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Major biochemical traits of kasthuri turmeric (*Curcuma aromatica* Salisb.). Influenced by organic manurial practices

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Abstract
The experiment was conducted with the main objective of studying the combined effect of organic manures and microbial inoculants on major biochemical traits in kasthuri turmeric (*Curcuma aromatica* Salisb.). The results revealed that application of treatment M_2 d (T_2) - Vermicompost (VC) 25.0 t ha^{-1} + mi recorded significantly superior values for rhizome biochemical traits like volatile oil (6.60 %), nonvolatile ether extract (6.73 %) and starch (22.58 %) followed by M_3 d (T_3) Neemcake (NC) 6.0 t ha^{-1} + mi and M_4 d (T_7) - FYM 20.0 t ha^{-1} + VC 6.25 t ha^{-1} + NC 1.5 t ha^{-1} + mi. The results convincingly proved the superiority of treatment M_2 d (T_2) - Vermicompost (VC) 25.0 t ha^{-1} + mi in case of volatile oil, nonvolatile ether extract and starch. Similarly, low crude fibre (2.13 %) was recorded in same treatment which is a very important quality attribute.

Keywords: *Curcuma aromatica* Salisb, organic manures, microbial inoculants, biochemical characters

1. Introduction
Kasthuri turmeric (*Curcuma aromatica* Salisb.) belonging to the family Zingiberaceae is a medicinal and aromatic plant with multiple uses. Several commercially produced cosmetics and ayurvedic preparations contain kasthuri turmeric. Skin care is the major domain of application of this aromatic plant. Rhizome of *Curcuma aromatica* are also used in medicines as a stomachic, carminative and emmenogogue, for skin diseases and also as a health food in Japan (Kojima *et al.*, 1998) [1]. Considering the world demand for organic food, the improvement of soil health and productivity and the availability of local resources, the organic farming practice can be encouraged. Our farmers can take advantage of this opportunity presently available in the international market by offering organically produced spice, aromatic and medicinal products. Use of biofertilizers for crop production is gaining momentum as they are environmentally safe when compared to chemical fertilizers. Though organic manures have beneficial effects on soil health and crop productivity, their limited nutrient content and requirement in large quantity is a constraint for their wider usage. Dwindling availability and huge cost of bulky organic manures warrants the need for reducing their quantity through appropriate substitutes. As a cost effective supplement to chemical fertilizers and as a renewable energy source, microbial inoculants can economize the high investment needed for fertilizer usage of N and P (Pandey and Kumar, 2002) [2]. Microbial inoculants like *Azospirillum*, *Phospho bacteria* and AMF are capable of enhancing the fertilizer use efficiently, soil fertility status and thus help in improving the yield and quality of crops. Since, kasthuri turmeric is a cosmetic cum medicinal plant, apart from yield, quality of rhizomes also assumes significant importance. The quality of any crop is determined by the biochemical constituents present in the economic part. Hence, the present investigation was undertaken to study the combined effect of organic manures and microbial inoculants on major biochemical traits in kasthuri turmeric (*Curcuma aromatica* Salisb.).

2. Materials and methods
The experiment was conducted at the farm attached to the College of Agriculture, Vellayani (Thiruvananthapuram). The area is situated at 8º 30' North latitude and 76º 54' East longitude at an altitude of 29 m above MSL. The treatments consist of different combinations of three organic manures and four microbial inoculants.

