J. Soils and Crops 32 (2) 373-377, December 2022

ISSN 0971-2836 (Print)
ISSN 2582-2756 (Online)

EFFICACY OF BIO-FERTILIZERS ON KHASI MANDARIN (*Citrus reticulata*, Blanco)

Arunima Gogoi[1] and A. C. Barbora[2]

ABSTRACT

Present study was conducted at Citrus Research Station, Tinsukia, Assam during 2013-2018 to find out the effect of biofertilizers on yield, quality and nutrient content of Khasi mandarin. The data showed that application of 75% Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant^{-1}) + Azadirachtin (1% at 3-4 ml litre^{-1} as spray) + *Pseudomonus fluorescence* (30-40 ml plant^{-1}) found to be effective in improving the yield, soil nutrient status and quality of Khasi mandarin with B: C ratio 2.94. Among the five different treatments tested, the treatment (T_4) having 75% Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant^{-1}) + Azadirachtin (1% at 3-4 ml litre^{-1} as spray) + *Pseudomonus fluorescence* (30-40 ml plant^{-1}) was found to be effective for improved vegetative growth as compared to other treatments. Results revealed that maximum plant height (4.51m) and canopy volume (33.16 m^3) was observed in treatment T_4. Regarding, fruit qualities, higher juice content (48.7%), TSS (11.2^{0}Brix) and maximum number of fruits tree^{-1} (483) were observed in above mentioned treatment (T4). Maximum soil nutrient status and higher organic carbon content (1.25 %) were recorded under the same treatment. Significantly higher soil fertility status and superior N, P, K content on leaf were observed under this treatment.

(Key words: Khasi mandarin, biofertilizers, vermicompost, yield, quality, nutrient status)

INTRODUCTION

Biofertilizers plays an important role in increasing availability of nutrients and productivity in sustainable manner.Vermicompost enhances plant growth, improve soil fertility status, increase production, suppresses disease in plants, increases porosity and microbial activity in soil, enhance enzymic activities and improves water retention and aeration.Vermicompost increase the chlorophyll content, carbohydrate and protein content and improve the quality of fruits and seeds (Moghadam *et al*.,2014, Asefa and Alemayehu, 2021). Vermicompost poses outstanding biological properties and have microbial populations significantly larger and more diverse compared to conventional compost (Singh *et al.*, 2021). *Trichoderma* fungi and *Pseudomonas* fluorenses bacteria which are soil borne microorganisms have the potential to be used to help plant growth. Trichoderma, besides having a role in protecting plants from pathogenic disorders in rhizofer, has the ability as a biofertilizer agent because of some of its superior characteristics, namely degrading organic matter to produce nutrients and produce growth regulating compounds for plants, (Srivastava *et al.*, 2010). *P. fluorescens* bacteria is one type of bacteria that has the ability to produce growth regulating compounds such as auxin, producing enzymes that are capable of working in the P-organic mineralization process into P-in organic which is available to plants. Both types of microbes can be used together to help the growth of vegetative plants and maintain plant health so that plants are able to produce well (Buysens *et al.*, 2016) Trichoderma produces several enzymes which degrade organic matter to produce nutrients and produce compounds that play a role in promoting plant growth. (Mei *et al.*, 2019).

Citrus is one of the largest fruit industries in the world having nutracutical properties. In India, citrus holds a prominent place among the major commercial fruits covering an area of about 1003 thousand ha with an annual production of 12546 thousand metric tons and productivity of 12.5 t ha^{-1} (Anonymous, 2018). Among the Citrus fruits, Khasi mandarin covering an area of 14.95 thousand ha, and production of 203.72 thousand metric tons in Assam (Anonymous, 2018), whereas it occupies 1.47 thousand ha area and 24.37 thousand metric tons production in Tinsukia with highest productivity of 15.8 t ha^{-1}(Anonymous, 2018).

Khasi mandarin (*Citrus reticulata* Blanco) is the most economically important citrus fruit crops available in north-eastern region. The Khasi mandarin (*Citrus reticulata*), commonly known as orange, produced in this region is famous in India for its superior quality in respect of its flavour, Juice content, soluble sugar and acidity ratio. The soil climatic conditions of this region are most suitable for its production and it has the potentiality to generate livelihood in the rural areas substantially.

