roots for assessment of some of their growth characteristics: stem length, number of branches per plant, number of leaves per plant and leaf area. The leaf area was measured with a portable area meter, LI-COR Model LI-3000, USA. Then, these parts were dried at 80°C for 48 h and weighed and calculated of stem dry weight per plant, leaf dry weight per plant, root dry weight per plant, total dry weight per plant, pod DW plant^{-1}, seed dry weight yield (g m^{-2}) and leaf dry weight yield.

Finally, the amounts of andrographolide and total lactone in the dry shoot biomass were determined by a method reported by Jain *et al.,* (2000) and Singh *et al.,* (2011). An analysis of variance was carried out on the obtained data, and the LSD ($p = 0.05$) was calculated (Gomez and Gomez, 1984).

Results and Discussion

Growth characteristics

Plant height

The plant heights at the time of harvest of the three kalmegh varieties varied table 1. The average plant height of Prachinburi variety was significantly higher than those of Phisanulok 5-4 and Phichit 4-4, by 43.25% and 61.28% respectively. Different periods of shading strongly influenced plant height. The average height of plants grown under shading at 30 DAP was higher than that of plants grown under shading at 60 DAP and 90 DAP, by 27.28% and 43.11% respectively. The kalmegh plants grown unshaded (control) had the lowest height. Increased treatment plant heights can be attributed to the favorable shade condition which might make the plants grow taller by increasing their cell division and elongation (Himbindu *et al.,* 2017; Saravanan *et al.,* 2008). A few previous studies reported that kalmegh grown under shade were taller than those under no shade (Saravanan *et al.,* 2008; Rosli *et al.,* 2018). In addition, shaded kalmegh grew taller with a larger canopy than those grown under no shade so that it could capture more light. The kalmegh plants grew taller because their stems elongated more in response to the low red to far-red ratio light that they were exposed to. Red to far-red ratio is the ratio of light at 655–605 nm to light at 725–735 nm. Red light suppresses stems from elongating while far-red light enhances elongation (Smith, 2000; Franklin and Whitelam, 2005). Shading decreases this ratio and stimulates the cells of the stems to make more phytohormones such as auxin, cytokinin and gibberellin (Müller and Leyse, 2011). These phytohormones cause the stems to elongate resulting in taller plants. This explains why the height and canopy of shaded kalmegh in this study were taller and more abundant. Several research results suggest that plant height depends on stem elongation (Liu *et al.,* 2016; Nagashima and Hikosaka, 2011).

Number of branches plant^{-1}

Among three kalmegh varieties (Table 1), the variety that yielded the highest number of branches plant^{-1} was Prachinburi, followed by Phisanulok 5-4 and Phichit 4-4 Table 1. The number of branches plant^{-1} was affected by the different periods of shading. The maximum of number of branches plant^{-1} was found in the plant grown under shading at 30 DAP, followed by those grown under shading at 60 DAP and 90 DAP in this order. The minimum of number of branches plant^{-1} was found in the plant grown under no shading (control). The number of branches plant^{-1} might depend on the height of the plant which favored formation of more lateral buds: a taller plant yielded a higher number of branches plant^{-1}. This phenomenon was also reported by Singh *et al.,* (2011) and Sunil Kumar *et al.,* (2011).

Number of leaves plant^{-1} and leaf area index

Significant variations of the number of leaves per

plant and leaf area index among the three local kalmegh varieties can be observed in table 1. The highest number of leaves per plant and leaf area index was yielded by the Prachinburi variety and the lowest was yielded by Phichit 4-4. Shading at different growth stages significantly affected the number of leaves per plant and leaf area index compared to the control (no-shading treatment). The numbers of leaves per plant shaded at 30 DAP were 9.39%, 32.36% and 40.05% greater than those shaded at 60, 90 DAP and no-shading, respectively. The results of leaf area index were similar to those of number of leaves plant^{-1}.

