Influence of soil drenching and foliar application of biostimulants on physiological and quality parameters in curry leaf (*Murraya koenigii* Spreng.).

Abstract

A field experiment was conducted to study the effect of soil drenching and foliar application of different biostimulants on physiological and quality parameters of curry leaf (*Murraya Koenigii* Spreng.) at Karamadai, Coimbatore during 2019 to 2021. This study was laid out in factorial randomized block design with control, factor 1 as soil drenching (D_1- humic acid @ 5 ml/plant and D_2- jeevamrutham @ 50 ml/plant), factor 2 as foliar spray of different biostimulants (S_1- Effective microorganism culture @ 2 %, S_2- Egg amino acid @ 1%, S_3- Panchagavya @ 3%, S_4- Sea weed extract @ 2% and S_5- Pink Pigmented Facultative Methylotrops (PPFM) @ 1 % and control as farmer practices. The experimental results revealed that significantly higher leaf area (9.88 cm^2), leaf area index (15.36), chlorophyll a (0.89 mg/g), chlorophyll b (0.66 mg/) and total chlorophyll (1.80 mg/g), relative water content (74.41%) and fresh herbage yield (840.18g) in the treatment D_1S_3 (Humic acid + Panchagavya @3 %). Quality parameters like essential oil (0.19%) and oleoresin (2.86%) were significantly higher in the treatment D_1S_4 (Humic acid + sea weed extract).

Key words: Curry leaf, Soil drenching, Foliar spray, Leaf area, Chlorophyll, Quality.

Introduction

Murraya koenigii is commonly known as curry leaf or Karipatta in Indian accent and also called as miracle plant for its importance. *Murraya* belongs to the family Rutaceae, which represents more than 150 genera and 1600 species. Among fourteen global species belongs to the genus of *Murraya*, only *Murraya koenigii* Spreng. and *Murraya paniculata* (Linn.) is available in India (Nayak *et al*., 2010). It is native to South Asia particularly India, Sri Lanka and Bangladesh and distributed throughout India. It is considered as the most important ingredients in South Asian cuisine for its fragrance and aroma due to presence of pinene, sabinene, caryophyllene, cardinol and cardinene (Dipika *et al*., 2021). Curry leaves are used as source of calcium to those with calcium deficiency besides, that it has Vitamin A, Vitamin B and B2, Vitamin C and iron. It has been used for centuries in the Ayurvedic System of Medicine. The leaves, bark and the roots of the plant are used in indigenous medicine as tonic, stomachic, stimulant and carminative (Singh et al., 2014). Curry leaf essential oil is used several industrial applications in the manufacturing of hair oils, soaps, perfumes, cosmetics, food processing and many others. Dried leaf powder, food preparations using leaf powder and essential oils are exported to several countries (Raghu et al. 2020). Curry leaf has richest source of carbazole alkaloids, which act as anti-tumor, anti-oxidative, anti-mutagenic and anti-inflammatory (Muthumani *et al*., 2010).

Biostimulants are environmental-friendly substances that can increase crop yield by acting on plant metabolism thus improving nutrient use efficiency (Yazdani *et al.,* 2014). Biostimulants are composed of bioactive compounds such as amino acids, peptides, humic

substances, seaweed extracts, Effective microorganism, Panchagavya, jeevamrutham etc. Seaweeds are used as nutrient supplements such as biostimulants or biofertilizers to increase the plant growth and yield (Khan *et al*., 2009). They can alter the biological, biochemical, and physical properties of the soil and enhance the performance of plants under abiotic stress. They can also give impact on the overall transcriptome profile by modifying the plant metabolism (Battacharyya *et al*., 2015). Zakaria Fouad Fawzy (2010) reported that the highest amount of humic acid sprayed @ 4 ml per litre was found to improve the growth, yield and quality. Egg amino @ 2 % showed higher chlorophyll a, chlorophyll b and total chlorophyll content than the control plants. Application of Egg amino and Jeevamrutha also recorded less infection on collar reported by Rini *et al*. (2016) in black pepper. Viji *et* al. (2018) reported that treatment combination of RDF @125% + Azospirillum + PPFM @ 1 % in moringa increase the plant height, number of branches per plant, number of leaves per plant, leaf area, chlorophyll content, chlorophyll stability index. Foliar application of panchagavya at 3 % to increased the growth and herbage yield was reported by Sharon *et al*. (2012) in curry leaf. Seaweed extracts are contain phytohormone such as cytokinins, isopentyladenine, dihydrozeatin, and cis-zeatin which have all been linked to positive plant growth in spinach (Werner, *et al.*,2001).

