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J Cheena
Medicinal and Aromatic Plant Research Station, Rajendranagar, Hyderabad, Sri Konda Laxman Telangana State Horticultural University, Mulugu (V & M), Siddipet, Telangana, India

V Krishna Veni
Medicinal and Aromatic Plant Research Station, Rajendranagar, Hyderabad, Sri Konda Laxman Telangana State Horticultural University, Mulugu (V & M), Siddipet, Telangana, India

M Sreenivas
College of Horticulture, Mojerla, Wanaparthy, Sri Konda Laxman Telangana State Horticultural University, Mulugu (V & M), Siddipet, Telangana, India

Corresponding Author:
J Cheena
Medicinal and Aromatic Plant Research Station, Rajendranagar, Hyderabad, Sri Konda Laxman Telangana State Horticultural University, Mulugu (V & M), Siddipet, Telangana, India

Effect of nitrogen and sulphur on growth, yield and essential oil of clove basil (*Ocimum grattissimum* L.)

J Cheena, V Krishna Veni and M Sreenivas

Abstract
An investigation was carried out at Medicinal and Aromatic Plant Research Station Rajendranagar, Sri Konda Laxman Telangana State Horticultural University, Mulugu (V & M), Siddipet Dist., Telangana State during the period of 2017-20 to determine the effect of Nitrogen and sulphur on growth, yield and essential oil of clove basil. The study was conducted with the application of different levels of Nitrogen i.e, N_1- 100 kg/ha., N_2- 150 kg/ha., N_3- 200 kg/ha and different levels of Sulphur i.e, S_1- 60 kg/ha., S_2-120 kg/ha., S_3- 180 kg/ha. All the parameters interms of plant height, no. of primary branches, fresh herbage yield, oil yield were significantly differed with respect to the different dosage of Nitrogen and sulphur. The maximum plant height (148.94cm) was found in N_3S_1, no. of primary branches (26.05), fresh herbage yield (34.61t/ha), dry herbage yield (12.08 t/ha) were found in N_2S_3 and oil yield (7.10 ml/kg) was found in N_2S_2 while the minimum plant height and oil yield was found in N_1S_2 and no. of primary branches, fresh herbage yield, dry herbage yield were found in N_3S_1.

Keywords: Clove basil, nitrogen, sulphur, essential oil, yield

Introduction
Ocimum gratissimum L. belongs to the Lamiaceae family and is commonly found in Africa, Asia, and South America. The *Ocimum* genus contains approximately 30 known species that are distributed across the warmest regions of the planet. This plant genus produces an essential oil that is valued by the perfumery, cosmetic, pharmaceutical, and food industries (Pandey *et al*., 2014) [12]. *O. gratissimum* is popularly known as clove basil, African basil, and in Hawaii as wild basil. It is characterized by a woody stem at the base and reaching up to 3 m in height. It has ovate leaves ranging from 5 to 13 cm in length and 3 to 9 cm in width, with petioles 1 to 6 cm in length. Its flowers are arranged in simple or branched bunches, ranging from 5 to 30 cm in length (Matasyoh *et al*., 2007) [11]. The leaves are used in medicine for treatment of rheumatism, paralysis, epilepsy and mental illness. Besides, the presence of active compounds also characterizes it as a natural insecticide, nematicide, fungicide and antimicrobial (Effraim *et al*., 2001) [6]. A good crop nitrogen nutritional status enhances crop tolerance to drought, and a moderate increase in nitrogen supply improves water use efficiency in semi-arid environments (Cossani *et. al*, 2012) [3]. Biomass production is a function of the relationship between nitrogen and water availability and this relationship has been described it as a co-limitation (Sadras, 2004) [14]. Nitrogen plays an important role in various physiological processes. Furthermore, application of nitrogen fertilizer increased the uptake and accumulation of other nutrients such as phosphorus and potassium (Baranauskiene *et. al*, 2003) [2]. The increase of linalool and engenol concentrations by the application of Sulphur treatments is considered important as these substances are widely used by agrifood, pharmaceutical and cosmetic industries (Marotti *et al*., 1996) [10]. The degree of plant supply with nitrogen is a major factor regarding yielding and affects the quantity and composition of volatile oils (Daneshian *et. al*, 2009) [5]. The essential oil of clove-basil can be classified in three different chemical groups: eugenol, thymol and geraniol. The eugenol chemotype is the most important economically, with a strong and aromatic perfume with reminiscences of clove, it is distilled in Brazil, Tahiti and Indonesia. Eugenol also attributes to clove-basil antiseptic and local anesthetic actions (Craveiro, 1981) [4], wherefore it is used as dental anesthetic, as a substitute to clove oil, vanillin synthesis and as insect attractive (Farmacopéia, 1959; Merck, 1996) [7].