The experiment was laid out in RBD with nine treatments and three replications. The treatments were based on prestandardised doses and included full dose of individual organic manures FYM (40 t ha^{-1}), Vermicomost (25 t ha^{-1}) and Neemcake (6 t ha^{-1}) in combination a common with microbial inoculants {*Azospirillum* + AMF + *Trichoderma* + *Pseudomonas* (mi)}, half dose of above organic manures individually in combination with mi, half FYM dose + quarter dose of Vermicompost and Neemcake in combination with mi and half FYM dose + 1/8 th dose of Vermicompost and Neemcake in combination with mi. The above treatments along with control formed the nine treatments which were repeated thrice in RBD. The treatments were M_1 d (T_1) - FYM 40.0 t ha^{-1} + mi, M_2 d (T_2) - Vermicompost (VC) 25.0 t ha^{-1} + mi, M_3 d (T_3) - Neemcake (NC) 6.0 t ha^{-1} + mi, M_1 d/2 (T_4) - FYM 20.0 t ha^{-1} + mi, M_2 d/2 (T_5) - Vermicompost 12.5 t ha^{-1} + mi, M_3 d/2 (T_6) - Neemcake 3.0 t ha^{-1} + mi, M_4 d (T_7) - FYM 20.0 t ha^{-1} + VC 6.25 t ha^{-1} + NC 1.5 t ha^{-1} + mi, M_4 d/2 (T_8) - FYM 20.0 t ha^{-1} + VC 3.125 t ha^{-1} + NC 0.75 t ha^{-1} + mi and M_0 d_0 (T_9) - Absolute control with no organic manures and microbial inoculants. The crop was sown in the first week of June. Rhizome bits were planted at a depth of 5 cm with buds facing upwards at a spacing of 30 x 30 cm and covered with soil. The crop was raised as rainfed but with need based life saving irrigations. IISR accession of kasthuri turmeric were used as a planting material. Plot size was 1.2 x 3 m with 27 plots. Full dose of organic manures as per the treatments were applied as basal dose at the time of planting. Commercial inoculum of AMF 2-3 g per pit was applied at the time of planting to all treatments excluding control. In the case of *Trichoderma*, slurry was prepared by dissolving 20 g in 1 liter of water and the rhizomes were dipped in it and then dried for 30 minutes under shade before planting. *Azospirillum* was mixed with the organic manures in a ratio of 1:25 and applied. Two per cent suspension of *Pseudomonas* was applied as soil drenching at the rate of 5-10 ml per pit just after planting. Hand weeding was done during first and second month and forth month whereas, earthing up and mulching were done at second and fourth months after planting. The crop was harvested when the above ground portion were completely dried up (at around 230 days after planting).

Biochemical analysis for rhizomes and leaf were done at the time of harvest and six months after planting respectively in kasthuri turmeric cultivation. Curcumin content of rhizomes was estimated by the official analytical method suggested by Sadasivam and Manickam (1991) [3]. The method adopted for determining volatile oil was hydro distillation using Clevenger distillation apparatus. Non volatile ether extract (NVEE) was estimated according to AOAC (1973) [4]. The fibre content of the rhizomes was estimated by acid and alkali digestion method (Sadasivam and Manickam, 1991) [3]. Starch content in dried rhizomes was estimated by potassium ferricyanide method (Ward and Pigman, 1970) [5]. Photosynthetic pigments namely, chlorophyll a, chlorophyll b and total chlorophyll were estimated by the method described by Sadasivam and Manickam (1991) [3].

3. Results and discussion

3.1. Curcumin content (%)

Generally, curcumin content in kasthuri turmeric rhizomes is less compared to turmeric and the value ranges from 0.05 to 0.10 per cent. In the present study the content ranged from 0.02 to 0.08 and no significant difference among the treatments was noticed. However, full dose of vermicompost and neemcake and combined application of organic manures + mi (M_2 d, M_3 d and M_4 d) recorded highest values whereas the control (M_0d_0) recorded the lowest curcumin content. In turmeric Rakhee (2002) [6] also recorded no significant difference in curcumin content by the application of different organic manures. However, Sadanandan and Hamza (2006) [7] reported highest curcumin recovery in turmeric by the application of neemcake

3.2. Volatile oil content (%)

Volatile oil in kasthuri turmeric was significantly increased by the application of organic manures and microbial inoculants. In this regard all treatments were found significantly superior than the control plants (M_0 d_0). Among the treatments, however full dose of vermicompost (M_2 d) and neemcake (M_3 d) were found to be superior (Table 1). Among the different organic manures tested, vermicompost was found to have most significant influence whereas, FYM was found to have the least influence. Similar observation in kasthuri turmeric was also reported by Nirmalatha (2009) [8]. An increase in the volatile oil content in turmeric by the application of vermicompost was reported by Rakhee (2002) [6]. Combined application of microbial inoculants also increased the volatile oil content in kasthuri turmeric as reported by Nirmalatha (2009) [8]. However, Sadanandan and Hamza (1998) [9] reported that in ginger, application of neem cake 2.5 t ha^{-1} enhanced volatile oil content and similar increase in volatile oil with neemcake application was reported by Sreekala (2004) [10] in ginger.

