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Effect of Bokashi on plant growth, yield and essential oil quantity and quality in Patchouli (*Pogostemon cablin* Benth.)

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ABSTRACT

The plant growth parameters of Patchouli (*Pogostemon cablin* Benth.) were significantly influenced by Bokashi treatment with maximum height, number of branches, plant spread, leaf size and leaf area index as 72.4 cm, 32.8 cm, 7818.63 cm², 64.88 cm² and 5.06 respectively. Application of Bokashi treatment provided the highest dry herbage yield of 4.67 t/ha against 3.96 t/ha in control by one time harvest. The essential oil content and oil yield per hector were also significantly influenced by the Bokashi treatment and it recorded 2.35 % (w/w) oil recovery when distilled after 30 days of curing. Essential oils from both the treatments of Patchouli were analyzed by GC-MS. Patchouli alcohol content both in Bokashi treatment and control are 45.330% and 44.669% respectively. Other major components are also higher in Bokashi treatment than control.

Key words: *Pogostemon cablin*, Bokashi, Herbage yield, Patchouli alcohol.

INTRODUCTION

Pogostemon cablin Benth. belongs to the family Lamiaceae and commonly known as Patchouli. It is native to subtropical Himalayas, South-East Asia and the Far East, and has been cultivated extensively in Indonesia, Malaysia, China and Brazil for the essential oil namely "patchouli oil". The essential oil contains various sesquterpenes and hydrocarbons such as; patchouli alcohol (patchoulol), patchoulene, bulnesene, guaiene, caryophyllene, elemene and copaene (Lawrence,1981; Hasegawa and Sugimura, 1992). This oil has been used in food industry as flavour and as fixative in perfumery raw material. It also has therapeutic properties, namely antidepressant, antiseptic, astringent, anti-inflammatory, fungicide, insecticide, sedative and tonic (Bunrathep *et. al.*, 2006).

The modern and intensive agriculture methods are not only costly, but also cause environmental pollution. Thus, by considering the recent concept of eco-friendly technology, application of Bokashi substitutes the above need in many crops. Bokashi is a fermented biomass. It acts as a store house for EM (effective microorganism). It gives sustained nutrition supply from organic matters and EM and produces growth regulator. Unlike traditional compost, where a lot of energy loss takes place due to heat generation, Bokashi production is able to conserve this energy.

Effective Microorganisms (EM) originated in Japan in the 1980s. Its originator or developer was Prof Teruo Higa. As the name suggests, it makes use of microorganisms, mainly lactic acid bacteria, photosynthetic bacteria, yeast, filamentous fungi and ray fungi. These microorganisms are both aerobic and anaerobic and are not genetically-modified. Effective Microorganisms, also called EM Technology, is a brand name for a series of products using a base culture called "EM•1 Microbial Inoculant". EM Technology is supposed to maintain sustainable practices such as farming and sustainable living, and also claims to support human health and hygiene, animal husbandry, compost and waste management, disaster clean-up and

generally used to promote functions in natural communities (Higa, Teruo; *et. al*., 1994.

Thus, keeping the above facts in view, the present study was undertaken to study effect of Bokashi as supplement of nutrient sources on growth, yield and essential oil content in patchouli

MATERIAL AND METHODS

A field experiment was conducted during 2008-09 at NEDFi R & D Centre, Khetri, Kamrup, Assam. Geographical situation is – Altitude: 169.7 ft, Latitude: N 26º06'957" and Longitude: E 092º05'522". The soil of the experiment field was acidic (pH 5.37), sandy clay loam in texture having 3.23% organic matter and available N,P & K were 1065.05, 18.85 and 112.80 kg/ha respectively. The experiment was conducted in RBD with three replications. The treatment was carried out as –
T1 = Bokashi Treatment (B.T.) + FYM (10t/ha).
T2 = Control + FYM (10t/ha).

Bokashi Preparation

S. No.	Components	Amount
1.	Rice Bran (Power)	2.5 kg
2.	Rice Husk	1 kg
3.	Molasses	400 ml
4.	EM solution	200 ml
5.	Curd	200 ml
6.	Mustard oil cake (powder)	500 gm
7.	Water	8 litre

Above mixture kept in air tight container for 15 days. Bokashi applied to the field after 15 days at the rate 200 gm/ 1 m² (20 q / ha).
EM solution produced by Maple Orgtech Ltd., Kolkata, India was taken.

