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Nutritional quality of bhendi as influenced by effective microorganism application

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ABSTRACT

The effect of different levels of fertilizers and frequencies of effective microorganisms (EM) spray on the quality attributes of bhendi was studied through a field experiment conducted in the Surakudy soil series (*Fluventic Haplustept*) at Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal. The field experiment was laid out in a factorial randomized block design with three replications. The experiment consisted of two factors, *viz.* fertilizer levels (F) and frequency of EM spray (E), consisting of nine treatments, including a control without any EM spray and fertilizer application. Three levels of fertilizers include; F_1- No fertilizer, F_2- 75% RDF and F_3- 100% RDF. Three levels of 1% EM spray include E_1- No spray, E_2- Weekly spray, and E_3- Fortnightly spray. The quality parameters of bhendi fruit, like chlorophyll, mucilage, protein, starch, and ascorbic acid content, were higher in the treatment which received 100 percent RDF and fortnightly spray of EM, and the same was high in fruits from mid-harvest compared to the initial and final stages of harvest. The crude fiber content and physiological loss in weight of the fruits were found to be low in the treatment that received 100 percent RDF, and the highest was noticed with no fertilizer, and the same trend was noticed with EM spray. Application of 100 percent RDF recorded the highest fruit yield, which was found to be 109.38 per cent more than the treatment that did not receive fertilizer (control). Foliar application of 1 percent EM at fortnightly intervals recorded higher fruit yield, and this was observed to be 18.02 percent more than no EM spray and was on par with weekly spray of 1 per cent EM. Among the nine treatment combinations tried, application of 100 per cent RDF and EM spray at fortnightly intervals was found to be beneficial in improving the yield and quality attributes of bhendi.

Keywords: Bhendi, effective microogranisms, mucilage, protein, crude fibre, quality attributes

INTRODUCTION

Vegetables play an important role in human beings' day-to-day dietary requirements. They supply protective substances like vitamins and minerals and energy-giving substances like carbohydrates, protein, and fats. Among the vegetables, bhendi is one of the important vegetable crops grown in India, and it finds a place in people's everyday food. Bhendi fruit is a good source of vitamins A, B and C. The calcium content in its fruits is very high (66 mg/100g of edible portion) compared to that of other fruit vegetables. It is an excellent source of iodine. It is also rich in protein and mineral nutrients. Among fruit vegetables, bhendi fruits have good demand throughout the year. India is the world's largest bhendi producer (National Horticulture database, 2020-21). The shrinking land and other agricultural resources make it imperative to find suitable technologies to enhance vegetable productivity and sustain production. Research has shown that adding organics and fertilizers would increase crop yields and sustain soil health (Bhardwaj *et al.*, 2023; Chejara *et al.*, 2021).

Professor Teruo Higa, University of the Ryukyus, Okinawa, Japan, developed the concept of effective microorganisms (EM). It consists of three organisms, *viz.,* lactic acid bacteria, phototropic bacteria, and yeast, which can enhance the beneficial microbes, sustain soil health, and enhance crop growth and yield. The application of EM on the phyllosphere of crops could increase nutrient absorption, hormone biosynthesis, and photosynthetic efficiency, leading to enhanced crop growth and yield. Foliar application of EM results in a large number of beneficial microorganisms at the leaf surface or phyllosphere. It is believed that certain microorganisms in the EM culture, including photosynthetic bacteria and N-fixing bacteria, can enhance the plant's photosynthetic rate and efficiency and its N-fixing capacity as well (Pati and Chandra, 1981; Atlas and Bartha, 1981). Generally, vegetable yields were higher with foliar-applied effective microorganisms than with chemical fertilizer (Widdiana and Higa, 1998; Singh *et al.*, 2022). Xiaohou *et al.* (2001) reported that sprinkling with effective microorganisms could increase the yield and quality of various crops, fruits, and vegetables. Foliar application of EM appears to suppress the occurrence of plant diseases and facilitates the uptake of simple organic molecules that can increase plant growth and yield. Considering the role of EM that it could play in crop improvement, the present study was undertaken to examine the effect of EM on improving the yield and quality of bhendi.

