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### Influence of PGPM and INM on essential oil content and its constituents of black turmeric (*Curcuma caesia* Roxb.)

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#### Abstract

Black turmeric (*Curcuma caesia* Roxb.) is a perennial herb belongs to Zingiberaceae family, usually found in N-E and Central India. It is an endangered species which includes many medicinal properties which possess several uses in pharmaceutical and cosmetic industry. The studies on agronomical requirements of the crop are scarce. Hence the present study on "Influence of PGPM and INM on yield and economics in black turmeric" was carried out at College of Horticulture, Bengaluru during the year 2021-22. The study includes 14 treatments comprises different combination PGPMs, FYM and chemical fertilizers with two replications. The maximum essential oil content (0.51%) was registered with the application of EMC3- AM fungi- *Glomus* spp (1 g plant<sup>-1</sup>), *Azospirillum, P. fluorescens, Trichoderma,* PSB and KMB @ 10 ml L<sup>-1</sup> each + FYM (10 t ha<sup>-1</sup>) + 125% of RDF. Further, the treatments consist both organic and inorganic fertilizers coupled with bio fertilizers proved best for enhanced secondary metabolites. In that, T<sub>10</sub> recorded maximum camphene (3.35%) content in the essential oil extracted from fresh rhizomes.

Keywords: Black turmeric, INM, PGPMs and EMC

#### 1. Introduction

Black turmeric (*Curcuma caesia* Roxb.) is a perennial herb belongs to Zingiberaceae family., usually found in N-E and Central India (Zaman *et al.*, 2013) <sup>[17]</sup>. It is an endangered species which includes many medicinal properties which possess several uses in pharmaceutical as well as cosmetic industry, is economically vital the growers (Mukunthan *et al.*, 2014) <sup>[7]</sup>. Rhizomes are bluish-black in colour which are bitter in taste and possess pungent smell and is largely exploiting in treatment of cancer, leprosy, haemorrhoids, asthma, fever, epilepsy, wound, vomiting, menstrual disorder, inflammation, skin diseases etc. (Zaman *et al.*, 2013) <sup>[17]</sup>. The oil of commerce contains about 30 constituents, representing 97.48 percent of the oil, major being camphor (28.3%), ocimene (8.2%), ar-turmerone (12.3%), 1, 8-cineole (5.3%), elemene (4.8%), bornylacetate (3.3%), borneol (4.4%), curcumene (2.82%) and ar- curcumene (6.8%) as the main constituents. (Kumar and Dewangan, 2014) <sup>[5]</sup>.

Black turmeric plants have short stem with large oblong leaves. It produces ovate pyriform or cylindrical or oblong rhizomes, which are often branched further having brownish yellow in colour exocarp (Swami *et al.*, 2021)<sup>[15]</sup>. The plant is native to India and South-East Asia and is being under cultivation in Ceylon, Belgium, Indonesia and India. In India its cultivation is confined to a small extent in West Bengal, Orissa, Madhya Pradesh, Uttar Pradesh, Chhattisgarh along with North Eastern Hilly Himalayan states (Nadkarni, 1976)<sup>[8]</sup>. It grows well in moist deciduous forest areas. It is flourishes in rich humid and clayey soils (Sahu *et al.*, 2016)<sup>[13]</sup>.

The combined application of organic and inorganic fertilizers known as "Integrated Nutrient Management" (INM) not only enhances the yield but also ensures the physical, chemical and biological property of soil which further add-on fertility, water holding capacity in addition to productivity of soil. The organic manures will aid to sustain nutrient equilibrium in soils while, the inorganic fertilizers readily furnish nutrient which might increase the initial growth in the crop eventually results in good growth, development and yield. Continuous use of inorganic fertilizers has emanated in ecological imbalance with consequent adverse effect to the soil. Moreover, in recent days, bio-fertilizers have come out as promising component of plant nutrient supply system. The micro-organisms contribute much towards enhancing the fertility

status of the soil besides augmenting yield as reported by Ray *et al.*  $(2000)^{[10]}$ .