All of these results indicate that shading at different growth stages treatments increased the number of leaves per plant compared to no-shading. The number of leaves per plant under a shade were influenced by the light intensity and exposure duration that the plant got. The plant that received an appropriate light intensity for a proper duration, especially intensity at 20% shading for a period of 30 DAP till harvest, achieved a proper balance between water transpiration from its leaves and water and mineral absorption by its roots, resulting in good photosynthesis and high accumulation of carbohydrate; thus, its growth and development was close to perfect. This result agrees well with those reported by Sulandjari *et al.,* (2005) and Purwanto (2011). A previous study also reported that the total leaf area per plant of shaded kalmegh was the highest, whereas plants grown under no shade produced the lowest total leaf area per plant (Saravanan *et al.,* 2008).

Table 1: Effects of different times of shading on plant height, number of branch plant^{-1}, number of leaves plant^{-1} and leaf areas of 3 local kalmegh cultivars at harvest (120 days after planting).

Treatments	Plant height (cm)	Number of branch plant^{-1} (branch)	Number of leaves plant^{-1} (leaves)	Leaf area index (LAI)
Cultivars (A)				
Prachinburi	56.41 A*	29.45 A	47.18 A	0.82 A
Phisanulok 5-4	32.04 B	18.20 BC	39.81 B	0.50 B
Phichit 4-4	21.84 B	15.22 B	28.65 C	0.33 C
Times of shading (B)				
30 DAP till harvest	58.10 a	25.20 a	25.28 a	0.64 a
60 DAP till harvest	42.25 b	22.48 b	42.17 ab	0.56 b
90 DAP till harvest	33.05 bc	20.26 b	38.21 b	0.52 bc
No shading (control)	20.31 c	18.55 c	28.52 c	0.48 c
LSD (A) (0.05)	5.14	4.73	5.32	0.09
LSD (B) (0.05)	5.70	2.40	5.09	0.07
LSD (AxB) (0.05)	ns	ns	ns	ns
C.V. (A) (%)	11.82	19.33	12.18	15.20
C.V. (B) (%)	14.98	11.22	13.34	12.73

ns = No significant at the 0.05 probability level; * = value within a column to followed by the different letters are significantly different by DMRT $p \leq 0.05$.

Stem dry weight

Stem dry weights were significantly different among the three kalmegh varieties table 2. The stem dry weight of Prachinburi variety was 41.06%, 51.40% greater than those of Phisanulok 5-4 and Phichit 4-4, respectively. The stem dry weight of the plant shaded at 30 DAP till harvest was higher than those of the plants shaded at 60 and 90 DAP till harvest by 9.63% and 18.94%, respectively. The lowest stem dry weight was from the plant grown under no shade (control).

Leaf dry weight

The leaf dry weights differed among the three kalmegh varieties table 2. In comparison, the leaf dry weight of Prachinburi variety was the highest, followed by those of Phisanulok 5-4 and Phichit 4-4 in this order. A significant increase in leaf dry weight was observed in the plant grown under a longer shading period. The kalmegh grown under shade at 30 DAP till harvest yielded the highest leaf dry weight while the kalmegh grown under no-shade (control) yielded the lowest leaf dry weight.

Our result indicates that a longer period of shading resulted in a remarkable increase in plant height, number of branches plant^{-1}, stem dry weight and leaf dry weight. This finding agrees well with the findings from a study by Detpiratmongkol and Liphan (2018) that a minimum continuous shading level (20%) at an early growth stage till harvest not only increased kalmegh plant height but also its growth parameters such as number of branches plant^{-1}, stem and leaf dry weights as well as seed and leaf dry weight yield (Detpiratmongkol and Liphan, 2018).