3.3. Non volatile ether extract (%)

The non volatile ether extract of kasthuri turmeric was significantly influenced by the application of organic manures and microbial inoculants. Full dose of vermicompost + mi (M_2 d) was the best treatment followed by neemcake + mi (M_3 d) and combined application of organic manures + mi (M_4 d) (Table 1). NVEE content in kasthuri turmeric is an indicative of its quality and quality improvement in kasthuri turmeric by the application of vermicompost, neemcake and also by the combined application of microbial inoculants has been reported by Nirmalatha (2009) [8]. Increase in the oleoresin content in turmeric by the application of organic manures has been reported by Nampoothiri (2001) [11]. Application of neem cake increased oleoresin content in ginger (Sadanandan and Hamza, 1998 [9]; Sreekala 2004 [10]). Positive effect of combined application *Azospirillum* and *Azotobacter* increased oleoresin content in turmeric has been reported by Mohan *et al*. (2004) [12]. Higher level application of organic manures recorded higher NVEE than their lower level application and similar findings were reported by, Maheswarappa *et al*. (2000) [13] in galangal which stated that higher level application of vermicompost (28 t ha^{-1}) and FYM (32 t ha^{-1}) gave the higher oleoresin content than their lower level. Dash *et al.* (2008) [14] reported that application of FYM 20 t ha^{-1} recorded the highest oleoresin content than its lower level in ginger.

3.4. Crude fibre (%)

Crude fibre content of kasthuri turmeric rhizome was significantly reduced by the application of organic manures in combination with microbial inoculants. Full dose of vermicompost + mi (M_2 d) was found to be the best treatment followed by neemcake + mi (M_3 d) and combined application of different organic manures + mi (M_4 d) and full dose of FYM + mi (M_1 d) (Table 1). When the vermicompost,

neemcake and FYM quantity was reduced to half the crude fibre content showed an increase. The control plants (M_0 d_0) recorded the highest crude fibre content in the rhizome. Nirmalatha (2009) [8] also reported similar observation in kasthuri turmeric. Combined application of microbial inoculants also recorded the lowest percentage of crude fibre in kasthuri turmeric as reported by Nirmalatha (2009) [8]. A significant negative correlation between crude fibre content and volatile oil, NVEE content was noticed in ginger by Pruthi (1989) [15] and in present experiment similar correlation was noticed and best treatments were found to have higher volatile oil, non-volatile ether extract and least crude fibre. In general, organic manures improves soil structure, physical properties and experts water holding capacity, leading to higher microbial activity which in turn result in better availability and uptake of nutrients leading to better yield and quality.

3.5. Starch (%)

Starch content in kasthuri turmeric was significantly increased by the application of organic manures and microbial inoculants and all treatments recorded significantly superior values than control (M_0d_0). Full dose of vermicompost + mi (M_2 d) and full dose of neemcake + mi (M_3 d) were the best treatments (Table 1). The property of organic manures along with microbial inoculants in increasing the yield and quality of crops might have lead to the higher starch content in these treatments. The report of Nirmalatha (2009) [8]. in kasthuri turmeric also corroborates the present finding. The positive influence of vermicompost in increasing starch content was reported by Sureshkumar (1996) [16]. in sweet potato and Sreekala (2004) [10]. in ginger.

3.6. Chlorophyll content (Chlorophyll a, b and total chlorophyll) (mg g^{-1})

No significant difference was noticed in the chlorophyll a, b and total chlorophyll content in the leaves of kasthuri turmeric by different treatments at 6 MAP (Table 2). All treatments including control recorded higher values for this character. However, plants treated with full dose of vermicompost and microbial inoculants (M_2 d) recorded the maximum values throughout the growth stages while the control plants (M_0 d_0) recorded the minimum values. Due to the decomposition of different organic manures by the microorganisms, the availability of nutrients such as N, P and K and Mg in soil increased resulting in more uptake of nutrients by the plants. The nutrients such as nitrogen and magnesium have a vital role in the formation of the structures of leaf chlorophyll (Evans and Sorger, 1966) [17].

