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# Effect of Bio-Fertilizers on Yield, Quality and Nutrient Content of Khasi Mandarin (*Citrus reticulata*, Blanco) in Assam

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# ABSTRACT

An experiment was carried out in 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 biofertilizers on yield, quality and nutrient content of Khasi mandarin. The experiment was laid out with 5 m  $\times$  5 m spacing along with 5 treatments, 4 replication and designed with RBD. The result revealed that application of 75% Vermicompost (on N equivalent basis of RDF) + Trichoderma harzianum (30-40 ml plant<sup>-1</sup>) + Azadirachtin (1% at 3-4 ml liter<sup>1</sup> as spray) + *Pseudomonus fluorescence* (30-40 ml plant<sup>-1</sup>) were found 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  $(T_A)$  having 75% Vermicompost (on N equivalent basis of RDF) + Trichoderma harzianum (30-40 ml plant<sup>-1</sup>) +

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Email : arunima\_gogoi123@yahoo.co.in \*Corresponding author Azadirachtin (1% at 3-4 ml liter<sup>1</sup> as spray) + *Pseudomonus fluorescence* (30-40 ml plant<sup>-1</sup>) 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<sup>3</sup>) were observed in treatment T<sub>4</sub>. Regarding, fruit qualities, higher juice content (48.7%), TSS (11.20Brix) and maximum number of fruits per tree (483) were observed in above mentioned treatment (T<sub>4</sub>). 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.

**Keywords** Khasi mandarin, Biofertilizers, <sup>v</sup>ermicompost, Yield, Quality.

# **INTRODUCTION**

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<sup>-1</sup> (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<sup>-1</sup> (Anonymous 2018)

Biofertilizers plays an important role in in-

creasing 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 Wagari 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 (Buysen 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).

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 flavor, 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.

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-fertilizers on yield, quality and nutrient content of Khasi mandarin. The experiment was laid out with 5 m  $\times$  5 m spacing along with five different treatments viz.,  $T_1$ : control,  $T_2$ = 00% Vermicompost (on N equivalent basis of RDF), T<sub>3</sub> =75% Vermicompost (on N equivalent basis of RDF) + Trichoderma harzianum (30-40 ml plant<sup>-1</sup>) + Azadirachtin (1% at 3-4 ml liter<sup>-1</sup> as spray),  $T_4 = 75\%$  Vermicompost (on N equivalent basis of RDF) + Trichoderma harzianum (30-40 ml plant<sup>-1</sup>) + Azadirachtin (1% at 3-4 ml liter<sup>-1</sup> as spray) + *Pseudomonus fluorescence* (30-40 ml plant<sup>-1</sup>) and  $T_{5} = 50$  % Vermicompost (on N equivalent basis of RDF) + Trichoderma harzianum (30-40 ml plant<sup>-1</sup>) +Azadirachtin (1% at 3-4 ml liter<sup>-1</sup> as spray) + Pseudomonus fluorescence + Azotobacter chroococcum  $(30-40 \text{ ml plant}^{-1})$ . 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). 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

Treatments		Soil properties						
	рН	OC %	Av. N	Av. $P_2O_5$ kg ha <sup>-1</sup>	Av.K <sub>2</sub> O			
T <sub>1</sub>	5.26	0.99	385	20.5	180.6			
T,	5.27	1.12	390	21.1	185.0			
$T_3^2$	5.64	1.18	442	22.2	195.5			
$T_4^{j}$	5.95	1.25	476	24.4	276.5			
$T_{5}^{\dagger}$	5.65	1.05	401	21.0	212.7			
SEm	0.04	0.03	1.9	1.4	2.5			
CD at 5%	0.12	0.09	5.7	4.2	7.5			
Initial	5.1	0.89	285	19.05	165.9			

Table 1. Effect of organic application on nutrient status of Khasi mandarin (compiled data).

content was estimated by Kjeldhal Method Jackson (1973), P content was estimated by vanadomolybdo phosphoric acid yellow color 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 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 fruit. 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 CD. and SE (m) were computed as described by Panse and Sukhatme (1954).

