

Liquid microbial consortia with graded level of inorganic fertilizers for leaf biomass and leaf quality attributes in moringa

V. Viji¹, R. Balakumbahan¹, V. Sivakumar^{2*} and V. Davamani³

¹Department of Vegetable Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam East, Theni district, Tamilnadu-625 604. ²Coconut research Station, Aliyarnagar; ³Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore -641 003. *E-Mail: hortibala@gmail.com

Abstract

Moringa oleifera, a tropical vegetable crop of India, has gained importance for its nutrient-rich leaf production. A study was conducted at the Department of Vegetable Crops, Horticultural College and Research Institute, Periyakulam, to increase leaf biomass productivity and quality attributes of moringa leaves using liquid microbial inoculum. Microbial inoculum included *Azospirillum*, phosphate solubilizing bacteria, potassium solubilizing bacteria and pink pigmented facultative methylotrophs along with graded level of inorganic water soluble fertilizer. The annual moringa var. PKM -1 seeds were sown at a spacing of 40 x 20 cm on well prepared raised beds. Among the different treatments and combinations tested, moringa plants that received RDF 125 % of the recommended dose of inorganic fertilizers (337.5: 56.25: 112.5 kg NPK ha⁻¹) along with a liquid form of bio inoculants viz., *Azospirillum*, phosphate solubilizing bacteria, potassium solubilizing bacteria @ 500 mL each ha⁻¹ through drip system and foliar application of pink pigmented facultative methylotrophs @ 500 mL ha⁻¹ produced higher biomass yield. Nitrogen, phosphorus, potassium, calcium, magnesium, iron, ascorbic acid, tocopherol, crude protein, crude fiber, and total carbohydrates were found to be higher in the same treatment.

Key words: Annual moringa, leaf, water soluble fertilizers, *Azospirillum*, PSB, KSB, PPFM

Introduction

Moringa oleifera Lam, an indigenous vegetable crop of India is known for its multifarious uses in the world. All the parts of this plant is used either as vegetable, greens, medicinal source (pods, leaves and flowers) or as industrial purpose (Benn oil, flocculent, pulp, paper cellophane, textile production etc.). Since it is a source of several nutrients, vitamins and minerals it is also known by other names viz., 'Mother's Best Friend', 'Horse Radish tree' and 'Miracle tree' (Ramachandran *et al.*, 1980; Nautiyal and Venkataraman, 1987). India is the world's major producer of moringa with an annual production of 2.2 million tonnes of tender pods from an area of 38,000 ha. Among the states, Andhra Pradesh leads in both the area and production (15,665 ha) followed by Karnataka (10,280 ha) and Tamil Nadu (7,408 ha) (Bhargave *et al.*, 2015). Tamil Nadu is the pioneer state as it has broad genetic diversity (annual and perennial moringa) and diversified geographical areas, as well as few introductions from Sri Lanka (Rajendran and Prahadeeswarran, 2014). It is one of the important commercial vegetable crops of Tamil Nadu because of its significant economic importance. Moringa has been cultivated mainly for its tender pods as commercial point of view and pods are exported to many parts of the world from India. After realising the nutrient density of the leaves of moringa and its demand in international market, cultivation of moringa for leaf purpose is gaining importance in the recent past. A 100 g of moringa leaves contain four times of vitamin A than carrots, four times of calcium in a cup of milk, iron more than 100 g of spinach, seven times of vitamin C in 100 g of oranges and three times of potassium in 100 g of bananas. The protein quality of moringa leaves also

rivals that of milk and eggs. Besides it provides a rich and rare combinations of nutrients, amino acids, antioxidants and anti-inflammatory properties used for nutrition and healing purpose. (Fahey, 2005; Fuglie, 2001; Ramachandran *et al.*, 1980). World Health Organization also promoted moringa leaf as an alternative food supplies to treat malnutrition (Johnson, 2005; Manzoor *et al.*, 2007). Many research works have been initiated with regard to moringa leaf production to meet out the demand. Integrated approach by combining the inorganic and microbial sources to meet out nutrient requirement of plants is one of the key areas to sustain the productivity of crops.

Incorporation of biofertilizers with the nutrient management of crop plants helps to keep the soil environment rich in all kinds of micro and macro nutrients via nitrogen fixation, phosphate and potassium solubilisation or mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Sinha *et al.*, 2014; Sivakumar *et al.*, 2013). With this view a study was conducted to know the effect of various microbial inoculants as liquid biofertilizers on growth and yield of moringa for leaf production.

