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INFLUENCE OF DIFFERENT SOIL MOISTURE REGIMES ON MORPHOLOGICAL ATTRIBUTES, YIELD AND ANDROGRAPHOLIDE CONTENT OF KALMEGH (*ANDROGRAPHIS PANICULATA* BURM F.)

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ABSTRACT

A field experiment was carried out at College of Horticulture, Gandhi KrishiVignana Kendra, Bengaluru,during rabi season of 2014 to know the effect of moisture regimes on morphological attributes, yield and andrographolide content of kalmegh (*Andrographis paniculata* Burm F.). The study consisted of seven moisture regimes based on pan evaporation, viz., watering to 100%, 90%, 80%, 70%, 60%, 50% and 40% pan evaporation. Among this 100% PE moisture regime significantly influenced the plant height (47.5 cm), plant spread in North-South (32.9 cm) and East-West direction (30.4 cm), days taken to first flowering (62.33), leaf area (276.67 sq. cm/pl), dry weight of leaves(4.9 g/pl) and dry weight of stem (9.1 g/pl). The highest dry weight of root(8.40 g), root length (20.67 cm), root volume (15.00 cc), andrographolide content in leaf (2.730% w/w) and stem (1.766% w/w) were recorded in severe moisture stress of 40% PE. Whereas, the lowest andrographolide in leaf and stem were recorded in control. The results indicated that the 100% PE moisture regime is optimum for obtaining higher herb yield coupled with higher potency of andrographolide yield required for drug industry. While, the highest moisture stress resulted in increase in andrographolide content in kalmegh.

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INTRODUCTION

Andrographis paniculata commonly known as "king of bitters" belongs to family Acanthaceae, is an annual herb widely used in tropical Asia. It is a hardy and erect plant which grows mainly as under shrub in tropical, moist deciduous forest. The leaves and aerial parts of the plant are used in Indian traditional medicine for the treatment of common cold, fever, malaria, sore throat, wounds, ulcers, leprosy, pharyngotonsillitis, diarrhoea and hypertension (Pandey and Mandal, 2010).Recently, it has been utilized as a treatment for HIV, hepatitis, diabetes, cancer and kidney disorders (Valdiani *et al.*, 2012).

The plant contains a number of diterpenoids. However, the major bitter constituent is andrographolide, which is diterpene lactone which contributes for therapeutic value of the kalmegh to cure various ailments. The leaves and stem contains 2 per cent and 0.1-0.4 per cent andrographolide respectively. Kalmegh has been existing in the list of highly traded Indian medicinal plants and also positioned as the 17th crop among the 32 prioritized medicinal plants of India with a demand of 2197.3 tons in the year 2005-2006 and annual growth of 3.1% according to the NMPB report (Kala *et al.*, 2006). The total production volume achieved from wild growing plants is about 5,000 tonnes per year, mainly from the states of Assam, Bihar, Karnataka, Kerala, Madhya Pradesh, Andhra Pradesh, Uttar Pradesh and West Bengal (Sanjay *et al.*, 2011). The heavy demand of andrographolide in India as well as international markets has motivated Indian farmers to start commercial cultivation of this medicinal plant (Zhu and Liu, 1984)

Considering its pharmaceutical values, the demand for this herb is increasing in recent time. The value of herb is dependent majorly on the andrographolide content. The production of secondary metabolites largely depends on the genotype and the environmental conditions. Water is an important element, drives various physiological processes like maintaining cell turgidity for cell functions, absorption, translocation, transpiration, photosynthesis, etc. besides multiplication plant growth and yield. Any practice which can improve the secondary metabolite production and herbage yield in kalmegh can improve the profit margin and livelihood of farmers. Creating moisture stress may improve the production of secondary metabolites. However, research studies on the influence of different levels of moisture stress on the morphology, herbage yield and secondary metabolite production are very meagure.

Hence, in light of this background, the present investigation was carried out to study the "Influence of different moisture regimes on morphological attributes, yield and andrographolide content of Kalmegh (*Andrographis paniculata* Burm. F Nees)".

MATERIALS AND METHODS

Present study was conducted at College of Horticulture, University of Horticultural

Sciences Campus, Gandhi Krishi Vignana Kendra, Bengaluru, during, rabi season of 2014. The details of the materials used and methods adopted during the course of study are detailed below.

Plant material

Kalmegh variety, Anand-kalmegh was collected from Directorate of medicinal and aromatic crops, Boriavi, Anand, Gujarath and were used for the present investigation.

The seeds were sown in nursery and seedlings of 45 days old were planted in the field at a spacing of 30 x 15 cm to evaluate morphological and biochemical parameters as influenced by different moisture regimes. The crop was grown as per the cultivation practices recommended by University of Horticultural Sciences, Bagalkot. The experiment consisted of 7 treatments *viz.*, T_1: Control (Watering to 100% pan evaporation), T_2: Watering to 90% pan evaporation, T_3: Watering to 80% pan evaporation, T_4: Watering to 70% pan evaporation, T_5: Watering to 60% pan evaporation, T_6: Watering to 50% pan evaporation, T_7: Watering to 40% pan evaporation which were replicated thrice in Randomized Complete Block Design (RCBD).