#### **RESULTS AND DISCUSSION**

## Soil characteristics

Initially soils were acidic (pH 5.1) in nature with high organic carbon contents (0.89%). Initial available N,  $P_2O_5$  and  $K_2O$  content in soils were found to be low (Table 1).

After application of above said treatments maximum available N,  $P_2O_5$  and  $K_2O$  content with higher organic carbon contents in soils were observed in  $T_4$ treatment involving application of 75% Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant<sup>-1</sup>) + Azadirachtin (1% at 3-4 ml liter<sup>-1</sup> as spray) + *Pseudomonus fluorescence* (30-40 ml plant<sup>-1</sup>). Statistical analysis revealed that significant differences was found in soil parameters i.e. organic carbon content, available N,  $P_2O_5$  and  $K_2O$  content in soil (Table 1).

Though leaf nutrient content of N, P and K and Zn, Ca were found maximum in  $T_4$  treatment (Table 2). In Khasi mandarin but no significant differences

Tree	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)
T,	1.81	0.17	0.98	0.74	0.34	0.07	90.6	36.6	2.6	9.7
$T_2^1$	1.88	0.18	0.89	0.74	0.35	0.06	87.2	37.5	3.1	9.7
$T_3^2$	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,	1.93	0.17	0.89	0.78	0.42	0.07	91.4	36.1	2.7	10.2
SÉm	0.03	0.01	0.04	0.04	0.03	0.01	0.40	0.40	0.30	0.4
CD at 5%	-	-	-	-	-	-	-	-	-	-

Table 2. Effect of organic application on leaf nutrient content of Khasi mandarin plants.

Trea- ments	Bacteria (CFUx10 <sup>6</sup> per gram of oven dry soil)		Fungi (CFUx10 <sup>3</sup> per gram of oven dry soil)		Actinomycetes (CFUx10 <sup>2</sup> per gram of oven dry soil)		Azospirillum (CFUx10 <sup>5</sup> per gram of oven dry soil)	
	Before	After	Before	After	Before	After	Before	After
T,	20.5	22.5	18.9	19.6	5.8	6.5	2.3	4.8
T,	21.4	24.5	18.4	20.2	6.1	7.9	4.4	7.2
T,	22.6	25.7	20.7	22.1	7.2	8.9	5.7	7.8
T,	27.6	32.9	22.5	24.9	8.4	10.8	6.9	12.4
Ţ	19.3	23.9	19.7	20.8	6.5	7.8	3.9	6.7

Table 3. Effect of bio-fertilizers on microbial population of bacteria, fungi, Actinomycetes and Azospirillum in soil for Tinsukia condition.

were observed in leaf nutrient content of N, P and K, Ca, Mg, S, Fe, Mn, Cu, and Zn.

Microbial population of bacteria, fungi, Actinomycetes and Azospirillum in soil were observed before flowering and after harvesting of fruit. Microbial population of bacteria, fungi, Actinomycetes and Azospirillum in soil were also found higher in the same treatment (Table 3). 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 soil-borne disease and arthropod pest attacks (Arancon and Edwards 2005).

## Growth

Maximum plant height (4.51m) and canopy volume (33.16 m<sup>3</sup>) was observed under the treatment ( $T_4$ ) involving with 75% Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant<sup>-1</sup>) + Azadirachtin (1% at 3-4 ml liter<sup>-1</sup> as spray) + *Pseudomonus fluorescence* (30-40 ml plant<sup>-1</sup>) (Table 4).This could be explained by the activities of

the biofertilizers viz. nitrogen fixation, release and solubilize the Pi from insoluble phosphate, mobilize the phosphate, production of phytohormones. 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). Trichoderma singly increased plant height, number of leaves, stover dry weight and root dry weight of soyabean 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-spec-

Table 4. Effect of organic application on growth parameter of Khasi mandarin (compiled data).