Materials and methods

Annual moringa variety PKM-1 seeds were obtained from the Department of Vegetable crops, Horticultural College and Research Institute, TNAU, Periyakulam. The experiment was conducted in the field situated at 77° E longitude, 10° N latitude and at an altitude of 300 m above mean sea level (MSL). The nature of soil in the experimental plot was sandy loam with the pH of 7.2 and electrical conductivity of 0.39 dSm⁻¹. Water soluble

fertilizers viz., urea (46 % N), mono ammonium phosphate (12 : 61 % of N and P₂O₅), potassium nitrate (13: 45 % of N and K₂O) and All 19 (19: 19: 19 % of N, P₂O₅ and K₂O) were used as inorganic sources of nutrients. *Azospirillum* (*Azospirillum brasilense*), phosphate solubilizing bacteria (*Bacillus megaterium*), potassium solubilizing bacteria (*Bacillus mucilaginosus*), pink pigmented facultative methylotrophs (*Methylobacterium* sp) were used as microbial inoculants. The experimental field was ploughed three times to bring the soil to fine tilt. Before last ploughing FYM at 25 t ha⁻¹ and *Pseudomonas fluorescens* at 2.5 kg ha⁻¹ were applied as basal and incorporated in the soil. Raised beds of 160 cm width and 20 cm height were prepared. Drip fertigation system was installed to supply irrigation water, water soluble fertilizer and liquid bio fertilizers. The seeds were sown in raised bed with spacing of 40 cm × 20 cm (Baby, 2012; Jaison, 2016) and the beds were irrigated.

The fertilizer doses for fertigation were worked out by taking account of the N, P₂O₅ and K₂O contents present in the fertilizers used. A dosage of 27.0 : 4.5 : 9.0 g of NPK / m² was taken as 100% dose (Jaison, 2016). Inorganic water soluble fertilizers were applied as per the treatment in four stages in all the treatments on 20, 50, 80 and 110 days after seed sowing as mentioned in Table-1.

Following treatments were applied: T₁ = RDF 100 % (control); T₂ = RDF 100 % + *Azos* + PSB + KSB; T₃ = RDF 125 % + *Azos* + PSB + KSB; T₄ = RDF 75 % + *Azos* + PSB + KSB; T₅ = RDF 100 % + PPFM; T₆ = RDF 100 % + *Azos* + PSB + KSB + PPFM; T₇ = RDF 125 % + *Azos* + PSB + KSB + PPFM; T₈ = RDF 75 % + *Azos* + PSB + KSB + PPFM (RDF – Recommended dose of fertilizers, *Azos* – *Azospirillum*, PSB – Phosphate solubilizing bacteria, KSB – Potassium solubilizing bacteria, PPFM – Pink Pigmented Facultative Methylotrophs)

Table 1. Schedule of inorganic water soluble fertilizer application through fertigation system

Nutrient	RDF	Time of application				Total (kg/ha)
		20 DAS (kg/ha)	50 DAS (kg/ha)	80 DAS (kg/ha)	110 DAS (kg/ha)	
N	100	67.50	67.50	67.50	67.50	270.00
	125	84.40	84.40	84.40	84.40	337.50
	75	50.60	50.60	50.60	50.60	202.50
P	100	36.00	4.50	2.25	2.25	45.00
	125	45.00	5.60	2.80	2.80	56.25
	75	27.00	3.40	1.70	1.70	33.75
K	100	22.50	22.50	22.50	22.50	90.00
	125	28.10	28.10	28.10	28.10	112.50
	75	16.80	16.80	16.80	16.80	67.50

DAS – Days after sowing. RDF: Recommended dose of fertilizer

Table 2. Effect of microbial consortia on fresh leaf weight of moringa var. PKM-1