MATERIALS AND METHODS

The field experiment was conducted in field number C_8 of the Eastern Farm of Agronomy Department at Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal. The farm is four meters from the mean sea level between 10°49′ and 11°01′ N latitude and 78°43′ and 79°52′ E longitude. The mean annual temperature ranges from a minimum of 24.22 °C to a maximum of 32.68 °C. The mean annual rainfall is 1450 mm. The field soil comes under the Surakudy soil series, grouped under *Fluventic Haplustept.*

Initial characterization of the experimental soil was done by adopting the standard procedures, and the results are presented in Table 1. The soil is sandy loam in texture belonging to the Surakudy soil series, taxonomically grouped under *Fluventic Haplustept*. Initial soil analysis revealed that the soil of the experimental site was neutral (pH = 7.01) with an EC of 0.052 dS m^{-1}. The cation exchange capacity (CEC) was 20.70 cmol (p^+) $kg^{-1,}$ with Ca^{2+} as the dominating cation, followed by Mg^{2+}, Na^+, and K^+. The organic carbon content was 0.55 percent. The $KMnO_4$-N, Olsen-P, and NH_4OAc-K status indicated that the soil possessed low N and medium P and K.

Table 1. Initial characteristics of the experimental soil

Sl. No.	Properties	Values
A. Physical properties		
1.	Texture	
	Clay (%)	16.65
	Silt (%)	6.25
	Fine sand (%)	33.78
	Coarse sand (%)	41.84
2.	Textural class	Sandy loam
3.	Taxonomic unit	*Fluventic Haplustept*
4.	Bulk Density (Mg m^{-3})	1.24
5.	Particle Density (Mg m^{-3})	2.22
6.	Pore space (%)	44.08
B. Physico-chemical properties		
7.	Loss on ignition (%)	4.72
8.	Electrical conductivity (dS m^{-1})	0.052
9.	pH (1:2.5 soil water suspension)	7.01
C. Chemical properties		
10.	Organic carbon (%)	0.55
11.	Cation Exchange Capacity [cmol (p^+) kg^{-1}]	20.70
12.	Exchangeable Calcium (cmol kg^{-1})	7.98
13.	Exchangeable Magnesium (cmol kg^{-1})	5.43
14.	Exchangeable Sodium (cmol kg^{-1})	4.05
15.	Exchangeable potassium (cmol kg^{-1})	0.42
16.	Total N (%)	0.064
17.	Total P (%)	0.191
18.	Total K (%)	0.320
19.	$KMnO_4$-N (kg ha^{-1})	221.2
20.	Olsen-P (kg ha^{-1})	18.4
21.	NH_4OAc-K (kg ha^{-1})	122

The field experiment was conducted during the *Kharif* (July - October) season. Bhendi F_1 hybrid Sakthi was used as a test crop to study the influence of effective microorganisms on the growth and yield of bhendi. The field experiment was laid out in a factorial randomized block design with three replications. The experiment consisted of two factors, *viz.,* fertilizer levels (F) and frequency of EM spray (E), consisting nine treatments, including a control without any EM spray and fertilizer application. Three levels of fertilizers include; F_1- No fertilizer, F_2- 75% RDF and F_3- 100% RDF. Three levels of 1% EM spray include E_1- No spray, E_2- Weekly spray, and E_3- Fortnightly spray.

The EM stock solutions marketed by Maple Orgtech (India), Limited, Kolkata, was used in the present study. EM solution contained Lactic acid bacteria ($10^5_<$), Photosynthetic bacteria ($10^1_<$), and yeast ($10^2_<$). EM solution at 1 percent concentration was sprayed weekly and fortnightly as per the treatment. A control without EM spray was also maintained. Spraying was taken up from the 15th day after sowing. Nitrogen was applied as urea in two equal splits as basal and as top dressing at 25 DAS. Full doses of phosphorus as super phosphate and potassium as muriate of potash were applied basally as per the treatment. All the intercultural operations were performed uniformly as recommended (Crop Production Techniques of Horticulture Crops, 2012).

The yield of fruits per plot recorded at each harvest was summed up and converted to yield per hectare per crop. Representative fruit samples collected at 5th, 12th, and 19th harvests were used for analysis; the mean value is presented in Table 2.

Plant analysis

Quality parameters *viz*., chlorophyll, starch, protein, ascorbic acid, and crude fiber content were determined using the procedure that Sadasivam and Manickam (1992) suggested. Fresh fruit was grinded using distilled water for mucilage content and kept for 24 hours. The content was then filtered using muslin cloth, and 50 ml of alcohol was added to the filtrate. Filtrate was then filtered through a pre-weighed filter paper. Filter paper and residue were dried, and mucilage content was expressed in%. PLW was measured as suggested by Sankaran (1999). Immediately after harvest, the fruit was weighed. The weight was recorded daily until complete loss of freshness, and weight loss was taken as PLW.