Root dry weight

Root dry weight varied significantly among the varieties table 2. The highest root dry weight was from Prachinburi variety. It was 37.50% and 53.13% greater than those of Phisanulok 5-4 and Phichit 4-4. Shading had a significant effect on the root dry weight of kalmegh. The plant grown under shading at 30 DAP till harvest had the highest root dry weight, followed by those grown under shading at 60 and 90 DAP till harvest in this order, whereas the plant grown under no shade (control) had the lowest. Previous tudies have reported that plants grown under a shading condition were taller and had a greater leaf, stem and root dry mass than those grown under no shade (Pitono *et al.,* 1996; Rosli *et al.,* 2018).

Table 2: Effects of different times of shading on stem, leaves, root, pod dry weight and total dry weight of 3 local kalmegh cultivars at harvest (120 days after planting).

Treatments	Stem DW. (g plant^{-1})	Leaves DW. (g plant^{-1})	Root DW. (g plant^{-1})	Pod DW. (g plant^{-1})	Total DW. (g plant^{-1})
Cultivars (A)					
Prachinburi	3.58 A*	2.84 A	0.32 A	0.25 A	5.30 A
Phisanulok 5-4	2.11 B	2.05 B	0.20 B	0.12 B	3.66 B
Phichit 4-4	1.74 C	1.29 C	0.15 C	0.05 C	1.86 C
Times of shading (B)					
30 DAP till harvest	3.22 a	2.42 a	0.26 a	0.16 a	4.34 a
60 DAP till harvest	2.91 ab	2.10 ab	0.23 ab	0.15 a	3.72 b
90 DAP till harvest	2.61 b	1.95 b	0.21 b	0.13 bc	3.43 bc
No shading (control)	1.14 c	1.72 c	0.17 c	0.12 c	2.93 c
LSD (A) (0.05)	0.32	0.28	0.02	0.01	0.49
LSD (B) (0.05)	0.35	0.25	0.03	0.01	0.36
LSD (AxB) (0.05)	ns	ns	ns	ns	ns
C.V. (A) (%)	11.28	11.94	14.55	10.91	12.10
C.V. (B) (%)	14.30	12.39	15.16	13.43	10.20

ns = No significant at the 0.05 probability level; DW = dry weight; DAP = days after planting; * = value within a column to followed by the different letters are significantly different by DMRT $p \leq 0.05$.

Pod dry weight

Pod dry weight varied among the three kalmegh varieties table 2. The pod dry weight of Prachinburi variety was 52.00%, 80.00% larger than those of Phisanulok 5-4 and Phichit 4-4 in this order. The pod dry weight of kalmegh grown under shading at 30 DAP till harvest was the highest; it was greater than those of kalmegh grown under shading at 60 and 90 DAP till harvest by 6.25% and 18.75%, in this order. The lowest pod dry weight was from kalmegh grown under no shade.

Total dry weight

The trend in total dry weight was similar for stem, leaf, root and pods table 2. The highest total dry weight was from Prachinburi variety; it was greater than those of Phisanulok 5-4 and Pichit 4-4 by 30.9% and 64.9%, in this order. The total dry weights of kalmegh shaded at different growth stages were greater than that of kalmegh grown under no shade. Kalmegh grown under 30 DAP till harvest gave the highest total dry weight; it was 14.29% and 20.96% higher than those yielded by kalmegh grown under shading at 60 and 90 DAP till harvest. Kalmegh grown under no shade gave the lowest total dry weight.

Yield and andrographolide content

Seed dry weight yield

Similar to the results for pod dry weight and total dry weight table 3, the highest seed dry weight was from Prachinburi variety while the lowest was from Phichit 4-4. The seed dry weight yield was greatly affected by shading at different growth stages. The highest seed dry weight yield was from the shading condition of 30 DAT till harvest, followed by shading at 60 and 90 DAP till harvest in this order, whereas the lowest was from kalmegh grown under no-shade condition.