1. Jr. Scientist, Regional Agricultural Research Station, Assam Agricultural University, Nagaon-782002
2. Retired Chief Scientist, Citrus Research Station, AAU, Tinsukia-786125

The nutritional requirement of Khasi mandarin varied widely owing to its perennial in nature. Mandarins, being a commercially important fruit crop, proper and correct dose of organic, inclusive of bio-fertilizers need to be evaluated to ensure quality of fruits, high economic productivity and sustaining the nutrition of the plant at a desirable level. Moreover, the quantification of most of the bio-fertilizers to substitute a unit quantity of chemical fertilizer are yet to be established in most of the fruit crops. Keeping all these aspects in view, the present study aims to find out the effect of bio-fertilizers on yield, quality and nutrient content of citrus.

MATERIALS AND METHODS

An experiment was carried out on twelve years old Khasi mandarin plot in the farm of Citrus Research Station, Tinsukia of Assam during 2013-2018 to find out the effect of bio-fertilisers on yield, quality and nutrient content of Khasi mandarin. The experiment was laid out with 5 m x 5 m spacing along with five different treatments viz., T1 : control, T2 = 100% Vermicompost (on N equivalent basis of RDF) ,T3 =75% Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant^{-1}) + Azadirachtin (1% at 3-4 ml litre^{-1} as spray), T4 =75% Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant^{-1}) + Azadirachtin (1% at 3-4 ml litre^{-1} as spray) + *Pseudomonus fluorescence* (30-40 ml plant^{-1}) and T5= 50 % Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant^{-1}) + Azadirachtin (1% at 3-4 ml litre^{-1} as spray) + *Pseudomonus fluorescence* + *Azotobacter chroococcum* (30-40 ml plant^{-1}). The treatments were applied in randomized block design with four replications having four plants each.

Biofertilizers except vermicompost were applied as single dose during the month of September. Vermicompost were applied in two equal splits in the month of February and September. Growth parameters (Plant height, Stem girth, East-West spread, North -South spread, canopy volume) were measured by using standard procedure. Soil chemical properties (pH, organic carbon, available nitrogen, available phosphorus, and available potassium) over the years were determined as per the method outlined by Jackson (1973). Organic carbon content was determined by wet oxidation method (Walkley and Black, 1934). Number of fruits, average fruit weight, and other quality parameters (juice content, TSS, acidity, Ascorbic acid, Shelf life, yield) were estimated by adopting the standard techniques. Leaf samples were collected during the month of March (after flowering).Leaf N content was estimated by Kjeldhal Method (Jackson, 1973), P content was estimated by vanadomolybdo phosphoric acid yellow colour method as described by Jackson (1973) and K contents were estimated by ammonium acetate extraction method using Flame photometer (Jackson, 1973). S content in leaf was estimated by Turbidimetric method outlined by Chesnin and Yien (1951).Ca, Mg were determined by using complexometric titration method (Baruah and Barthakur, 1998). Fe, Mn, Cu and Zn were measured by using DTPA extractable method by using Atomic Absorption Spectrophotometer (Soltanpour and Schuwab ,1977).Microbial population of bacteria, fungi, Actinomycetes and Azospirillum in soil were observed before fruiting and after harvesting of fruits. Microbial population of bacteria, fungi, Actinomycetes and Azospirillum in soil were counted by serial dilution method (Baruah and Barthakur, 1998).Benefit: Cost ratio was determined after pooling the data over the years of experiment. The data generated in five consecutive years *viz.*, 2013 to 2018 were pooled and used to prepare analysis of variance table and accordingly C.D. and SE(m) were computed as described by Panse and Sukhtame (1954).

RESULTS AND DISCUSSION

Soil characteristics

Initially soils were acidic (pH5.1) in nature with high organic carbon contents (0.89%). Initial available N, P_2O_5 and K_2O content in soils were found in Table 2.

After application of above said treatments significantly maximum available N, P_2O_5 and K_2O content with higher organic carbon contents in soils were observed with the application of 75% Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant^{-1}) + Azadirachtin (1% at 3-4 ml litre^{-1}as spray) + *Pseudomonus fluorescence* (30-40 ml plant^{-1})

In Khasi mandarin, nosignificant differences were observed in leaf nutrient content *viz.*, N, P and K, Ca, Mg, S, Fe, Mn, Cu and Zn.

Microbial population of bacteria, fungi, Actinomycetes and Azospirillum in soil were also found higher in the same treatment. The application of vermicomposts in the field enhances the quality of soils by increasing microbial activity and microbial biomass which are key components in nutrient cycling, production of plant growth regulators and protecting plants from soil-borne diseases and arthropod pest attacks (Arancon and Edwards, 2005).