Material and Methods
The experiment entitled "Effect of Nitrogen and sulphur on growth, yield and essential oil of Clove basil (*Ocimum grattissimum L.*)" was carried out for three years (2017-2020) at Medicinal and Aromatic Plant Research Station Rajendranagar, SKLTSHU, Hyderabad.

The meteorological data were collected from the Agricultural Research Institute, Rajendranagar for the experimental period. It was normal weather data on total rainfall, maximum and minimum temperature, relative humidity, that prevailed during the period of experimentation.
The land used under the experimental layout was red with good drainage and low water holding capacity with uniform texture. To grow necessary seedling for the experiment, seeds were sown in June, 30 days old seedlings of 10cm height were transplanted in the field. The experiment was designed in Factorial Randomised Block Design with three replications with the spacing of 50*45cm. In the experiment, different levels of Nitrogen i.e, 100kg/ha, 150kg/ha, 200kg/ha, different levels of Sulphur 60kg/ha, 120kg/ha, 180kg/ha was applied. The following agronomic practices of clove basil were recorded as follows,

Plant height (cm): The plant height was recorded 90 days after sowing, in each plot taking 5 plants into consideration.

No. of primary branches: The number of primary branches were recorded 90 days after sowing, in each plot taking 5 plants into consideration.

Fresh herbage yield (t/ha): In each plot, the plants were harvested with sickle 10cm above the ground level and immediately weighted for obtaining plot yield. Then plot yield was transferred to yield per hectare.

Dry herbage yield (t/ha): In each plot, the plants were harvested with sickle 10cm above the ground level and placed the plants in sun till it dried completely, the plot yield was transferred into yield per hectare.

Oil yield (ml/kg herbage): The essential oil extraction was done at laboratory of Medicinal and Aromatic Plant Research Station, Rajendranagar. Fresh leaves of 500g were taken from each treatment and subjected to steam distillation for 3 hours in Heating mantle apparatus. The content was calculated using the formula: Content = v (ml) x 100 m-1(g), where v is the volume obtained in milliliters, and m is the dry weight in gram.

Results and Discussion

Plant height (cm)

A significant difference was observed on plant height due to application of different dosage of Nitrogen. The maximum plant height (146.23cm) was recorded with Nitrogen of 200 kg/ha while the minimum plant height (117.42cm) was recorded with Nitrogen of 100 kg/ha. Plant height was found significant due to application of different dosage of sulphur. The maximum plant height (134.04cm) was recorded with Sulphur of 60kg/ha while the minimum plant height (132.73cm) was recorded with Sulphur of 120 kg/ha. The interaction effect of Nitrogen and Sulphur showed significant variation on plant height (Table 1). The highest plant height (148.94cm) was recorded with Nitrogen of 200 kg/ha and Sulphur of 60kg/ha (N3S1) which was on par (148.56) with Nitrogen of 200 kg/ha and Sulphur of 120kg/ha (N3S2) while the minimum plant height (113.4cm) was obtained with the application of Nitrogen of 100 kg/ha and Sulphur of 120kg/ha (N1S2).