#### 2. Material and Method

#### 2.1 Experimental details

The study was conducted in college of Horticulture, Bengaluru-65 in the year 2021-22. The area was ploughed well and divided into 28 raised beds with the size of 8.25m<sup>2</sup>. The design used for the study was randomized complete block design with 14 treatments along with 2 replications.

Healthy well matured seed rhizomes of about 20-40 g are treated with Bavistin (3g/ L of water) and Chloropyriphos (2 ml L<sup>-1</sup> of water) for 1 hour and shade dried the day before planting. The planting was done in the *karif* season by giving respective treatments according to the treatment details and spacing maintained was 45 cm X 30 cm.

The treatment details include, T<sub>1</sub> - EMC1- AM fungi- Glomus spp (1 g plant<sup>-1</sup>), Azospirillum, Pseudomonas fluorescens, PSB (Bacillus megaterium) and KMB (Frateuria aurantia) @ 10 ml L<sup>-1</sup> each + FYM @ 10 t ha<sup>-1</sup>, T<sub>2</sub> - EMC2- AM fungi-Glomus spp (1 g plant<sup>-1</sup>), Azospirillum, Trihcoderma, PSB and KMB @ 10 ml  $L^{-1}$  each + FYM (10 t ha<sup>-1</sup>), T<sub>3</sub> - EMC3- AM fungi- Glomus spp (1 g plant<sup>-1</sup>), Azospirillum, P. fluorescens, Trichoderma, PSB and KMB @ 10 ml L<sup>-1</sup> each + FYM (10 t ha<sup>-1</sup>), T<sub>4</sub> - RDF as per turmeric POP + (FYM @ 10 t ha<sup>-1</sup>), T<sub>5</sub> - $T_1$  + 100 percent RDF,  $T_6$  -  $T_2$  + 100 percent RDF,  $T_7$  - $T_3$  + 100 percent RDF,  $T_8$  - $T_1$  + 75 percent RDF,  $T_9$  -  $T_2$  + 75 percent RDF, T<sub>10</sub> - T<sub>3</sub> + 75 percent RDF, T<sub>11</sub> - T<sub>3</sub> + 125 percent RDF,  $T_{12}$   $T_3$  + 150 percent RDF,  $T_{13}$ -  $T_4$  + Trichokavach (T. asperellum, P. fluorescens, Paecilomyces *lilacinus* and 2% chitosan- soil application @ 75 g plot<sup>-1</sup> for 2 times),  $T_{14}$  - FYM @ 10 t ha<sup>-1</sup> (control).

Incorpoation of FYM at the rate of 10 tonnes per hectare is common for all the treatment while preparing the beds. *Glomus spp* (1 g plant<sup>-1</sup>) and trichokavach (at planting and 120 DAP at 75 g plot<sup>-1</sup>) as soil application and all other liquid bio fertilizers were used to treat the rhizomes for 1 hour before planting at the concentration of 10 ml per L of water and RDF (NPK @ 150:125: 150 kg ha<sup>-1</sup> as Urea, SSP and MOP) was practiced as per the treatment details in 4 splits at 30, 60, 90 and 120 DAP. Turmeric special (1kg 300 L<sup>-1</sup>) was sprayed as common application for all the treatments at 120 DAP.

The first irrigation was given immediately after planting and whenever there was no occurrence of rain, the crop was irrigated once in 3 days. Weeding operation carried out through hand weeding once in a month up to the crop covers the ground area completely, totally 4 weedings and 2 earthing ups at 30 and 150 DAP were done. The crop comes to harvesting in the month of December.

#### 2.2 Estimation of essential oil

The cleaned and washed rhizomes after harvesting taken from five tagged plants were sliced and used for oil extraction through hydro-distillation for 6 hours (Singh *et al.*, 2020)<sup>[14]</sup>.

#### 2.3 Analysis of volatile compounds by GC-MS method

The GC-MS analysis of hydro distilled essential oil was subjected to GC-MS analysis at bioenergy research and quality assurance laboratory University of Agricultural Sciences, Gandhi Krishi Vigyana Kendra, Bengaluru. Gas chromatography mass spectroscopy is an analytical method that combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. Gas chromatography has a mobile phase and a stationary phase. The mobile phase is helium and the stationary phase is the column. When a sample is injected then it is carried by the mobile phase across the stationary phase and based on the mobility of different hydrocarbons in the stationary phase, they get separated and are detected at different time intervals by Flame Ionization Detector.