Irrigations through drip was given as per treatments based on the pan evaporation values from USWB class A open pan evaporimeter. The duration of drip irrigation was calculated using the formulae, T = IW/ Oem × Nem × 60**,** Where, T = Duration of Irrigation in minutes, IW = Irrigation water (litre) = depth of irrigation x plot area (m^2)**,** Oem = Mean output of emitter (litre hr^{-1}), Nem = Number of emitters per plot. The depth of irrigation was worked by taking the cumulative pan evaporation (CPE) for the irrigation cycle according to the treatments and irrigation were given once in two days.

Morphological parameters

Various morphological parameters were recorded *viz.*, plant height, plant spread, number of primary and secondary branches, leaf area, leaf area index (Sestak *et al.*, 1971), days to first and fifty per cent flowering, fresh and dry weight of leaf, stem and herb and leaf to stem ratio, fresh and dry weight of roots, root volume and root density of kalmegh treated with different moisture regimes.

Biochemical parameter

For assessment of biochemical variability the fully grown kalmegh plants of each treatment were tested for the active principle (andrographolide) content. The methanolic extract of the shade dried leaves and stem were analysed by HPLC to estimate its andrographolide concentration. Andrographolide content was estimated with necessary minor modifications as per the methods of Agarwal and Murali (2010). Analysis was carried out with Shimadzu, Nexera X_2 Ultra High Performance Liquid Chromatographic system with SPD-M20A Photo diode array detection combination with Lab solutions software. The Flow rate of each sample was at 0.4 ml/minutes at a detection wavelength of 223nm using Shim packs XR-ODS-III 2x150mm column with injection volume of 4μL.

Analysis of variance and interpretation of the experimental data was carried out as suggested by Panse and Sukhatme (1967). The level of significance used in 'F' P=0.05. Critical difference (CD) values were calculated at 5 per cent level, wherever 'F' test was significant.

RESULTS AND DISCUSSION

Morphological attributes in kalmegh

Among the different moisture regimes, the important growth traits like plant height, plant spread, number of branches and leaf area influenced the growth and productivity of the crop. These growth parameters were differentially influenced by the different moisture regimes, which contribute to the growth, yield and quality of the crop. The significantly maximum plant height (47.5 cm), plant spread in North-South (32.9 cm) and East-West direction(30.4 cm), number of primary branches (20.1) , secondary branches (33.3), leaf area (276.67 cm^2/pl) and leaf area index (0.61 leaf area index $plant^{-1}$) at harvest were recorded with the moisture regime of 100% PE. Growth parameters decreased with decrease in water regimes and found least with 40% PE (Table 1). Similar results were obtained by Priyanka *et al.* (2009) in kalmegh. The enhanced growth under 100 % PE might be due to better turgidity of the cells, leading to cell enlargement and better cell wall development. Such increments may be due to the increasing water supply and in turn improves root function, consequently enhance nutrient uptake and metabolic processes in kalmegh. These findings are in line with the work of (Jaleel *et al.*, 2008) and (Sonal *et al.,* 2010) in *Catharanthus roseus* and *Withania somnifera,* respectively.

Root parameters

Fresh root weight (15.70 g), dry weight of root (8.40 g), root length (20.67cm) were found to be maximum in the plants which were subjected to severe stress condition *ie.*, at 40 % PE and the lowest was recorded with 100% PE. Irrigation regime

Table 1: Growth parameters of kalmegh at harvest as influenced by different soil moisture regimes

Treatments	Plant height (cm)	Plant spread (cm) NS	EW	Number of branches Primary	Secondary	Leafarea (sq. cm/pl)	Leaf area Index	Days to first flowering	Days to fifty per cent flowering
T_1	47.5	32.9	30.4	20.1	33.3	276.67	0.61	62.33	74.00
T_2	42.0	30.4	27.9	19.3	31.6	265.00	0.59	54.67	68.33
T_3	40.3	26.2	27.1	18.2	28.1	262.00	0.58	52.33	64.33
T_4	39.7	25.6	26.9	15.2	27.3	246.33	0.55	49.33	64.33
T_5	38.1	25.0	25.6	15.1	26.9	232.67	0.52	49.33	63.00
T_6	37.9	24.4	25.2	14.2	22.0	201.00	0.45	45.33	61.00
T_7	36.6	23.9	24.0	13.8	21.5	196.33	0.44	42.33	59.67
S. Em. ±	2	1.7	1.4	0.7	1.5	8.43	0.02	2.09	1.72
CD@ 5%	6.5	5.1	4.5	2.3	4.7	25.97	0.06	6.43	5.3

*Significant at p=0.05 level; NS- North South, EW- East West

Table 2: The effect of different soil moisture regimes on growth of root in kalmegh at harvest