Table 2. Effect of treatments on bhendi fruit yield (t ha^{1})

	E_1	E_2	E_3	Mean
F_1	6.42	7.45	7.90	7.25
F_2	12.85	13.79	14.86	13.83
F_3	13.72	15.68	16.14	15.18
Mean	10.99	12.31	12.97	
	F	E	F X E	
S. Ed	0.39	0.39	0.69	
CD (0.05)	0.84	0.84	NS	

Statistical analysis

The analytical data were subjected to statistical scrutiny following the procedure Gomez and Gomez (1976) outlined, using AGRES package. The data on yield and quality attributes were statistically analyzed using analysis of variance (ANOVA) in a randomized block design and compared by critical difference at 5 percent significance level. Pearson's coefficient analysis was used for correlation.

RESULTS AND DISCUSSION

The quality of fruit is as important as that of yield since it determines the product's value, particularly vegetable crops. A few quality parameters, like the greenness and freshness of the fruit, are morphologically expressed, and certain others are hidden but important. For example, the chlorophyll content indicates the greenness of the fruit, while the physiological loss in weight (PLW) could serve as an index to show how long the fruit could remain fresh in the market. Similarly, the protein, starch, and ascorbic acid contents reflect the nutritional value, the mucilage, the taste of the fruit, and the crude fiber content, which indicates the tenderness of the fruits. The present investigation revealed the significant influence of fertilizer levels and frequencies of EM spray on all the quality parameters studied.

Chlorophyll content

The results show that applying RDF and EM spray could produce fruits with higher chlorophyll content (Table 3). The interaction between fertilizer and EM was significant, and the chlorophyll content of the fruit ranged from 0.123 mg g^{-1} (F_1E_1) to 0.250 mg g^{-1} (F_3E_3). This could be attributed to a balanced supply of nutrients with RDF essential for chlorophyll synthesis. Kuppusamy (2008) observed results similar to those of Nibin *et al*. (2016a) and Rajakumar and Bagavathi-Ammal (2017). An increase in chlorophyll content with EM spray has been reported earlier by Muthaura *et al*. (2010) in pigweed and Chantal *et al*. (2010) in cabbage. Yeast, an important constituent of EM, enriches cytokinin, which has stimulatory effect on chlorophyll formation, as Thomas (1996) and Ibreheim (2014) reported. Increased chlorophyll content and delayed senescence resulting from the beneficial microbes would have contributed to better fruiting and increased yield in the present study, as Chantal et al. (2010) reported. The correlation analysis result

Table 3. Quality attributes of bhendi as influenced by different treatments

Treatments	Chlorophyll (mg g^{-1})	Mucilage (%)	Protein (%)	Starch (%)	Ascorbic acid (mg 100 g^{-1})	Crude fibre (%)	PLW (%)
F_1E_1	0.123	1.707	0.618	2.331	8.92	14.84	33.74
F_1E_2	0.150	2.085	0.749	2.503	10.51	13.25	32.76
F_1E_3	0.164	2.434	0.777	3.427	11.54	13.06	32.72
F_2E_1	0.175	2.657	0.810	3.834	13.37	12.06	32.25
F_2E_2	0.190	2.977	0.825	3.875	13.52	12.33	30.65
F_2E_3	0.200	3.388	0.870	4.128	14.02	12.13	29.95
F_3E_1	0.192	3.226	0.851	4.006	13.74	11.94	29.30
F_3E_2	0.228	3.598	0.897	3.990	14.17	11.56	28.10
F_3E_3	0.250	3.898	0.941	4.403	14.49	10.96	27.06
S.Ed	0.003	0.078	0.014	0.155	0.14	0.25	0.19
CD (0.05)	0.006	NS	0.029	0.312	0.28	0.50	0.38

Table 4. Correlation analysis between yield and various biochemical characters of the fruit (n=9)

Variables	Yield	Chlorophyll	Mucilage	Protein	Starch	Ascorbic acid	Crude fibre	PLW
Yield	1.000							
Chlorophyll	0.917**	1.000						
Mucilage	0.953**	0.979**	1.000					
Protein	0.912**	0.963**	0.966**	1.000				
Starch	0.930**	0.887**	0.931**	0.923**	1.000			
Ascorbic acid	0.964**	0.903**	0.943**	0.955**	0.974**	1.000		
Crude fibre	-0.912**	-0.938**	-0.934**	-0.982**	-0.921**	-0.960**	1.000	
PLW	-0.898**	-0.964**	-0.963**	-0.900**	-0.820**	-0.836**	0.873**	1.000

** = Significant at 1 % level * = Significant at 5 % level NS = Non-significant

showed that (Table 4) the chlorophyll content of the fruit was positively and significantly related to yield, mucilage, protein, starch, and ascorbic acid and was negatively correlated with crude fiber and PLW.