45 days old seedlings were transplanted in the plot of 12m X 2m on 18th Dec, 2008. The well rotten FYM was applied at the time of field preparations in both the treatments and irrigation and intercultural operations were given as and when required. Single harvest was done after 160 days of planting. Observations were recorded on growth, yield and quantity attributes. Leaves along with the stem were dried under shade for 7 days and essential oil was extracted after 30 days of curing, using Clevenger's apparatus to estimate the oil recovery percentage and to calculate the oil yield. The oils were dried over anhydrous sodium sulphate and GC-MS analysis was done.

GC-MS Analysis

The essential oil was analyzed by a Network GC-MS system of Agilent GC/MS-6890 gas chromatograph equipped with a FID, using DB-5 MS / 60 m column. The oven temperature was programmed 70°C to 300°C at 2.0°C/min, injector temperature 250°C, detector temperature 280°C, carrier gas helium. Peak identification was based on computer matching of mass spectra libraries and retention indices. The compound identification was confirmed by comparison of their relative retention indices (Davies, 1990) with literature values.

RESULTS AND DISCUSSION

Growth parameters

Application of Bokashi influenced the morphological characters of plants resulting in the improved growth parameters at 160 days after planting (DAP) (Table-1).Plant was significantly influenced by Bokashi Treatment with maximum height of 72.4 cm against 65.28cm in control. The number of branches per plant was maximum with the application of Bokashi (32.8) as compared to the control (21.75).Plant spread (7818.63 cm²), leaf size (64.88 cm²) and leaf area index (5.06) were maximum in the Bokashi treatment , where as in control these parameters were 5969.01 cm² , 58.09 cm² and 4.5 respectively. After 90 DAP, it was observed that Bokashi also controlled the flowering in Patchouli. The percentage of flowering was found to be less in Bokashi treatment (4.46 %) and highest in control (9.82 %). In patchouli, flowers have not any fertile activity and on the other hand suppressed the vegetative growth. Root to shoot ratio in Bokashi treatment was found to be 1: 9.79 and in control 1:11.86 (Table-2). By Bokashi treatment the root shoot ratio was observed less then the control, its mean that the root distribution or system is good as compared to the control. Enhanced growth parameters like plant height, number of branches, plant spread, leaf size and leaf area index due to the application of Bokashi might be due to increased availability of nutrients to the plants.

Table 1: Plant growth Data

Treatments	Plant Height (cm)	No. of Branches (per plant)	Plant spread (cm^2)	Leaf size (L×B) cm^2	Leaf Area Index	Flowering (%) After 90 days
B.T.	72.4	32.8	7818.63	64.88	5.06	4.46
Control	65.28	21.75	5969.01	58.09	4.5	9.82
SEm±	1.67	2.47	278.08	1.54	0.15	1.09
CV%	5.96	19.21	13.44	6.15	7.66	37.5

Mean value of three replicated plot.

Table 2: Root to shoot ratio per plant

Treatments	Dry weight of root (gm)	Dry weight of Shoot (gm)	Root shoot ratio
B.T.	24.65	241.419	1 : 9.79
Control	12.561	148.96	1 : 11.86

Mean value of three replicates

Table 3: Herbage yield: - (single harvest)

Treatments	Fresh Yield (t/ha)	Dry Yield (t/ha)	Fresh & dry yield ratio
B.T.	27.07	4.67	5.79 : 1
Control	21.44	3.96	5.41 : 1
SEm±	1.27	0.21	
CV%	12.84	12.14	

Mean value of three replicated plot

Table 4: Essential oil content and oil yield: Oil was extracted using Clevenger's apparatus after 30 days of curing

Treatments	Essential oil content (%) (w/w)	Essential oil yield (kg/ ha)
B.T.	2.35	109.78
Control	2.16	85.83
SEm±	0.06	2.42
CV%	6.16	13.58

Mean value of three replicates

Yield parameters

The Bokashi treatment recorded significantly higher cumulative fresh herb yield of 27.07t/ha and dry herb yield of 4.67t/ha, where as control recorded lower fresh herb yield of 21.44 t/ ha and dry herb yield of 3.96 t/ha (Table-3). In that way, the essential oil content was significantly higher (2.35%) in Bokashi treatment where as in control recorded oil content of 2.16% (Table-4).