Treatments	Fresh leaf weight g plant ⁻¹			Fresh leaf weight kg plot ⁻¹			Dry leaf weight g plant ⁻¹			Dry leaf weight kg plot ⁻¹		
	I harvest	II harvest	III harvest	I harvest	II harvest	III harvest	I harvest	II harvest	III harvest	I harvest	II harvest	III harvest
T ₁	43.84	51.57	54.48	7.89	9.28	9.81	8.60	9.73	10.48	1.55	1.75	1.89
T ₂	52.71	58.86	63.33	9.49	10.59	11.40	10.34	11.11	12.18	1.86	2.00	2.19
T ₃	65.00	65.64	70.16	11.70	11.82	12.64	12.38	12.74	13.50	2.23	2.29	2.43
T ₄	44.50	54.31	56.95	8.01	9.78	10.26	8.72	10.25	10.96	1.57	1.84	1.97
T ₅	47.70	56.36	57.15	8.59	10.14	10.29	9.35	10.63	10.99	1.68	1.91	1.98
T ₆	56.32	60.74	68.67	10.14	10.93	12.36	11.04	11.46	13.21	1.99	2.06	2.38
T ₇	67.31	70.08	72.34	12.12	12.61	13.01	13.20	13.22	13.90	2.34	2.38	2.50
T ₈	53.01	54.91	61.00	9.54	9.88	11.09	10.39	10.36	11.85	1.87	1.86	2.13
Mean	53.80	59.06	63.01	9.69	10.63	11.36	10.50	11.19	12.13	1.89	2.01	2.18
SE (d)	4.97	3.27	2.77	0.90	0.59	0.50	1.02	0.60	0.53	0.18	0.11	0.96
CD (0.05)	10.67	7.00	5.94	1.93	1.26	1.07	2.20	1.28	1.14	0.40	0.23	0.20

Liquid form of bio inoculants viz., *Azospirillum*, PSB, KSB were applied through the fertigation system 15 days after the application of inorganic fertilizers in four stages viz., 35, 65, 95 and 125 DAS. (Khan and Chattopadhyay, 2009; Doifode and Nandkar, 2014). The standard inoculum of PPFM isolates was diluted at 1:100 ratio of sterile water and 0.1% surfactant (Tween-20) and sprayed using hand sprayer on the leaves @ 500 litres of spray fluid per hectare (Holland and Polacco, 1994) during 35, 65, 95, 125 DAS. First harvesting of moringa leaf was commenced 60 days after sowing at the height of 45 cm above the ground level and subsequent leaf harvests were done at every 45 days intervals at the same height of first harvest and leaf yield was calculated (Newton *et al.*, 2006; Baby, 2012; Jaison, 2016).

Estimation of nutrients, mineral and other quality parameters:

The nitrogen content in the plant sample on dry weight basis was estimated by Micro Kjeldhal method (Humphries, 1956) and expressed in percentage. The phosphorus, potassium, calcium and magnesium content of dried leaf sample were estimated as per the method proposed by Jackson (1973) and expressed in percentage.

The iron content of leaves was estimated as per the method described by Jackson (1973) and expressed in parts per million (ppm). The ascorbic acid content of dried leaf sample was estimated as per the method described by Harris (1935) and expressed in mg 100 g⁻¹. The tocopherol was estimated in the dried leaf samples by the Emmerie-Engel reaction as reported by Rosenberg (1992) and expressed in mg 100 g⁻¹.

Data analysis: The data recorded were subjected to statistical analysis as per the method suggested by Panse and Sukhatme (1985). The significance of the mean difference between different treatments was determined by computing the standard error and critical difference.

Results and discussion

Effect on leaf yield: Achieving maximum yield per unit area without affecting the quality is the primary goal in any production system. Effect of microbial consortia on leaf fresh weight per plant was found to be significantly different among the various treatments. Application of 125% RDF along with *Azos* (500 mL ha⁻¹), PSB (500 mL ha⁻¹), KSB (500 mL ha⁻¹) through fertigation system and foliar application of PPFM (500 mL ha⁻¹) recorded the highest fresh and dry leaf weight and this finding is in accordance with the reports of Makinde (2013); Abdullahi *et al.* (2013); Jaison (2016) in moringa. Availability of photosynthates and the

Table 3. Effect of microbial consortia on N,P,K content in leaves of moringa var. PKM-1

Treatments	Nitrogen content (%)			Phosphorus content (%)			Potassium content (%)		
	I harvest	II harvest	III harvest	I harvest	II harvest	III harvest	I harvest	II harvest	III harvest
T ₁	2.36	2.38	2.39	0.15	0.16	0.17	0.15	0.16	0.17
T ₂	2.39	2.40	2.40	0.17	0.18	0.18	0.17	0.18	0.18
T ₃	2.40	2.42	2.42	0.19	0.19	0.19	0.19	0.19	0.19
T ₄	2.37	2.38	2.39	0.16	0.17	0.18	0.16	0.17	0.18
T ₅	2.38	2.38	2.40	0.16	0.18	0.19	0.16	0.18	0.19
T ₆	2.42	2.43	2.44	0.18	0.19	0.19	0.18	0.19	0.19
T ₇	2.43	2.45	2.47	0.19	0.19	0.20	0.19	0.19	0.20
T ₈	2.38	2.39	2.40	0.16	0.18	0.19	0.16	0.18	0.19
Mean	2.39	2.40	2.41	0.17	0.18	0.19	0.17	0.18	0.19
SE (d)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
CD (0.05)	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02