Mucilage, protein, starch, and ascorbic acid content

Application of recommended doses of fertilizer resulted in higher mucilage, protein, starch, and ascorbic acid (Table 3) contents. Such an increase in these quality parameters is quite expected as the recommended doses of fertilizer application could have supplied required nutrients in a balanced way, which helped in higher chlorophyll content and photosynthetic activities, resulting in higher sugar content and is duly accompanied by an increase in mucilage, protein, starch and ascorbic acid contents. These findings are in accordance with Kuppusamy (2008), Nibin *et al*. (2016a), and Rajakumar and Bagavathi-Ammal (2017) in bhendi.

Similarly, the foliar application of EM solution gave higher values for the above parameters. The important constituent of EM is yeast, which enriches cytokinin, stimulating chlorophyll formation (Ibreheim, 2014). This would have enhanced the photosynthetic efficiency of leaves (Wididana and Higa, 1993), resulting in a higher accumulation of starch, protein, mucilage, and ascorbic acid. An increase in ascorbic acid content duly accompanies higher sugar/starch accumulation. The result is in line with the findings of Sankaran *et al.* (2005) who reported that sugar is converted into ascorbic acid. The significant and positive relationship of ascorbic acid content with fruit's starch, protein, mucilage, and chlorophyll content supports the above inference (Table 4). Kuppusamy (2008), Nibin *et al*. (2016a), and Rajakumar and Bagavathi-Ammal (2017) also reported a similar positive relationship of ascorbic acid with starch, protein and mucilage content. Similar results of the superiority of EM spray were also reported in the protein content of snap beans (Midan and Sorial, 2011) and soya beans (Yue *et al.*, 2014). Spraying of yeast, which is a constituent of EM, gave higher starch and protein content in potatoes (Ahmed *et al.*, 2011), protein in soya beans (Dawood *et al.*, 2013), and tomatoes (El-Yazied and Mady, 2011), ascorbic acid in tomato (El-Yazied and Mady, 2011) and in snap bean (Fawzy *et al.*, 2010).

The correlation analysis showed that the mucilage content was significantly and positively related to protein and starch (Table 3). It is quite possible since the mucilage consists of acidic polysaccharides with associated proteins and minerals (Woolf *et al*., 1976), and the bhendi mucilage polysaccharide is composed of galacturonic acid, galactose, rhamnose, and glucose, which are all the products of carbohydrate metabolism. Crude fiber and PLW were negatively and significantly correlated with mucilage content. The crude fiber may be the indigestible fraction of the carbohydrates consisting of cellulose, hemicelluloses, and lignin of the cell wall. Both the mucilage and crude fiber are the derivatives of carbohydrates. In the plant system, if the photosynthetic carbon source synthesizes mucilage content by using any metabolites from the organic sources, it results in low crude fiber in the fruit. The mucilage is a viscous medium, and it will not easily lose its weight through dehydration under a normal environmental condition, which resulted in low physiological loss in weight (or) better keeping quality (Kuppusamy, 2008; Nibin *et al*. (2016a) and Rajakumar and Bagavathi-Ammal (2017).

Crude fibre content

The crude fiber content of the fruit at different stages has shown that as the crop matures, the crude fiber content increases. Among the treatments, the application of 100 percent RDF and fortnightly spray of EM resulted in lower crude fiber content (Fig. 16). The enhanced metabolic activity of the plant by fertilizer and EM spray stimulated the synthesis of protein, starch, and mucilage in the fruit thus resulting in a reduced availability of metabolites for crude fiber accumulation. This result followed Raj and Kumari (2001), Kuppusamy (2008), Rajakumar (2014), and Sreenadha Reddy (2014), and they reported that the increased protein synthesis had resulted in reduced crude fiber content in bhendi. El-Tohamy and El-Greadly (2007), Fawzy *et al*. (2010), and Abdel-Hakim *et al*. (2012) reported a similar decrease in crude fiber with yeast application in snap beans. The significant and negative relation of crude fiber with mucilage, protein, starch, and ascorbic acid content also supports the above findings (Table 3). A similar negative relation of crude fiber was reported earlier by Kuppusamy (2008), Nibin *et al*. (2016a), and Rajakumar and Bagavathi-Ammal (2017).