No. of primary branches

A significant difference was observed on number of primary branches due to application of different dosage of Nitrogen. The maximum number of primary branches (25.23) was recorded with Nitrogen of 150 kg/ha and the minimum number of primary branches (21.25) was recorded with Nitrogen of 200 kg/ha. Number of primary branches was found significant due to application of different dosage of sulphur. The maximum number of primary branches (23.48) was recorded with Sulphur of 180kg/ha while the minimum number of primary branches (23.23) was recorded with Sulphur of 60 kg/ha.
The interaction effect of Nitrogen and Sulphur showed significant variation on number of primary branches (Table 1). The highest number of primary branches (26.05) was recorded with Nitrogen of 150 kg/ha and Sulphur of 180 kg/ha (N2S3) which was on par (25.51) with Nitrogen of 150 kg/ha and Sulphur of 60kg/ha (N2S1) while the minimum number of primary branches (20.24) was obtained from Nitrogen of 200 kg/ha and Sulphur of 60kg/ha (N3S1).
The number of branchings significantly depended upon the dose of applied nitrogen. The basil plants had different numbers of branchings (5.2–45.5 pcs per plant) that coincides with Kandil *et al*. 2009 [9], Said- Al Ahl and Mahmoud 2010 [15], which was related to their height and habit. The applied nitrogen doses differently stimulated basil branching, and the greatest number of branchings (12.8 pcs per plant) was reported at medium and the highest nitrogen doses. The increased amount of NPK causes the increased number of basil branching (Kandil *et al.* 2009) [9].

Fresh herbage yield (t/ha)

A significant difference was observed on fresh herbage yield due to application of different dosage of Nitrogen. The maximum fresh herbage yield (32.29 t/ha) was received with Nitrogen of 150 kg/ha while the minimum fresh herbage yield (23.09t/ha) was received with Nitrogen of 200 kg/ha. Fresh herbage yield was found significant due to application of different dosage of sulphur. The maximum fresh herbage yield (27.66 t/ha) was received with Sulphur of 180kg/ha while the minimum fresh herbage yield (25.50 t/ha) was recorded with Sulphur of 60 kg/ha.
The interaction effect of Nitrogen and Sulphur showed significant variation on fresh herbage yield (Table 2). The highest fresh herbage yield (34.61t/ha) was recorded with Nitrogen of 150 kg/ha and Sulphur of 180 kg/ha (N2S3) which was on par (32.31t/ha) with Nitrogen of 150 kg/ha and Sulphur of 60kg/ha (N2S1) while the minimum fresh herbage yield (21.41t/ha) was obtained from Nitrogen of 200 kg/ha and Sulphur of 60kg/ha (N3S1).
The nitrogen dosage of 150 kg/ha recorded highest fresh herbage yield which maybe due to more vegetative growth. This result is in accordance with Arabaci and Bayram, 2004 [1]. On the other hand, however, the increased level of NPK fertilization significantly increases the biomass of basil (Kandil *et al.* 2009, Sharafzadeh *et al.* 2011) [9, 17], like the increased nitrogen dose stimulates fresh weight of basil overground parts (Golcz *et al.* 2006, Sifola and Barbieri 2006) [8, 16].

Dry herbage yield (t/ha)

Dry herbage yield was influenced by the application of different dosage of Nitrogen. The maximum dry herbage yield (10.62 t/ha) was received with Nitrogen of 150 kg/ha and the minimum dry herbage yield (7.78t/ha) was recorded with Nitrogen of 200 kg/ha. Dry herbage yield was not significantly influenced the application of different dosage of

sulphur. The maximum dry herbage yield (9.37t/ha) was recorded with Sulphur of 180kg/ha while the minimum dry herbage yield (8.08t/ha) was recorded with Sulphur of 60 kg/ha.
The interaction effect of Nitrogen and Sulphur application has shown the variation on dry herbage yield (Table 2). The highest dry herbage yield (12.08 t/ha) was recorded with Nitrogen of 150 kg/ha and Sulphur of 180 kg/ha (N2S3) while the minimum dry herbage yield (6.61t/ha) was obtained from Nitrogen of 200 kg/ha and Sulphur of 60kg/ha (N3S1).