The main objective of using biostimulants are to reduce the chemical fertilizers and to accelerate the organic products for healthy life. The present study focused on determining the influence of soil drenching and foliar application of biostimulants on physiological and quality parameters in curry leaf.

Materials and methods

A field experiment was conducted in the farmer's field practicing organic farming, Karamadai, Coimbatore, during 2019-20 to 2020-21. The experiments were laid out in Factorial Randomized Block Design with control and three replication. Factor 1 as soil drenching (D_1- humic acid @ 5 ml/plant and D_2- jeevamrutham @ 50 ml/plant); factor 2 as foliar spray of different biostimulants (S_1- EM culture {Effective microorganism}@ 2 %,S_2-Egg amino acid @ 1%, S_3-Panchagavya @ 3 %,S_4-Sea weed extract @ 2% and S_5- PPFM {Pink Pigmented Facultative Methylotrops}@ 1 % and control is farmer practices(tank silt @ 25 t/ ha. as a basal doses + fish oil resin soap +Ebomidin @ 3% and Mixed herbal leaf extract @ 2% as foliar application). The local cultivar Senkambu (eight years old) with spacing 1m x 1m is used for this study. One week after pruning soil drenching was given and the foliar applications at 30, 50 and 70 days after pruning were imposed. Physiological parameters like leaf area (cm), leaf area index, chlorophyll a, chlorophyll b and total chlorophyll, relative water content and quality parameters like essential oil (%) and oleoresin (%) were recorded 80 days after pruning. The harvesting was done four times per year with the harvest intervals of three months.

Table. 1 Treatment details

Treatments	**Treatment combination**	
	Factor 1 (Soil drenching)	**Factor 2 (foliar application)**

D_1S_1	Humic acid @ 5 ml	EM culture @ 2%
D_1S_2		Egg amino acids @ 1%
D_1S_3		Panchagavya @ 3%
D_1S_4		Sea weed extract @ 2%
D_1S_5		PPFM @ 1%
D_2S_1	Jeevamrutham @50 ml	EM culture @ 2%
D_2S_2		Egg amino acids @ 1%
D_2S_3		Panchagavya @ 3%
D_2S_4		Sea weed extract @ 2%
D_2S_5		PPFM @ 1%
Check (farmer's practice)	Tank silt @ 25 t/ ha. as a basal doses + fish oil resin soap + Ebomidin @ 3% and Mixed herbal leaf extract @ 2%	

Preparation of Egg amino acid

Ripened lemon (20 numbers) is squeezed and juice is taken in a plastic container. Then 10 eggs were kept inside the lemon juice till the eggs were soaked completely and kept undisturbed for 10 days. After 10 days, eggs were smashed well and 250 g jaggery is added and kept for 10 days. Filter the content after 10 days and liquid portion is collected and stored in a separate container for foliar spray (Anonymous, 2015).

Leaf area

The leaf area of the plant was estimated by destructive sampling method. The compound leaves are feed into the photosensitive, automatic portable leaf area meter (Model L.I. 3000) at 45 days after imposing the treatments and the mean value was calculated and expressed in cm^2.

Leaf area index

The leaf area index was calculated as per the procedure suggested by Williams (1946), using the following formula.

$$LAI = \frac{\text{Leaf area of plant } (cm^2)}{\text{Ground area occupied } (cm^2)}$$

Chlorophyll content

One gram of the fresh leaf sample was collected and macerated in 10 ml of the 80 per cent acetone and centrifuged at 3000 rpm for 10 minutes. After centrifugation the supernatant was collected and made up to 25 ml using 80 per cent acetone.

The intensity was observed as OD values at 645 nm, 652 nm and 663 nm using spectrophotometer. The content of chlorophyll 'a', chlorophyll 'b' and total chlorophyll were estimated and expressed as mg per g (Yoshida *et al.*, 1971).

Relative water content

The relative water content was worked out using the formula by Barrs and Weatherly (1962) and expressed in per cent.

$$\text{Relative water content} = \frac{\text{Fresh weight- Dry weight}}{\text{Turgid weight- Dry weight}} \times 100$$

Quality parameters

Essential oil content

Fresh curry leaves were used for extraction of essential oil with Clevenger apparatus and total essential oil was expressed in percentage (Anonymous, 1968).

Procedure

A quantity of 100 mg of fresh curry leaf was taken and transferred to volumetric flask and 500 ml of water was added. The flask was heated and maintained at a reflux rate of 1 to2 drops per second. Thus it refluxed until two consecutive readings were taken at one hour interval which shows change in oil volume in the trap. After cooling, the values of essential oil were noted and expressed in percentage.