GC-MS analysis

Quality attributes were also significantly influenced by the Bokashi treatment (Table-5). The presence of patchouli alcohol and alpha bulnesene of the first and second major components (that indicate quality oil) with other components are reported. It is observed from the data that the major components are varying in their composition. The major components in sample1 (B.T.) are patchouli

Table 5:GC/MS analysis report showing- chemical composition (in %) of the essential oil of *Pogostemon cablin*

S. No.	Ingredients	Area (%) Sample 1 (B.T.)	Area (%) Sample 2 (control)
1.	Alpha Pinene	0.029	0.035
2.	Beta Pinene	0.072	0.083
3.	Limonene	0.018	0.018
4.	Delta Elemene	0.166	0.168
5.	Eugenol	0.023	-
6.	Beta Patchoulene	1.338	1.370
7.	Beta Elemene	1.478	1.680
8.	Caryophyllene	3.120	3.000
9.	Alpha Guaiene	10.31	10.26
10.	Seychellene	4.111	3.880
11.	Alpha Humulene	-	1.057
12.	Alpha Patchoulene	5.027	4.260
13.	Gamma Patchoulene	-	0.942
14.	Allo Aromadendrene	.0833	-
15.	Beta Selinene	0.405	0.356
16.	Neoisolongifolene	-	0.074
17.	Germacrene D	0.058	0.058
18.	Alpha Selinene	0.385	0.398
19.	Aciphyllene	1.425	-
20.	Alpha Bulnesene	15.95	15.74
21.	Delta Cadinene	0.034	-
22.	Alpha Panasinsen	0.211	-
23.	Delta Cadinene	0.032	-
24.	Elemol	0.094	0.108
25.	Nerolidol	0.055	-
26.	Norpatchoulenol	1.144	1.195
27.	Caryophyllene Oxide	0.926	1.100
28.	Spathulenol	0.244	-
29.	Isospathulenol	0.305	0.403
30.	Patchouli Alcohol	45.33	44.669
31.	Farnesol	0.462	0.592
32.	Phytol	0.033	0.036
33.	Others	7.14	8.52

alcohol (45.33%), alpha bulnesene (15.95%), alpha guaiene (10.31%), alpha patchoulene (5.027%) and caryophyllene (3.120%). The percentage of major components in sample2 (control) are patchouli alcohol (44.669), alpha bulnesene (15.74), alpha guaiene (10.26), alpha patchoulene (5.027) and caryophyllene (3.000).

By the application of Bokashi it was possible to get an increased fresh herb yield as compared to control. This might be due to enhanced ability of nutrients and synthesis of some growth regulators which in turn might have contributed to the better growth, yield and quality. In the present study the higher oil yield may be attributed to maximum herb yield (Sirohi and Singh, 1983 and Maheshwari *et. al*., 1991).

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REFERENCES

1. Ahmed, M., Deka, P.and Gogoi, P., *Commercialization of patchouli*,NEDFi, Guwahati, 1st Edition, pp- 16-29 (2002).
2. Bunrathep, S., Lockwood, G.B., Songsak, T. and Ruangrungsi, N., Chemical constitute from leaves and cell cultures of *Pogostemon cablin* and use of precursor feeding to improve Patchouli alcohol level. *Science Asia*, **32:** 293-296 (2006).
3. Davies, N.W., *Journal Chromatography*, **503**: 1-24 (1990).
4. Hasegawa, Y., Tajima, K., & Sugimura, Y., An additional constituent occurring in the oil from a patchouli cultivar. *Flavour Frag. J.*, 7: 333-335 (1992).
5. Higa,T., and Parr, J. , "Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment." (PDF). *Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment.*: 7, Atami, Japan: International Nature Farming Research Center. Retrieved on 21 January 2008 (1994).
6. Lawrence, B. M., Progress in essential oils. *Perfumer and Flavorist* , **6**: 73-76(1981).
7. Maheshwari, S.K., Gangrade, S.K. and Trivedi, K.C., Comparative responses of palmarosa to *Azobacter* and nitrogen under rained and irrigated shade. *Indian Perfume,* **35**(2): 198-211(1991).
8. Sirohi, S.S. and Singh, O.S., Relationship of Endomycorrhizal association in unsterilised soil with available soils phosphorus, plant growth, phosphorus uptake and synthesis in peppermint. *Science Hort.*, **20**: 185-191(1983).