Oil yield (ml/kg herbage)

A significant difference was observed on oil yield due to application of different dosage of Nitrogen. The maximum oil yield (6.68 ml/kg) was recorded with Nitrogen of 150 kg/ha while the minimum oil yield (5.69 ml/kg) was recorded with Nitrogen of 200 kg/ha.
Fresh herbage yield was found significant due to application of different dosage of sulphur. The maximum oil yield (6.34 ml/kg) was recorded with Sulphur of 120kg/ha while the minimum oil yield (5.99 ml/kg) was recorded with Sulphur of 60 kg/ha. The interaction effect of Nitrogen and Sulphur showed significant variation on oil yield (Table 2).
The highest oil yield (7.10 ml/kg) was recorded with Nitrogen of 150 kg/ha and Sulphur of 120 kg/ha (N2S2) which was on par (6.80 ml/kg) with Nitrogen of 150 kg/ha and Sulphur of 180kg/ha (N2S1) while the minimum oil yield (5.35 ml/kg) was obtained from Nitrogen of 100 kg/ha and Sulphur of 120kg/ha (N1S2). The essential oil yield was in accordance with application of nitrogen interms of green herbage yield i.e, 150 kg/ha level of nitrogen. Generally, the essential oil yield depends on different environmental conditions also.
Sulphur has a broad spectrum of action that improves the general appearance of the plant, strengthens its natural defense, increases resistance to nutritional and climatic stresses and pest attack, and also influences positively other important aspects for essential oil production and development of shoots (Rezende, 2003) [13].

Conclusion

From this investigation it can be concluded that the highest oil yield (7.10 ml/kg) was recorded in the treatment combination of Nitrogen of 150 kg/ha and Sulphur of 120 kg/ha (N_2S_2). The application of high dosage of Nitrogen and Sulphur did not influence the herbage and oil yield hence, the moderate application of Nitrogen i.e,150 kg/ha and Sulphur i.e, 120 kg/ha is suitable to get the better yield in clove basil.

Table 1: Standardization of Nitrogen and sulphur on plant height and no. of branches in Clove basil (*Ocimum gratissimum L.*)

	Plant height (cm)				**No. of primary branches**			
	S1	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**
N1	118.20	113.4	120.68	117.42	23.96	24.13	23.05	23.71
N2	134.99	136.24	138.14	136.45	25.51	24.13	26.05	25.23
N3	148.94	148.56	141.19	146.23	20.24	22.15	21.36	21.25
Mean	134.04	132.73	133.33	133.37	23.23	23.47	23.48	23.39
Factors	SE(M)+-		C.D at 5%		SE(M)+-		C.D at 5%	
N	8.39		4.50		1.42		1.40	
S	8.21		4.62		1.39		1.41	
N*S	8.36		4.45		1.38		1.36	

Table 2: Standardization of Nitrogen and sulphur on fresh herbage yield, dry herbage yield and oil yield in Clove basil (*Ocimum gratissimum L.*)

	Fresh herbage yield (t/ha)				**Dry herbage yield (t/ha)**				**Oil yield (ml/kg herbage)**			
	S1	S2	S3	Mean	S1	S2	S3	Mean	S1	S2	S3	Mean
N1	22.78	22.64	25.37	23.59	6.61	8.84	8.20	7.88	5.92	5.35	5.80	5.69
N2	32.31	29.97	34.61	32.29	10.51	9.28	12.08	10.62	6.15	7.10	6.80	6.68
N3	21.41	24.88	23.00	23.09	7.12	8.39	7.85	7.78	5.90	6.59	6.09	6.19
Mean	25.50	25.83	27.66	26.33	8.08	8.83	9.37	8.76	5.99	6.34	6.23	6.18
Factors	SE(M)+-		C.D at 5%		SE(M)+-		C.D at 5%		SE(M)+-		C.D at 5%	
N	1.80		4.90		0.40		NS		0.18		0.50	
S	1.75		4.85		0.45		NS		0.21		0.21	
N*S	1.69		4.62		0.51		NS		0.19		0.49	

Factor I : Levels of Nitrogen N1- 100 kg/ha., N2- 150 kg/ha., N3- 200 kg/ha.
Factor II : Levels of Sulphur S1- 60 kg/ha., S2- 120 kg/ha., S3- 180 kg/ha.

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