Calculation

$$\text{Essential oil} = \frac{\text{Volume of oil (ml)}}{\text{Weight of the sample}} \times 100$$

Oleoresin content

Accurately ten gram of curry leaf powder was weighed and filled up in the burette column with (3 times weight of powder) acetone or ethylene dichloride. Then allowed to percolate overnight and drained into a pre weight beaker (A). Then the residue was washed once or twice with acetone and the extract as pooled. For evaporation of the solvent, the beaker was kept over a water bath at 80°C until constant weight was obtained (B). The oleoresin content in the sample was calculated and expressed in percent (Anonymous, 1975).

$$\text{Oleoresin content} = \frac{W_2 - W_1}{\text{Weight of the sample taken}} \times 100$$

Where,

W_1 = Weight of empty beaker

W_2 = weight of beaker with air dried oleoresin

Statistical analysis

The data recorded were subjected to statistical analysis using TNAUSTAT software. The critical difference was worked out for five per cent (0.05) probability.

Results and discussion

Physiological parameters

Leaf area and leaf area index

The effect of soil drenching and foliar application of biostimulants on leaf area and leaf area index are given in table 2. The foliar spray of biostimulnats showed significant differences in leaf area. The highest leaf area (9.81 cm^2) was recorded in panchagavya @ 3% (S_3) compared to check (9.57 cm^2) lowest leaf area (7.48 cm^2) in PPFM (S_5). The leaf area did not show significant differences in soil drenching treatments. Whereas, the interaction effect showed significant differences in leaf area among the treatments.

The leaf area index was significant difference between foliar spray and soil drenching. Foliar spray of Panchagavya @ 3 % recorded the highest leaf area index (15.18) and lowest (9.74) recorded in PPFM @ 1 %. The interaction effect showed significant differences in leaf area index among the treatments, highest leaf area index (15.36) was recorded in treatment combination of Panchagavya @ 3 % + Humic acid (D_1S_3) and lowest (9.72) in PPFM @ 1 % + Jeevamrutham (D_2S_5). Availability of nutrients, would have aided in increased the number of leaves, leaf area, leaf area index, photosynthetic rate. Turkmen *et al.* (2005) reported that application of humic acid increased the nitrogen content of shoot and root and which may lead to increase biomass of the crops. Similar observation for increase in leaf area was studied by Medeiros *et al*. (2001) in lettuce. Beaulah (2001) reported that spraying with panchagavya produced bigger leaves and denser canopy in moringa. Similarly Suba *et al.* (2010) reported that foliar application of panchagavya @ 3% to increase the leaf area and leaf area index in curry leaf. Sanjutha et al., (2008) concluded that the application of FYM @ 15 t/ha + panchagavya @ 3% foliar spray recorded the number of leaves (105.67) and highest Leaf Area Index (LAI) (1.03) when compared to other treatments in Kalmegh.

Chlorophyll

The data with respect to chlorophyll a, chlorophyll b and total chlorophyll is presented in table 3. Chlorophyll a was significantly influenced by foliar spray with different biostimulants. Among the foliar treatments, Panchagavya @ 3 % (S_3) recorded maximum chlorophyll a (0.88mg/g) and minimum (0.62 mg/g) in PPFM @ 1 % (S_5). The chlorophyll a showed non-significant effect with respect to soil drenching. Furthermore, interaction effect also showed non-significant difference between foliar spray and soil drenching.

Chlorophyll b differed significantly for foliar application of biostimulants. Among the different foliar sprays, the chlorophyll b was highest (0.65 mg/g) in Panchagavya @ 3 % (S_3) and lowest (0.39 mg/g) in PPFM @ 1 % (S_5). Chlorophyll b showed non-significant effect on soil drenching. The interaction effect also showed non-significant difference between foliar spray and soil drenching.

Soil drenching and foliar application has shown significant differences on total chlorophyll content. In foliar spray maximum total chlorophyll (1.70 mg/g) were recorded in