Table 4. Effect of microbial consortia on Ca, Mg and Fe content in leaves of moringa var. PKM-1

Treatments	Calcium (%)			Magnesium (%)			Iron (ppm)		
	I harvest	II harvest	III harvest	I harvest	II harvest	III harvest	I harvest	II harvest	III harvest
T ₁	2.10	2.12	2.15	0.56	0.60	0.61	122	128	131
T ₂	2.17	2.18	2.20	0.59	0.62	0.62	137	138	145
T ₃	2.37	2.38	2.40	0.60	0.64	0.64	152	159	162
T ₄	2.16	2.17	2.20	0.57	0.60	0.61	133	131	142
T ₅	2.17	2.18	2.21	0.58	0.60	0.62	128	132	134
T ₆	2.28	2.30	2.32	0.62	0.65	0.66	143	143	148
T ₇	2.39	2.42	2.44	0.63	0.67	0.69	160	161	169
T ₈	2.14	2.17	2.20	0.58	0.61	0.62	135	139	140
Mean	2.22	2.24	2.26	0.59	0.62	0.63	139	141	146
SE (d)	0.02	0.02	0.02	0.01	0.01	0.01	0.12	0.17	0.35
CD(0.05)	0.04	0.04	0.04	0.02	0.02	0.02	0.26	0.34	0.76

energy conversion throughout the crop growth period has the direct relation with the biomass yield (Hedge and Srinivas, 1989). Nitrogen, phosphorus and potassium has a dramatic effect on photosynthetic efficiency of the crop and supports the vegetative growth. Besides fixation of atmospheric nitrogen, production of siderophores that chelates ions and make it available to the plant root, solubilization of minerals such as phosphorus and potassium and synthesis of phytohormones by the microbes might have also played a nominal role in better yield (Glick, 1995), which corroborates with the findings of Singh *et al.* (2012) and Das Ranjtkumar *et al.* (2015) in kasuri methi and kale, respectively. Moreover, acceleration of these growth parameters might be due to influence of nitrogen, which is the chief constituent of proteins. It is essential for the formation of protoplasm which might have lead to cell division and cell enlargement and thereby the increased biomass yield. Phosphorus is the second most important macro nutrient limiting the growth of crops. Plants acquire P from soil but, approximately 95–99 % might be in the form of insoluble phosphates (Abou El-Yazeid *et al.*, 2007). As a result, the amount of available 'P' to plants is usually a small proportion of the total phosphorous. Soil and seed inoculation with phosphate solubilizing bacteria (PSB) improves solubilization of fixed soil phosphorus and of applied phosphates, resulting in higher crop yields as reported earlier (Toro *et al.*, 1997; Bhattacharya and Jain, 2000 ; Chen *et al.*, 2006). This could be one of the reason for better crop growth and leaf yield in moringa. This is in accordance with the findings of Poonia and Dhaka (2012) in tomato.

Potassium plays significant roles in the activation of several metabolic processes, including photosynthesis, protein synthesis, and enzymes, as well as in resistance to diseases and insects in plants (Rehm and Schmitt, 2002). Though Indian soils are referred as potassium cake, only 1-2 % of this is available to plants, the rest being bound to other minerals and therefore unavailable to plants. The potassium is made available to plants when the minerals are slowly weathered or solubilized (Bertsch and Thomas, 1985). Mineral potassium solubilization by microbes (KSB) enhances crop growth and yield (Rajan *et al.*, 1996). It is evident from the results of this study that the uptake and translocation of nutrients in moringa was more pronounced when biofertilizers were applied with inorganic nutrients, there by confirming the synergistic interaction between biofertilizers and inorganic nutrients. This result is in line with the findings of Verma *et al.* (2011) in cauliflower. Biofertilizer inoculation enhanced phytohormone production (Anjappa *et al.*, 2012) in cucumber, nitrogen fixation (Bashyal, 2011) in cauliflower, phosphate and potassium solubilization (Azza *et al.*, 2014) in moringa, nutrient absorption (Deshmukh *et al.*, 2014) in cluster bean and specific activities of enzymes (Shams *et al.*, 2013) in lettuce involved in the metabolic pathway might be the reason behind growth and yield improvement in moringa. These results are in conformity with the findings of Anurajan (2003) in tomato.