The sample was analysed using Shimadzu QP2020 series gaschromatograph using, SH-Rtx Wax 30 m  $\times$  0.50 µm  $\times$  0.25 µm diameter column. Helium was used as the carrier gas at a flow rate of 1.5 ml per minute at constant pressure. Injection volume was 1 µL and a spilt ratio of 1: 100 was used. The pressure was maintained at 35.6 kPa. Detection was done with a flame ionization detector at 240 °C. The oven program was as follows, set point 50 °C was held for one minutes and further increased to 220 °C at the rate of 10 °C per minute and finally held at 240 °C for 3 minutes at 5 °C per minute. All samples were analyzed and values were reported. The mass spectrum of the sample was identified by computer comparison against a mass spectral library.

Parameter	Description				
GC column	SH-Rtx_Wax				
Column dimensions	0.50 μm T, 30.0 m L & 0.25 μm Dia				
Initial oven temperature	50 °C				
Ramp rate & hold time	Up to 240 °C with 5 °C ramp and 5 min hold				
Oven final temperature	240 °C				
Total run time	43.00 min				
Injection mode	Split				
Inlet temperature	220 °C				
Injection volume	1.0 µl				
Carrier gas	Helium				
Flow rate	1.5 ml min <sup>-1</sup>				
Split ratio	1:100				
Detector	MS				
Ion source temperature	220 °C				
Solvent cut time	3.5 minutes				
Acquisition mode	SCAN				
Blank solvent	Ethyl acetate/ methanol				

#### **3. Results and Discussion 3.1 Essential oil content**

**3.1 Essential oil content** The maximum essential oil content (0.51%) was found in  $T_{11}$ 

which was *on par* with  $T_{13}$  (0.45%),  $T_{12}$  (0.44%) and  $T_{10}$  (0.41%). Whereas, the lowest value (0.28%) was shown in control as well as  $T_6$  (Fig 1). Research reports also confirmed that, majorly the oil content is controlled only by genetic factors, but the availability of essential elements in the critical stage of plant growth can influence the rate of photosynthesis and plant metabolites production and ultimately help to accumulate oil (Nikolova and Popp, 2013 and Kaluzewicz *et al.*, 2017) <sup>[9, 4]</sup>. The increased quality might be due to increased content and uptake of macro and micro nutrients by the plants especially P, K, Zn and Mo.



T<sub>1</sub> - EMC1- AM fungi- *Glomus* spp (1 g plant<sup>-1</sup>), *Azospirillum, Pseudomonas fluorescens*, PSB (*Bacillus megaterium*) and KMB (*Frateuria aurantia*) @ 10 ml L<sup>-1</sup> each + FYM @ 10 t ha<sup>-1</sup>

T<sub>2</sub> - EMC2- AM fungi- *Glomus* spp (1 g plant<sup>-1</sup>), *Azospirillum*, *Trihcoderma*, PSB and KMB @ 10 ml L<sup>-1</sup> each + FYM @ 10 t ha<sup>-1</sup>

T<sub>3</sub> - EMC3- AM fungi- *Glomus* spp (1 g plant<sup>-1</sup>), *Azospirillum*, *P. fluorescens*, *Trichoderma*, PSB and KMB @ 10 ml L<sup>-1</sup> each + FYM @ 10 t ha<sup>-1</sup>

T<sub>4</sub> - RDF as per turmeric POP + FYM @ 10 t ha<sup>-1</sup>

 $T_5 - T_1 + 100$  percent RDF

 $T_6$  -  $T_2$  + 100 percent RDF

T<sub>7</sub> - T<sub>3</sub> + 100 percent RDF

 $T_8 \text{-} T_1 \text{+} 75 \text{ percent RDF}$ 

T<sub>9</sub> - T<sub>2</sub> + 75 percent RDF

 $T_{10}$  -  $T_3$  + 75 percent RDF

 $T_{11} - T_3 + 125$  percent RDF

 $T_{12}$  T<sub>3</sub> + 150 percent RDF

 $T_{13}$  -  $T_4$  + Trichokavach (*T. asperellum*, *P. fluorescens*, *Paecilomyces lilacinus and* 2 percent chitosan) -soil application @ 75 g plot<sup>-1</sup> for 2 times.