Leaf dry weight yield

The results on leaf dry weight yield are presented in table 3. They indicate that the leaf dry weight yield strongly depended on the kalmegh variety. The maximum leaf dry weight yield was observed in Prachinburi variety, followed by Phisanulok 5-4 and Phichit 4-4. Shading at different growth stages also strongly affected leaf dry weight yield compared to the no-shading condition. The highest leaf dry weight yield was obtained with the kalmegh grown under a condition of shading at 30 DAP till harvest, which was higher than those grown under a 60 or 90 DAP till harvest condition by 7.71% and 15.44% respectively. Kalmegh grown under no-shading condition gave the lowest leaf dry weight yield.

Table 3 : Effects of different times of shading on seed and leaf dry weight yield and andrographolide content in leaf of 3 local kalmegh cultivars at harvest (120 days after planting).

Treatments	Seed DWY. (g m^{-2})	Leaf DWY. (g m^{-2})	Andrographolide content (%)
Cultivars (A)			
Prachinburi	3.57 A*	40.44 A	2.39 A
Phisanulok 5-4	2.11 B	29.40 B	2.02 B
Phichit 4-4	1.74 C	18.29 C	1.66 C
Times of shading (B)			
30 DAP till harvest	3.22 a	33.74 a	2.33 a
60 DAP till harvest	2.91 ab	31.14 b	2.16 ab
90 DAP till harvest	2.61 b	28.53 b	1.99 b
No shading (control)	1.14 c	24.10 c	1.60 c
LSD (A) (0.05)	0.32	3.66	0.24
LSD (B) (0.05)	0.35	2.97	0.24
LSD (AxB) (0.05)	ns	ns	ns
C.V. (A) (%)	11.28	11.01	10.46
C.V. (B) (%)	14.30	10.21	11.98

ns = No significant at the 0.05 probability level; DWY = dry weigh yield; * = value within a column to followed by the different letters are significantly different by DMRT $p \leq 0.05$.

Andrographolide content (%)

The results of the determination of andrographolide content are shown in table 3. Significant variations were found with respect to andrographolide content among the kalmegh varieties. The Prachinburi variety had a 15.48% and 30.54% higher andrographolide content than those of Phisanulok 5-4 and Phichit 4-4, respectively. The andrographolide content in leaf also varied with the condition of shading. The highest andrographolide content was recorded for the plant grown under the shading condition of 30 DAP till harvest, followed the conditions of 60 and 90 DAP till harvest in this order, whereas the lowest content was observed in the plant grown under a no-shading condition.

Some researchers have studied the agronomic requirements for enhancing the growth and quality of kalmegh (Ramesh *et al.,* 2011; Mishra and Jain, 2013). They found that yield improvement and quality of crops correlated with optimum level and duration of its exposure to light. There has been a report that kalmegh responded positively to light (Niranjan *et al*., 2010). In addition, light strongly affected plant growth and yield potential (Zhu *et al.,* 2012). Kalmegh responded to variations in light intensity and exposure duration by changing their morphology and developing its growth characteristics such as taller height as well as higher leaf, stem and root dry weights and yields (Kumar *et al.,* 2009).

Shading reduces the active primary radiation in photosynthesis, resulted in a decrease net assimilation of light (Lambers and Poorter, 1992), an increase in stored photosynthetic products in the storage organs such as root (Schaffer, 1996), and a decrease in plant dry weight (Purwanto *et al.,* 2011). The authors of this last reference also reported that kalmegh needed shading in the range of 25% to 50% for optimum growth and andrographolide production. In this study, the maximum growth and yield parameters were observed under the condition of 20% shading at 30 DAP till harvest. The timing of shading clearly affected the growth and yield. The no-shading condition caused the greatest growth and yield reduction. Rosli *et al.,* (2018) reported that the growth as well as the quantity and quality of kalmegh grown under 40% shading at an early growth stage of 60 days after transplanting were enhanced.

Conclusion

It could be concluded that the Prachinburi variety was the best variety compared to Phisanulok 5-4 and Phichit 4-4. Shading at different growth stages improved its growth and yield. The best shading condition was 20% shading at 30 days after planting till harvest which improved its growth and yield the most.

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