Effect on quality parameters: Nitrogen, phosphorus, potassium, calcium, magnesium, iron, ascorbic acid, Vitamin E, crude protein, crude fibre, total carbohydrate and total phenols are the

important quality parameter of moringa leaves estimated in this study. Application of higher dose of inorganic fertilizers along with the microbial inoculum showed higher values of leaf quality parameters. Since application of higher dose of nitrogen (125 %) and *Azospirillum* fixes the native, added and atmospheric nitrogen which in turn might have enhanced the nutrient uptake and leaf nitrogen content. Increased nutrient status in different plant parts are due to accumulation of carbohydrates, which may take place gradually with the advancement of crop growth. The results are similar with the findings of Azza *et al.* (2014); Attia *et al.* (2014) in leaves of moringa and Syed *et al.* (2016) in peas leaves.

Phosphorus is very important for energy transfer in plant system. The highest concentration of phosphorus in leaves might be due to application of phosphate solubilizing bacteria which mobilized the non labile iron and aluminium phosphates as well as added insoluble phosphate by bringing about changes in the soil environment by producing chelating agents and organic acids. Alternatively, higher application of P may expose the plant nutrient to larger surface area that can enhance fixation of the nutrient and higher nutrient uptake efficiency. Moreover application of P might have helped in the root proliferation leading to the formation of more number of feeder roots aiding in the uptake of available nutrients, resulted in higher nutrients content in leaves. These results are in conformity with the findings of Ahmed *et al.* (2016) in moringa.

Potassium being a protoplasmic factor is an essential plant nutrient. Many enzymes are activated by potassium and it is also involved in photo and oxidative phosphorylation thus augmenting the energy required for growth and development. Application of RDF 125% + *Azospirillum* + PSB + KSB + PPFM registered the highest concentration of potassium in leaves. These results are in agreement with the findings of Ahmed *et al.* (2016) and Attia *et al.* (2014) in moringa.

The minor elements *viz.*, calcium, magnesium and iron showed significant variation among the treatments and found to be highest in the treatment RDF 125% + *Azospirillum* + PSB + KSB + PPFM. It is well known that the external addition of microbial consortium

Table 5. Effect of microbial consortia on ascorbic acid and vitamin E content in leaves of moringa var. PKM-1

Treatments	Ascorbic acid (mg 100g ⁻¹)			Vitamin E (mg 100g ⁻¹)		
	I	II	III	I	II	III
T ₁	12.71	12.83	12.76	46.05	46.75	45.36
T ₂	13.05	13.48	14.05	49.17	48.54	48.99
T ₃	14.44	14.68	15.12	50.62	51.16	52.18
T ₄	12.75	12.88	13.16	46.11	46.75	46.90
T ₅	12.78	13.10	13.22	47.20	47.16	47.23
T ₆	13.46	13.61	13.93	48.34	48.58	50.35
T ₇	14.73	15.11	15.35	51.14	52.58	53.70
T ₈	12.88	12.93	13.12	46.23	46.81	46.74
Mean	13.35	13.58	13.84	48.11	48.54	48.93
SE (d)	0.10	0.20	0.18	0.60	0.19	0.43
CD (0.05)	0.20	0.44	0.40	1.30	0.41	0.93

*: I, II, III harvest

with inorganic certainly increase the leaf nutrients and vitamin status. This might be due to exposure of plant with more of nitrogen, which increases protein production. The increase in quality due to application of microbial consortium with 125% RDF could be attributed to the enhanced photosynthetic and metabolic activities which resulted in higher amounts of nutrients. The increase in ascorbic acid content in microbial consortium with 125% RDF inorganic fertilizers treated plants might be ascribed to better availability and uptake of nutrients from soil, which helps in the synthesis of chlorophyll and enhanced ascorbic acid content. Similar findings were also reported by Singh *et al.* (2012) in kasuri methi leaves and Mishra *et al.* (2014) in snow pea pods.

In conclusion, moringa plants that received RDF at 125 percent of the recommended dose of inorganic fertilisers (337.5: 56.25: 112.5 kg NPK ha⁻¹) through drip irrigation and a liquid form of bio inoculants, namely *Azospirillum*, phosphate solubilizing bacteria, and potassium solubilizing bacteria @ 500 mL each ha⁻¹, as well as foliar application of pink pigmented facultative methylotrophs @ 500 mL ha⁻¹ produced higher biomass yield.

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