The decomposition of organic matter in the soil due to the microbial inoculants liberates CO_2, CH_4 etc. which were taken up by the plants very easily for their growth. Due to biological activities of the bioinoculants, the uptake of N and P increases which might have helped in building up of more chlorophyll in the organic manures and microbial inoculants applied plants. Mulching with the plant materials has got significant influence on the chlorophyll content of turmeric leaves as reported by Sanyal and Dhar (2008) [18]. In the present experiment the control plots were also provided with green mulching at 2 MAP and 4 MAP. The favorable effect of green mulching and regular after cultivation practices followed might have resulted in better chlorophyll build up and consequent yield maintains in the control plots also.

Table 1: Effect of organic manures and microbial inoculants on rhizome biochemical analysis in *Curcuma aromatica* Salisb. at harvest.

Treatments / Manures	Curcumin content (%)	Volatile oil (%)	Non volatile ether extract (%)	Crude fibre (%)	Starch (%)
M_1 d (T_1)	0.0605	5.96	6.33	2.67	21.93
M_2 d (T_2)	0.0853	6.60	6.73	2.13	22.58
M_3 d (T_3)	0.0729	6.50	6.63	2.27	22.44
M_1 d/2 (T_4)	0.0441	5.59	5.10	3.03	20.66
M_2 d/2 (T_5)	0.0580	5.98	5.70	2.66	20.81
M_3 d/2 (T_6)	0.0526	5.70	5.60	2.98	20.75
M_4 d (T_7)	0.0665	6.20	6.39	2.48	22.17
M_4 d/2 (T_8)	0.0565	5.92	5.60	2.81	21.83
M_0 d_0 (T_9)	0.0288	5.10	4.97	3.06	20.15
CD (Treatments)	0.0658	0.110	0.405	0.106	0.140
M_1 (FYM + mi)	0.0520	5.77	5.72	2.85	21.29
M_2 (VC + mi)	0.0720	6.29	6.22	2.39	21.70
M_3 (NC + mi)	0.0630	6.10	6.12	2.62	21.60
M_4 (FYM + VC+ NC + mi)	0.0620	6.06	5.99	2.64	22.00
CD (Manures)	0.0465	0.078	0.286	0.075	0.098
$F_{1,16}$ (M_0T_0 Vs M)	2.046 ^{NS}	601.395 **	53.121 **	1 35.524 **	916.037 **

Table 2: Effect of organic manures and microbial inoculants on leaf biochemical Analysis (Chlorophyll content) in *Curcuma aromatica* Salisb. 6 MAP.

Treatments / Manures	Chlorophyll 'a' (mg g^{-1})	Chlorophyll 'b' (mg g^{-1})	Total chlorophyll (mg g^{-1})
M_1 d (T_1)	0.416	0.300	0.715
M_2 d (T_2)	0.734	0.368	1.102
M_3 d (T_3)	0.427	0.317	0.745
M_1 d/2 (T_4)	0.290	0.216	0.506
M_2 d/2 (T_5)	0.449	0.300	0.749
M_3 d/2 (T_6)	0.347	0.222	0.569
M_4 d (T_7)	0.464	0.313	0.777
M_4 d/2 (T_8)	0.389	0.289	0.678
M_0 d_0 (T_9)	0.278	0.195	0.473
CD (Treatments)	0.359	0.236	0.443
M_1 (FYM + mi)	0.353	0.258	0.611
M_2 (VC + mi)	0.592	0.334	0.926
M_3 (NC + mi)	0.387	0.270	0.657
M_4 (FYM + VC+ NC + mi)	0.427	0.301	0.727
CD (Manures)	0.254	0.167	0.314
$F_{1,16}$ (M_0T_0 Vs M)	1.609 ^{NS}	1.328 ^{NS}	2.687 ^{NS}

^{NS}- Non significant ** - significant at 1 per cent

4. Conclusion

Kasthuri turmeric is a cosmetic cum medicinal plant, apart from yield, quality of rhizomes also assumes significant importance. The quality of any crop is determined by the biochemical constituents present in the economic part. In the present study, vermicompost + mi (M_2 d) produced best quality rhizomes with highest volatile oil, starch and NVEE and with less fibre content.

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