Freatments	Plant height (m)	Stem girth (cm)	E-W spread (m)	N-S spread (m)	Canopy volume (m <sup>3</sup> )	
T,	3.90	37.33	2.13	2.32	20.14	
T <sub>2</sub>	4.15	37.77	2.24	2.30	22.30	
T,	4.39	39.65	2.44	2.65	29.66	
T,	4.51	40.50	2.56	2.75	33.16	
T <sub>s</sub>	4.08	37.67	2.13	2.28	20.69	
SĔm	0.03	1.1	0.09	0.08	1.1	
CD at 5%	NS	NS	NS	NS	3.3	

Treatments	Average fruit weight	Juice (%)	Acidity (%)	TSS ( <sup>0</sup> Brix)	Ascorbic acid (mg/100 ml)	Nos. of fruit per tree	Shelf life (days)	t/ha	B:C ratio
T,	98.67	42.7	0.42	7.5	43.2	311.8	14	12.31	1.17
T,	110.50	43.9	0.41	9.0	44.7	366.2	15	16.19	1.73
T <sub>3</sub>	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	17	23.23	2.94
T <sub>5</sub>	101.67	42.6	0.40	7.8	44.1	319.6	16	13.0	1.45
SEm	0.5	0.03	0.03	0.04	0.03	8.0	0.03	1.4	-
CD at 5%	1.5	-	-	-	-	24.0	-	4.2	-

Table 5. Effect of organic application on quality attributes and B:C ratio of mandarin (compiled data).

trum 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

The higher yield (23.23 t ha<sup>-1</sup>) was found in  $T_{A}$ treatment with 75% Vermicompost (on N equivalent basis of RDF) + Trichoderma harzianum (30-40 ml plant<sup>-1</sup>) + Azadirachtin (1% at 3-4 ml liter<sup>-1</sup> as spray) + Pseudomonus fluorescence (30-40 ml plant<sup>-1</sup>) followed by T<sub>2</sub> treatment with 100% Vermicompost (on N equivalent basis of RDF) (Table 5). Statistical analysis revealed that 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 of 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. Changkija et al. (2018) reported that the application of Vermicompost in combination with biofertilizers are more effective in enhancing yield of Palak in Nagaland.

#### Quality attributes of mandarin

The fruit obtained under the treatment  $T_{4}$ , having

75% Vermicompost (on N equivalent basis of RDF) + *Trichoderma harzianum* (30-40 ml plant<sup>-1</sup>) + Azadirachtin (1% at 3-4 ml liter<sup>-1</sup> as spray) + *Pseudomonus fluorescence* (30-40 ml plant<sup>-1</sup>) was also found significantly superior in quality. Higher juice content (48.7%), maximum TSS (11.2 °Brix), Ascorbic acid, 46.1%; and lowest acidity, 0.38% (Table 5) were observed in above mentioned treatment (T<sub>4</sub>). Shelf life was the highest (17 days) 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 observed under this treatment (T<sub>4</sub>).

### CONCLUSION

From the results it is concluded that application of 75% Vermicompost (on N equivalent basis of RDF) + Trichoderma harzianum (30-40 ml plant<sup>-1</sup>) + Azadirachtin (1% at 3-4 ml liter<sup>1</sup> as spray) + Pseudomonus fluorescence (30-40 ml plant<sup>-1</sup>) was found to be effective in improving the yield and quality of mandarin compared to the rest of the treatments. Maximum plant height (4.51m), stem girth (40.50 cm) and canopy volume (33.16) were observed in treatment T<sub>4</sub>. Regarding, fruit qualities, higher juice content (48.7%), TSS (11.2 <sup>o</sup>Brix) and maximum number of fruits per tree (483) were observed in above mentioned treatment (T<sub>4</sub>). Maximum soil nutrient status and higher organic carbon content (1.25%) were recorded under the same treatment. Microbial population of bacteria, fungi, Actinomycetes and Azospirillum in soil were also found higher in the

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