Panchagavya @ 3 % (S_3) compared to the check (1.40 mg/g) and minimum (0.85 mg/g) in PPFM @ 1 % (S_5). Significant differences were observed in total chlorophyll on soil drenching of biostimulants. Among the soil drenching treatments the highest value for total chlorophyll (1.27 mg/g) was recorded in humic acid (D_1) and lowest (1.24 mg/g) in jeevamrutham (D_2). The interaction effect was significantly different for both foliar spray and soil drenching. The higher total chlorophyll content was recorded in D_1S_3 (1.80 mg/g) compared to check (1.40 mg/g) and lower in D_2S_5 (0.80mg/g). Kaur *et al.* (2014) humic acid, panchagavya also have cytokinin which can influence various physiological activities such as chlorophyll synthesis leading to increase in photosynthesis and as a result induces growth of the plant. Suba *et al.* (2010) reported that foliar application of panchagavya @ 3% to increase the chlorophyll content in curry leaf. *Azospirillum* present in panchagavya might have also increased the chlorophyll content of leaves which might be attributed to the N fixing ability of *Azospirillum* coupled with its ability to synthesis growth hormones besides other enzymes. Ping *et al*, (2001) reported that the enhanced uptake of Mg^{2+} and Fe^{2+} in the presence of humic acid resulted in enhanced chlorophyll synthesis. Sreenivasa *et al.* (2010) reported that panchagavya sprayed on chilli produced dark green colour in leaves.

Relative water content

The effect of soil drenching and foliar spray of biostimulants on relative water content is given in table 4. Significant differences were recorded in foliar spray treatments of biostimulants. Foliar spray of Panchagavya @ 3 % recorded the highest relative water content (74.01%) and lowest (61.74%) in PPFM @ 1 % compare to check. Soil drenching of biostimulants were significant differences for relative water content the highest (67.56 %) in Humic acid (D_1) and lowest (66.33) in Jeevamrutham (D_2). While, the interaction effect showed non significant differences among the treatments.

Fresh herbage yield

The data with respect to fresh herbage yield are presented in Table 4. The fresh leaf yield was significantly different for foliar spray of biostimulants. Foliar spray of Panchagavya @ 3 % recorded the highest fresh leaf yield per plant (840.18g) and lowest (808.10 g) in PPFM @ 1 %. Soil drenching treatment was significantly not different for fresh leaf yield per plant. Interaction effect was significantly different for fresh leaf yield per plant. Sharon *et al.*(2012) found that application of *Azospirillum* (2.5 kg/ha)+Phosphobacteria (2.5 kg/ha)+Panchagavya @ 3% recorded the maximum fresh leaf weight and fresh herbage yield in curry leaf. The increased synthesis of cytokinin and auxin in the root tissue by their enhanced activity due to the application of biofertilizers and Panchagavya and their simultaneous transport to the auxillary buds would have resulted in better vegetative growth. Sanjutha et al., (2008) concluded that the application of FYM @ 15 t ha-1 +panchagavya @ 3% foliar spray recorded significantly higher leaf yield and herbage yield as compared to other treatments in Kalmegh (Andrographis paniculata).

Quality parameters

The effect of soil drenching and foliar spray of biostimulants on essential oil is given in table 5. The foliar spray of biostimulants showed significant differences on essential oil. The higher essential oil content (0.18%) was recorded in sea weed extract (S_4) followed by EM culture (S_1) and lower (0.11%) in PPFM (S_5). The interaction effect between the soil drenching and foliar spray showed significant differences in essential oil. The higher essential oil observed in humic acid + sea weed extract (D_1S_4) compared to check (0.12 %) and the lower (0.13%) in PPFM (S_5).

The effect of soil drenching and foliar spray of biostimulants on oleoresin is given in table 5. Significant differences were recorded in foliar spray treatments of biostimulants. Foliar spray of sea weed extract @ 2 % recorded the highest oleoresin content (2.83%) and lowest (2.30%) in PPFM @1 %. A significant difference for oleoresin was observed in soil drenching of biostimulants alone. While, the interaction effect showed non significant differences in oleoresin among the treatments. Hamidreza Bayat (2019) reported that seaweed contains all required trace elements and plant growth hormones and sea weed manure is also rich in potassium and poor in nitrogen and phosphorus. The increased efficiency of translocation due to foliar spray of seaweed extract and humic acid in turn contributed to higher uptake of nutrients resulting in better quality. Similar findings were reported by Maheshwari *et al*. (2004) in chilli.

Conclusion

The present study revealed that soil drenching with humic acid @ 5 ml/plant and foliar application with panchagavya @ 3% at 30 days, 50 days and 70 days after pruning, has significantly increased the physiological characters and yield. Whereas foliar application of sea weed extract @ 2% showed better performance for quality characters than control in curry leaf. Hence, biostimulants can be used as an organic based compound which improves curry leaf production with increased quality under organic farming.