Physiological loss in weight

Applying 100 percent RDF and spray of EM significantly lowered PLW at different stages of picking (Table 3). Such results could be attributed to the phenomenon that the altered physiology and biochemistry of fruit as influenced by fertilizer and EM spray could have helped in the synthesis of hormones and vitamins, which might have led to reduced respiration and evapotranspiration, which in turn resulted in low PLW. It was also reported by Nanthakumar and Veeraragavathatham (1999) that better uptake and accumulation of Ca and P might have helped, to a certain extent, maintain cell wall turgidity so that the produce could have been stored better.

The negative and significant relationship of PLW with chlorophyll, protein, starch, mucilage, and ascorbic acid content and the positive relation with crude fiber support the above finding (Table 4). Raj and Kumari (2001), Kuppusamy (2008), Nibin *et al*. (2016a), and Rajakumar and Bagavathi-Ammal (2017) reported that the low crude fiber content had a positive influence in improving keeping quality.

Fruit yield

Applying different fertilizer and EM spray levels significantly improved the yield. The yield was found to range from 6.42 t ha^{-1} in the treatment that received no fertilizer and no spray of EM (F_1E_1) to 16.14 t ha^{-1} in the treatment that received 100 percent RDF with fortnightly spray of EM solution (F_3E_3). Of the three different fertilizer treatments studied, the application of 100 percent RDF (F_3) was significantly superior, with a maximum yield of 15.18 t ha^{-1}. The lowest yield (7.25 t ha^{-1}) was recorded in the treatment, and no fertilizer was received (F_1). The yield recorded with 100 percent RDF was 109.38 percent more than the treatment, which did not receive fertilizer. Among different frequencies of EM sprays, the maximum yield (12.97 t ha^{-1}) was recorded with a fortnightly spray of EM solution, which was on par with weekly EM spray. The yield recorded in E_3 was 18.02 percent more than no EM spray.

The increase in yield due to applying 100 percent RDF over 75 percent RDF and fertilizer control is quite expected. The yield increase with the application of 100 percent RDF might be due to an increase in metabolic activity in the plant system because of the balanced and increased availability of nutrients required for normal growth, which would have resulted in enhanced carbohydrate synthesis and effective translocation of photosynthates to the developing sink. Similar results were also reported by Ayub *et al*. (1993); Arora *et al*. (1991), Mal *et al*. (2013), Venkadeswaran *et al*. (2014a), Nibin *et al*. (2016) and Rajakumar *et al*. (2017)

The higher yield recorded with 1 percent EM spray at fortnightly intervals could be attributed to the buildup of beneficial microbes in the phyllosphere as EM constitutes lactic acid bacteria, photosynthetic bacteria, and yeast, as reported by Chantal *et al.* (2010) in cabbage. The activity of beneficial microbes could have been higher, which helped synthesize growth-promoting substances and, in turn, increased fruit yield and DMP. This aligns with the findings of Wididana and Higa (1993) and Vennila and Jayanthi (2010) in tomato and bhendi, respectively.

The presence of yeast in EM solution could also enrich vitamins, phytohormones, enzymes, amino acids and minerals, thereby stimulating cell division and enlargement as well as protein and nucleic acid synthesis and chlorophyll formation. This, in turn, is reflected in plant growth. This corroborates with the findings of Ibreheim (2014) in pea. Further, the presence of biologically active substances of EM capable of producing antimicrobial substances from amino acids secreted by photosynthetic bacteria (Mbouobda *et al*., 2013) could suppress harmful microbes and thus provide a favorable environment for the growth of plants, as reported by Shokouhian *et al*. (2013) in almond. The increased yield and DMP due to the application of EM might be due to enhanced sub-stomatal CO_2 in the leaf, as Abbas et al. (2014) reported, which supports the present finding.

CONCLUSION

The study concluded that among the different treatment combinations, the application of 100 percent RDF and EM spray at fortnightly intervals was found to be beneficial in recording higher fruit yield and increasing the nutritional quality parameters in bhendi.

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