Growth

Plant height (4.51m) and canopy volume (33.16 m^3) were maximum by the application of 75% Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant^{-1}) + Azadirachtin (1% at 3-4 ml litre^{-1} as spray) + *Pseudomonus fluorescence* (30-40 mlplant^{-1}) (Table1). This might be due to activities of the biofertilizers *viz.,*nitrogen fixation, release and solubilize the Pi from insoluble phosphate, mobilize the phosphate, production of phytohormones etc. with simultaneous uptake of nutrients. Application of vermicompost increased the growth, yield and quality of beans because vermicompost improved the physical conditions of the soil which support better aeration to plant root, drainage of water, facilitation of actions N+, P+ and K+ exchange, sustained availability of nutrients, and thereby the uptake by the plants resulting in better growth (Mahmoud and Gad, 2020; Manivannan, 2009).Trichoderma singly increased plant height, number of leaves, stover dry weight and root dry weight of soybean even under shade conditions; (Miftahurrohmat and Sutarman, 2019). *P. fluorescens* is a bacterium that is capable of producing compounds that can facilitate the process of phosphate release in the soil. This bacterium also produces metabolites which act as regulators of plant growth. In this trial, until the vegetative final phase, it appears that bacteria behave as users of the resources produced by Trichoderma activity(Attarzadeh *et al*., 2019). *Pseudomonas fluorescens* is one among PGPR which promotes plant growth, leaf nutrient contents and yield of banana (Ramesh and Ramassamy , 2015). Azadirachtin is a broad-spectrum insecticide, its acts as a feeding deterrent, insect growth disruptor (IGD), and sterilant and is used to control various agricultural pest species, including Coleoptera, Heminoptera, Diptera, Orthoptera, and Isoptera (Morgan, 2009).

Yield

Application of 75% Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant^{-1}) + Azadirachtin (1% at 3-4 ml litre^{-1}as spray) + *Pseudomonus fluorescence* (30-40 ml plant^{-1}) gave highest yield of 23.23 tha^{-1} followed by the application of 100% Vermicompost (on N equivalent basis of RDF) (Table 3).Considering the statistics significant differences were observed in yield of Khasi mandarin**.** Improved yield might be due to application of biofertilizers as a result of availability of major and minor nutrients at all the essential stages of growth and development and improvement in physio-chemical properties of soil; increase in enzymatic activity, microbial population and also increase in plant growth hormones and it also helps to increase the biological nitrogen fixation, and availability of phosphorus which is required for strong vegetative growth and upon decomposition-release nitrogen and phosphorus contents and allele-chemicals leading to disease suppression.

Quality attributes of mandarin

Treatment T_4, i.e., application of 75% Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant^{-1}) + Azadirachtin (1% at 3-4 ml litre^{-1} as spray) + *Pseudomonus fluorescence* (30-40 ml plant^{-1}) was found significantly superior in quality. Higher juice content (48.7%), maximum TSS (11.2^{0}Brix), Ascorbic acid, 46.1%; and lowest acidity, 0.38% (Table 3) were observed in above mentioned T4 treatment. Shelf life was the highest (17 days) in this treatment over other treatment (though not significant). Better quality in this treatment might be due to positive effect of vermicompost on assimilates translocation, activation photosynthetic enzyme, chlorophyll formation and improvement of plant growth (Kohnaward *et al*.,2012) Maximum B: C ratio (2.94) was also recorded by this treatment (T_4).

From the results it is concluded that application of 75% Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant^{-1}) + Azadirachtin (1% at 3-4 ml litre^{-1} as spray) + *Pseudomonus fluorescence* (30-40 ml plant^{-1}) was found to be effective in improving the yield and quality of mandarin as compared to remaining treatment under study. Maximum plant height (4.51m), stem girth (40.50cm) and canopy volume (33.16 m^3) was observed in treatments T_4. Regarding, fruit qualities, higher juice content (48.7%), TSS (11.2^{0}Brix) and maximum number of fruits tree^{-1} (483) was observed in above mentioned treatment (T_4). Maximum soil nutrient status and higher organic carbon content (1.25%) were also recorded by the same treatments. Microbial population of bacteria, fungi, Actinomycetes and Azospirillum in soil were also found higher in the same treatment. The maximum B: C ratio (2.94) was found under this treatment(T_4) i.e., application of 75% Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant^{-1}) + Azadirachtin (1% at 3-4 ml litre^{-1} as spray) + *Pseudomonus fluorescence* (30-40 ml plant^{-1}).