Table 2. Effect of soil drenching and foliar spray of biostimulants on leaf area (cm^2) and leaf area index

Treatment	Leaf area (cm^2)			Leaf area index		
	D_1	D_2	Mean	D_1	D_2	Mean
S_1	8.11	8.25	8.18	14.12	13.60	13.85
S_2	8.86	8.32	8.59	12.73	11.63	12.18
S_3	9.88	9.75	9.81	15.36	15.01	15.18
S_4	7.89	7.88	7.88	11.36	11.53	11.44
S_5	7.51	7.46	7.48	9.77	9.72	9.74
Mean	8.45	8.33	8.39	12.66	12.29	12.47
Check	9.57			14.55		
	S	D	S x D	S	D	S x D
SE	0.07	0.04	0.10	0.10	0.06	0.14

SEd	0.10	0.06	0.14	0.14	0.09	0.21
C.D(5%)	0.22*	0.13	0.31*	0.31*	0.19*	0.43*

*-Significant, SE -Standard Error, SEd - Standard Error of Difference and CD- Critical Difference (P=0.05)

Table 3. Effect of soil drenching and foliar spray of biostimulants on chlorophyll a (mg/g), chlorophyll b (mg/g) and total chlorophyll (mg/g)

Treatment	Chlorophyll a (mg/g)			Chlorophyll b (mg/g)			Total chlorophyll (mg/g)		
	D_1	D_2	Mean	D_1	D_2	Mean	D_1	D_2	Mean
S_1	0.77	0.78	0.77	0.48	0.49	0.48	1.30	1.30	1.30
S_2	0.73	0.73	0.73	0.51	0.50	0.50	1.19	1.30	1.24
S_3	0.89	0.88	0.88	0.66	0.65	0.65	1.80	1.60	1.70
S_4	0.72	0.69	0.70	0.46	0.47	0.46	1.19	1.20	1.19
S_5	0.63	0.62	0.62	0.39	0.39	0.39	0.90	0.80	0.85
Mean	0.74	0.74	0.74	0.50	0.50	0.50	1.27	1.24	1.25
Check	0.85			0.53			1.40		
	S	D	S x D	S	D	S x D	S	D	S x D
SE	0.008	0.005	0.113	0.005	0.003	0.008	0.145	0.009	0.020
SEd	0.011	0.007	0.016	0.008	0.005	0.011	0.020	0.013	0.029
C.D(5%)	0.024*	0.015	0.034	0.016*	0.010	0.024	0.043*	0.027*	0.060*

*-Significant, SE -Standard Error, SEd - Standard Error of Difference and CD- Critical Difference (P=0.05)

Table 4. Effect of soil drenching and foliar spray of biostimulants on relative water content (%) and fresh herbage yield / plant (g)

Treatment	Relative water content (%)			Fresh herbage yield /plant (g)		
	D_1	D_2	Mean	D_1	D_2	Mean
S_1	71.24	69.35	70.29	820.10	817.50	818.80
S_2	65.29	64.31	64.80	817.91	810.31	814.11
S_3	74.41	73.62	74.01	845.57	834.80	840.18
S_4	64.28	63.48	63.88	814.62	810.87	812.74
S_5	62.59	60.89	61.74	810.60	805.60	808.10
Mean	67.56	66.33	66.93	821.76	815.81	818.78
Check	67.31			820.81		

	S	D	S x D	S	D	S x D
SE	0.60	0.38	0.85	5.64	3.57	7.98
SEd	0.85	0.54	1.21	7.98	5.05	11.29
C.D(5%)	1.79*	1.13*	2.53	16.69*	10.56	23.61*

*-Significant, SE -Standard Error, SEd - Standard Error of Difference and CD- Critical Difference (P=0.05)

Table 5. Effect of soil drenching and foliar spray of biostimulants on essential oil (%) and oleoresin (%)

Treatment	Essential oil (%)			Oleoresin (%)		
	D_1	D_2	Mean	D_1	D_2	Mean
S_1	0.15	0.15	0.15	2.48	2.48	2.48
S_2	0.13	0.13	0.12	2.31	2.34	2.32
S_3	0.12	0.13	0.12	2.34	2.45	2.39
S_4	0.19	0.17	0.18	2.86	2.81	2.83
S_5	0.12	0.12	0.11	2.23	2.37	2.30
Mean	0.14	0.13	0.13	2.44	2.48	2.46
Check	0.12			2.33		
	S	D	S x D	S	D	S x D
SE	0.002	0.001	0.003	0.023	0.015	0.033
SEd	0.003	0.002	0.004	0.033	0.021	0.047
C.D(5%)	0.006*	0.004	0.009*	0.044*	0.070*	0.136

*-Significant, SE -Standard Error, SEd - Standard Error of Difference and CD- Critical Difference (P=0.05)

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