 $T_{14}$  - FYM @ 10 t ha<sup>-1</sup> (control).

Fig 1: Effect of PGPM and INM on essential oil content (%)

## **3.2** Active constituents of essential oil extracted from rhizomes (%)

The data regarding active constituents of oleoresin from mother rhizomes (Table 1). Major constituent camphene (4.28%) was highest in the treatment  $T_8$  which was followed by  $T_{10}$  (3.79%),  $T_6$  (3.35%) and  $T_{13}$  (3.24%). The treatment  $T_1$  recorded the highest curcumenol (18.13%) followed by  $T_6$  (18.06%),  $T_7$  (17.91%) and  $T_2$  (17.48%). Epicurzerenone was found maximum in  $T_1$  (14.21%) which was followed by  $T_2$  (11.87%),  $T_7$  (11.80%) and  $T_{14}$  (9.87%).

However, D- limonene was found maximum in  $T_7$  (2.58%) followed by  $T_6$  (1.49%),  $T_{13}$  (1.45%) and  $T_{10}$  (1.34%). Eucalyptol was found highest in  $T_{12}$  (22.61%) followed by  $T_{11}$  (21.91%),  $T_8$  (21.40%) and  $T_6$  (20.38%). The treatment  $T_{11}$  resulted higher (23.75%) (+)-4-bornanone which was followed by  $T_{12}$  (23.29%),  $T_8$  (21.84%) and  $T_{14}$  (20.94%).

Furthermore, isoborneol was found maximum in  $T_{11}$  (8.69%) followed by  $T_{12}$  (8.56%),  $T_{14}$  (7.93%) and  $T_8$  (7.88%). D-carvone was found highest in  $T_{11}$  (0.59%) followed by  $T_{12}$  (0.53%),  $T_2$  (0.53%) and  $T_{10}$  (0.48%). The treatment  $T_1$  resulted higher (0.84%) caryophellene which was followed by  $T_{13}$  (0.68%),  $T_2$  (0.67%) and  $T_4$  (0.62%).

Most of the active constituents are found to be maximum in the treatments supplemented with combined application of organic and inorganic fertilizers coupled with bio fertilizers and this might be due to application of *Frateuria aurentia* as a KMB plays a vital role in mobilisation of K and increases the K content in leaves. Potassium is the key component involved in curcumin formation in turmeric. The maximum content of curcumin is also attributed to greater availability of micronutrients from different organic sources supplied in the form of FYM. Among the micronutrients particularly Zn which is responsible for translocation of carbon metabolites, sugar, amino acid, organic acids from source to sink and their exertion for biosynthesis of curcumin (Kumar et al., 2004)<sup>[6]</sup>. Sadanandan et al., 2002 [12] also reported that application of organic manures and bio fertilizer increased the curcumin content in turmeric. Paecilomyces lilacinus helped in solubilizing essential nutrients, such as phosphorus and zinc and helps in secreting various secondary metabolites (Constantin et al., 2022)<sup>[3]</sup> The results in this study are in accordance with the observation of earlier workers like Chandrashekar and Hore, 2019<sup>[2]</sup> in ginger, Roy and Hore, 2011(b)<sup>[11]</sup>, Anusuya and Sathiyabama (2016)<sup>[1]</sup> in turmeric.