Treatments	Fresh weight of root(g)	Dry weight of root(g)	Root length(cm)	Root volume(cc)	Root density(g/cc)
T_1	11.67	6.20	12.00	9.00	0.69
T_2	11.80	6.73	13.27	10.33	0.65
T_3	12.93	7.47	15.53	12.00	0.62
T_4	13.57	7.47	16.97	13.00	0.57
T_5	13.67	7.93	17.40	14.00	0.57
T_6	15.27	8.13	18.03	14.33	0.57
T_7	15.70	8.40	20.67	15.00	0.56
S. Em. ±	1.11	0.72	1.27	1.07	0.10
CD@ 5%	3.42	-	3.91	3.29	-

*Significant at p = 0.05 level; NS- Non significant

Table 3: The effect of different soil moisture regimes on yield and andrographolide content of kalmegh at harvest

Treatments	Fresh weight(g/pl)		Total fresh herb yield(g/pl)	Dry weight(g/pl)		Total dry herb yield(g/pl)	Leaf to stem ratio	Andrograp holidein leaf (% w/w)	Andrograp holidein stem (% w/w)
	Leaf	Stem		Leaf	Stem				
T_1	14.3	29.8	44.1	4.9	9.1	14.0	0.59	1.065	0.961
T_2	13.0	29.8	42.8	3.8	9.0	12.8	0.59	1.318	1.102
T_3	12.6	25.5	38.1	3.7	8.5	12.3	0.58	1.393	1.164
T_4	12.1	25.3	37.3	3.5	8.3	11.8	0.57	1.690	1.405
T_5	11.7	24.6	36.3	3.0	8.0	11.0	0.56	1.892	1.484
T_6	11.0	22.1	33.1	2.7	6.8	9.5	0.52	2.287	1.765
T_7	10.2	21.7	31.9	2.6	6.5	9.1	0.47	2.730	1.766
S. Em. ±	0.8	1.1	1.6	0.2	0.3	0.4	0.05	-	-
CD @ 5%	2.4	3.3	4.9	0.7	0.9	1.3	NS	-	-

*Significant at p = 0.05 level; NS- Non significant

Note: The replicated pooled samples of leaf and stem were used for estimation of andrographolide content. Hence, could not be analyzed statistically and only mean values have been presented.

of 100 % PE recorded significantly maximum root density of (0.69 g/cc). On the contrary, the least root density of 9.00 g/cc was observed with 40% PE (Table 2). During water deficit condition in plants, the ABA signalling helps in the elongation of roots which helps plants to draw water from deeper layer. These findings are in support with the work carried out by Jaleel *et al.* (2008) in two varieties of *Catharanthus roseus* under soil water deficits and Abdolmajid *et al.* (2010) in *Papaver somniferum* L., where, in case of water deficit there was evidence of root water uptake deeper than 1.5 m.

Yield and yield attributes in Kalmegh

Among the different moisture regimes, the irrigation at 100% PE recorded better yield attributes like fresh weight of leaf(14.3 g/pl), fresh weight of stem (29.8 g/pl), dry weight of leaf (4.9 g/pl) and dry weight of stem (9.1 g/pl) at harvest. While, the least yield attributes were recorded in moisture regime of 40% PE followed by 50% PE, 60% PE (Table 3).

This reduction could be because of allocation of photosynthetic material vary with different moisture regimes in shoot. In addition, nutrient absorption in water deficit condition is very limit which ultimately ends up with the reduced yield in the severely water stressed plants. These results are in line with(Abbaszadeh *et al.*, 2008); (Ashok *et al.*, 2014); (Acharya *et al.*,2013) and (Suman *et al.*,2013)in mint, mung bean, lettuce and guava respectively.The moisture regimes with 40% PE took least number of days to first flowering (42.33 days) and fifty per cent flowering (59.67 days). In *Papaver somniferum* L., the water deficit hastened the flowering by 15 and 21 days, for irrigated and deficit treatments respectively. Abdolmajid *et al.* (2010). It is evident that drought stress reduced vegetative growth period and plant move to flowering stage. Therefore, quantity characteristics of medicinal plants decreased under drought conditions sorely (Mohamed *et al.*, 2002).

Andrographolide content

The active principle in kalmegh is diterpenoid lactone andrographolide and was found to be influenced by moisture regimes. The highest leaf(2.730%) and stem(1.766%) andrographolide content was recorded in moisture regime of 40% PE and the lowest was recorded in 100 % PE (1.065% and 0.961) in leaf and stem, respectively (Table 3).

Drought stress increases the essential oil percentage of medicinal and aromatic plants, because in case of stress, more metabolites are produced in the plants and these substances prevent oxidization of the cells, but essential oil content reduced under drought stress, because the interaction between the amount of the essential oil percentage and shoot yield is considered to be two important components of the essential oil content and by exerting stress, increases the essential oil percentage but shoot yield decreases by the drought stress, therefore essential oil content reduces (Aliabadi *et al.*, 2009). These findings are in support with the work carried out by (Baher *et al.*, 2002); Rajeswara (2002); Delfine *et al.* (2005); in Iranian *Satureja hortensis* L, rose-scented geranium,rosemary and spearmint plants, respectively.

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