Table 1. Effect of organics on growth parameters of Khasi mandarin (pooled data)

Treatments	Plant height (m)	Stem girth (cm)	E-W spread (m)	N-S spread (m)	Canopy volume (m^3)
T_1	3.90	37.33	2.13	2.32	20.14
T_2	4.15	37.77	2.24	2.30	22.30
T_3	4.39	39.65	2.44	2.65	29.66
T_4	4.51	40.50	2.56	2.75	33.16
T_5	4.08	37.67	2.13	2.28	20.69
SE(m)±	0.03	1.10	0.09	0.08	1.10
CD at 5%	-	-	-	-	3.30

Table 2. Effect of organics on nutrient status of Khasi mandarin(pooled data)

Treatments	Soil properties				
	pH	OC %	Av. N	Av. P_2O_5 (kg ha^{-1})	Av.K_2O
T_1	5.26	0.99	385	20.5	180.6
T_2	5.27	1.12	390	21.1	185.0
T_3	5.64	1.18	442	22.2	195.5
T_4	5.95	1.25	476	24.4	276.5
T_5	5.65	1.05	401	21.0	212.7
SE(m)±	0.04	0.03	1.90	1.4	2.5
CD at 5%	0.12	0.09	5.70	4.2	7.5
Initial	5.10	0.89	285	19.05	165.9

Table 3 : Effect of organics on quality attributes and B:C ratio of khasi mandarin (pooled data)

Treatments	Average fruit weight	Juice (%)	Acidity (%)	TSS (^{0}Brix)	Ascorbic acid (mg 100 ml)	No. of fruit tree^{-1}	Shelf life (days)	t ha^{-1}	B:C ratio
T_1	98.67	42.7	0.42	7.5	43.2	311.8	14	12.31	1.17
T_2	110.50	43.9	0.41	9.0	44.7	366.2	15	16.19	1.73
T_3	114.55	45.6	0.39	9.5	45.2	347.4	17	15.92	1.63
T_4	120.25	48.7	0.38	11.2	46.1	483.0	17	23.23	2.94
T_5	101.67	42.6	0.40	7.8	44.1	319.6	16	13.00	1.45
SE(m)±	0.50	0.03	0.03	0.04	0.03	8.0	0.03	1.40	-
CD at 5%	1.50	-	-	-	-	24.0	-	4.20	-

Table 5. Effect of organics on leaf nutrient content of Khasi mandarin plants (pooled data)

Treatments	N (%)	P (%)	K (%)	Ca (%)	Mg(%)	S (%)	Fe (ppm)	Mn(ppm)	Cu(ppm)	Zn(ppm)
T_1	1.81	0.17	0.98	0.74	0.34	0.07	90.6	36.6	2.6	9.7
T_2	1.88	0.18	0.89	0.74	0.35	0.06	87.2	37.5	3.1	9.7
T_3	1.85	0.18	0.89	0.78	0.38	0.06	89.6	37.4	2.6	9.8
T_4	1.98	0.20	0.96	0.86	0.43	0.07	91.5	36.7	3.6	10.5
T_5	1.93	0.17	0.89	0.78	0.42	0.07	91.4	36.1	2.7	10.2
SE(m)±	0.03	0.01	0.04	0.04	0.03	0.01	0.40	0.40	0.30	0.40
CD at 5%	-	-	-	-	-	-	-	-	-	-

Table 6. Effect of bio-fertilizers on microbial population of bacteria, fungi,Actinomycetes and Azospirillum in soil for Tinsukia condition

Treatments	Bacteria (CFUx10^6 gram^{-1} of oven dry soil)		Fungi (CFUx10^3 gram^{-1} of oven dry soil)		Actinomycetes (CFUx10^2 gram^{-1} of oven dry soil)		Azospirillum (CFUx10^5 gram^{-1} of oven dry soil)	
	Before	After	Before	After	Before	After	Before	After
T_1	20.5	22.5	18.9	19.6	5.8	6.5	2.3	4.8
T_2	21.4	24.5	18.4	20.2	6.1	7.9	4.4	7.2
T_3	22.6	25.7	20.7	22.1	7.2	8.9	5.7	7.8
T_4	27.6	32.9	22.5	24.9	8.4	10.8	6.9	12.4
T_5	19.3	23.9	19.7	20.8	6.5	7.8	3.9	6.7

REFERENCES

Anonymous, 2018. Horticultural Statistics at a glance, Government of India, Ministry of Agriculture and Farmers welfare, Department of Agriculture, Cooperation and Farmers Welfare. Horticulture Statistics Division.

Arancon, N.Q. and C. A. Edwards, 2005. Effects of vermicomposts on plant growth paper presented during the International Symposium Workshop on Vermi Technologies for Developing Countries (ISWVT, 2005), Los Banos, Philippines. APA Article. Soil Ecology Laboratory.