Table 1: Effect of PGPM and INM on active constituents of oil (%) extracted from rhizomes	
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Treatments	Camphene (%)	Curcumenol (%)	Epicurzerenone (%)	D- Limonene (%)	Eucalyptol (%)	(+)-4- Bornanone (%)	Isoborneol	D- Carvone	Caryophyllene	Isospathulenol
$T_1$	1.85	18.13	14.21	-	12.74	10.77	4.17	0.25	0.84	1.23
$T_2$	0.11	17.48	11.87	0.14	8.84	19.41	7.55	0.53	0.67	-
T <sub>3</sub>	2.93	14.74	8.91	-	19.43	15.50	5.84	0.38	0.40	-
<b>T</b> 4	2.42	14.86	9.42	-	17.53	17.91	6.95	0.43	0.62	-
T <sub>5</sub>	3.01	11.75	9.72	0.96	19.47	20.31	7.61	0.46	0.46	1.11
T <sub>6</sub>	3.35	18.06	-	1.49	20.38	19.25	7.14	0.46	0.61	1.19
<b>T</b> <sub>7</sub>	4.28	17.91	11.80	2.58	16.73	11.92	5.43	0.26	0.59	-
$T_8$	1.55	10.56	9.53	1.23	21.40	21.84	7.88	0.47	0.50	1.12
T9	1.73	9.92	8.44	1.10	18.29	14.41	7.39	0.45	0.40	1.03
T <sub>10</sub>	3.79	13.30	8.74	1.34	20.39	20.08	7.41	0.48	0.47	1.10
T <sub>11</sub>	2.86	9.34	8.29	0.68	21.91	23.75	8.69	0.59	0.38	1.10
T <sub>12</sub>	2.76	7.89	8.26	0.70	22.61	23.29	8.56	0.53	-	7.89
T <sub>13</sub>	3.24	4.25	9.77	1.45	17.60	17.69	6.43	0.39	0.68	0.93
T <sub>14</sub>	2.87	10.99	9.87	1.01	18.60	20.94	7.93	0.50	0.50	-

T<sub>1</sub> - EMC1- AM fungi- *Glomus* spp (1 g plant<sup>-1</sup>), *Azospirillum*, *Pseudomonas fluorescens*, PSB (*Bacillus megaterium*) and KMB (*Frateuria aurantia*) @ 10 ml L<sup>-1</sup> each + FYM @ 10 t ha<sup>-1</sup>

T2 - EMC2- AM fungi- Glomus spp (1 g plant<sup>-1</sup>), Azospirillum, Trihcoderma, PSB and KMB @ 10 ml L<sup>-1</sup> each + FYM @ 10 t ha<sup>-1</sup>

T<sub>3</sub> - EMC3- AM fungi- *Glomus* spp (1 g plant<sup>-1</sup>), *Azospirillum*, *P. fluorescens*, *Trichoderma*, PSB and KMB @ 10 ml L<sup>-1</sup> each + FYM @ 10 t ha<sup>-1</sup> T<sub>4</sub> - RDF as per turmeric POP + FYM @ 10 t ha<sup>-1</sup>

 $T_5 - T_1 + 100$  percent RDF

 $T_6 - T_2 + 100$  percent RDF

 $T_7 - T_3 + 100$  percent RDF

 $T_8 - T_1 + 75$  percent RDF

 $T_9 - T_2 + 75$  percent RDF

 $T_{10}$  -  $T_3$  + 75 percent RDF

 $T_{11}$  -  $T_3$  + 125 percent RDF

T<sub>12</sub> - T<sub>3</sub> + 150 percent RDF

 $T_{13}$  -  $T_4$  + Trichokavach (*T. asperellum*, *P. fluorescens*, *Paecilomyces lilacinus and* 2 percent chitosan) -soil application @ 75 g plot<sup>-1</sup> for 2 times. T<sub>14</sub> - FYM @ 10 t ha<sup>-1</sup> (control)

#### 4. Conclusion

From the results, it is evident that the essential oil content was significantly influenced the combined application of NPK (150: 125: 150 kg ha<sup>-1</sup>) + FYM (10 t ha<sup>-1</sup>) + Trichokavach (*T. asperellum, P. fluorescens, Paecilomyces lilacinus and* 2 percent chitosan- soil application @ 75 g plot<sup>-1</sup> for 2 times at the time of planting and 120 DAP). The treatments consist both organic and inorganic fertilizers coupled with bio fertilizers proved best for enhanced secondary metabolites.

#### Abbreviations

INM: Integrated nutrient management.PGPM: Plant growth promoting microorganism.EMC: Effective microorganism combinationDAP: Days after plantingFYM: Farm Yard Manure.

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