AsefaFayera and Wagari Alemayehu, 2021.Effect of organic and chemical fertilizers on growth, yield and yield component of soyabean. J. Soils and Crops,**31** (1):1-6.

Attarzadeh, M., H. Balouchi, M. Rajaie, M. M. Dehnavi and A. Salehi, 2019. Growth and nutrient content of *Echinacea purpureaas* affected by the combination of phosphorus with arbuscular mycorrhizal fungus and *Pseudomonas florescent* bacterium under different irrigation regimes. J. Environ.Manag.**231**:182–88.

Baruah, T.C. and H.P. Barthakur, 1998.A textbook of Soil Analysis.Vikash Publishing House Pvt. Limited. New Delhi.

Buysens, C., V. César, F. Ferrais, De Boulois, H. D. and S. Declerck, 2016. Inoculation of Medicago sativa cover crop with Rhizophagus irregularis and *Trichoderma harzianum* increases the yield of subsequently-grown potato under low nutrient conditions. App. Soil Ecolo.**105:**137–43.

Chesnin, L. and C.H.Yien, 1951. Turbidimetric Determination of Available Sulphur.Proceedings of Soil Science Society of America.**15**:149-151.

Jackson, M. L. 1973. Soil Chemical Analysis.Prentice Hall of India Pvt. Ltd., New Delhi.

Kohnaward, P., J. Jalilian and A. Pirzad, 2012.Effect of foliar application of micronutrients on yield and yield components of safflower under conventional and ecological cropping systems. I. R.J. App. and Basic Sci. **3** (7):1460-1469.

Manivannan, S., M. Balamurugan, K. Parthasarathi, G. Gunasekaran and L.S. Ranganathan, 2009.Effect of vermicompost on soil fertility and crop productivity-beans (*Phaseolus vulgaris*). J. Environ.Biol.**30**(2): 275-281.

Maskar, S.L.,P.B. Manjare, and M.G.Tonde, 2018. Effect of Biofertilizers and inorganic fertilizers on growth and yield of Sapota (*Manikara achras* (Mill) Forseberg) cv. Kalipatti. Int. J. Curr. Microbiol.App.Sci. **6:** 2606-2613.

Mei, L. M. Guang-shu, L. Hua, S Xiao-lin, T. Ying, H. Wen-kun, M. Jie and J .Xiliang, 2019. The effects of Trichoderma on preventing cucumber fusarium wilt and regulating cucumber physiology. J. Integrative Agric.**18**(3):607–17.

Miftahurrohmat, A. and Sutarman,2019. Local Soybean (*Glycine max* (L)) Stomatas' morphological and anatomic response in 3rd vegetation stage towards light intensity stress. IOP Conf. Series: J. Physics: Conf. Series 1232. 012043 doi:10.1088/1742- 6596/1232/1/012043.

Moghadam, M.K., H.H. Darvishi and M. Javaheri, 2014.Evaluation agronomic traits of soybean affected by vermicompost and bacteria in sustainable agricultural system.Int. J. Biosci.IJB**5** (9). 406-413

Morgan, E. D. 2009. Azadirachtin, a scientific goldmine.Bioorgan. Med. Chem. **17**: 4096–4105.

Panse, V. G. and P. V. Sukhatme, 1954.Statistical Methods for Agriculture Workers.Indian Coun. Agric. Res., New Delhi.

Ramesh, Y. and V. Ramassamy, 2015. Influence of pseudomonas fluorescens as biofertilizer in secondary hardening of tissue cultured banana var. poovan. Int. J.Appl.Sci. and Biotechnol.**3**(1):38-41.

Mahmoud, S.O. and D.A.M. Gad, 2020. Effect of vermicompost as fertilizer on growth, yield and quality of bean plants (Phaseolus vulgaris L.). Middle East J. Agric. Res.**9** (1): 220-226.

Singh Amritpal, Ram Pratap and Jagdeep Singh 2021, Response of maize (*Zeamaizs.* L.). to Integrated nutrient management. J. Soils and Crops,**31** (2) :237-42.

Soltanpour, P.N. and S.P. Schuwab, 1977. A new soil test for simultaneous extraction of macro and micro nutrients in alkaline soils. Comm. Soil. Sci. Plant Anal.**8**:195-207.

Srivastava, R., A.Khalid, U. S. Singh, and A. K .Sharma, 2010.Evaluation of arbuscular mycorrhizal fungus, fluorescent Pseudomonas and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. Biol. Control. **55**:24-31.

Walkley, A. J. and I.A. Black, 1934. Estimation of organic carbon by chromic acid titration method.Soil Sci. **37:**29-38.

Rec. on 28.06.2022 & Acc. on 04.07.2022