ASIAN JOURNAL OF AGRICULTURE
Volume 7, Number 2, December 2023
Pages: 88-97

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g070204

Integrated nutrient management enhances the growth efficiency and productivity of *Tagetes erecta* cv. Sirakole

ANITA MOHANTY[1], SRINIVAS ACHARYA[2], BARSHA BHUSHAN SWAIN[3], SMRUTIREKHA MISHRA[3], DEBASISH MOHAPATRA[3], PRADIPTA KUMAR MOHAPATRA[3,♥]

[1]Department of Horticulture, College of Agriculture, Orissa University of Agriculture and Technology. Bhubaneswar 51003, India
[2]Government Autonomous College. Phulbani 762001, India
[3]Department of Botany, Ravenshaw University. Cuttack 753003, India. Tel.: +91-09437356901, ♥email: pradiptamoha@yahoo.com

Manuscript received: 5 March 2023. Revision accepted: 18 July 2023.

Abstract. *Mohanty A, Acharya S, Swain BB, Mishra S, Mohapatra D, Mohapatra PK. 2023. Integrated nutrient management enhances the growth efficiency and productivity of* Tagetes erecta cv. *Sirakole. Asian J Agric 7: 88-97.* The African marigold (*Tagetes erecta* L. cv. Sirakole) is a highly significant plant because it can be cultivated year-round, produce large blooms, have a long post-harvest life, and compactness. To determine the optimal fertilizer and manure combination, we conducted a study to explore the plant's morphological, yield, and physiological characteristics under different combinations of fertilizers, Vermicompost (VC), Poultry Manure (PM), and biofertilizer (*Azospirillium* + phosphate solubilizing bacteria). The most successful combination for promoting vigorous growth, dense canopy, prolific branching, longer flowering duration, large-sized flowers, and higher yield was achieved with 50% Recommended Dose of Nitrogen and Phosphorus (RDNP) + VC equivalent to 25% Recommended Dose of Nitrogen (RDN) + PM equivalent to 25% RDN + biofertilizers (@400 gha^{-1}). Additionally, plants in this combination exhibited higher chlorophyll content (Chl) and photosynthesis (P_N) than those in plots with RDNP alone or other amendments. While photosynthetic fluorescence yield (φP_0) and energy dissipation (φD_0) did not vary, plots with RDNP had lower excitation (Ψ_0) and electron movement (φE_0), as well as lower photosynthesis performance indices (PIs), compared to the nutrient combinations. Additionally, the organically amended plots showed higher microbial density and soil enzyme activities than those with RDNP alone. Our findings indicate that using a combination of fertilizer, manure, and biofertilizers is the most effective way to improve the photosynthetic capacity and flower yield of *T. erecta* cv. Sirakole.

Keywords: Nutrient management, OJIP fluorescence rise, photosynthesis, productivity, *Tagetes erecta*

INTRODUCTION

In recent decades, commercial horticulture has emerged as a promising avenue for supplementing the income and livelihood of small and marginal farmers in India. Commercial flower cultivation, in particular, has developed due to its short crop duration, consistent income for an extended period, and higher returns than other non-conventional crops. This has led to a continuous increase in the area under floriculture and loose and cut flower production for export in India. One of India's most commercially exploited flower crops is the African marigold (*Tagetes erecta* L., Family: Asteraceae), with several varieties such as giant double yellow, giant double orange, early yellow, early orange, and Sirakole being grown. Sirakole is particularly significant due to its all-season cultivation, compact flower clusters, long post-harvest life, and large attractive blooms. The crop can be propagated through seeds and cuttings and grows well in all soil types. For the winter crop, seeds are sown from October to November, and the seedlings are transplanted when they reach a height of 5-7 cm or through transplanting of cuttings in the first week of November. For the summer crop, cuttings are transplanted in early February. In addition to being used as loose flowers, African marigolds are a source of xanthophylls and a major pigment source for the poultry industry, food color, and dyeing of fabrics (Attokaran 2017).

Commercial marigold cultivation in India is more organized in Tamil Nadu, Karnataka, and West Bengal (Kaur et al. 2022). The quality of marigold flowers is strongly influenced by the nutrient levels in the soil, particularly nitrogen and phosphorus (Acharya and Dashora 2004). However, the selection and management of soil can be poor, and overuse of synthetic fertilizers and chemicals leads to environmental pollution and potential harm to soil microflora and fauna, affecting the overall benefit-cost ratio. Therefore, there is a need for combination farming, which involves using biological, organic, and balanced nutrient amendments for integrated soil fertility management and sustained commercial and profitable flower production.

Marigold is a high-demand crop for nitrogen and potassium, especially during its early growth stages. Farmers often use high nutrient dosages to improve growth and yield and separately apply inorganic fertilizers, organic manures, and biofertilizers. Previous studies have evaluated the effects of these nutrient regimes on marigold performance using growth and yield parameters (Borah et al. 2020; Choudhury et al. 2020; Reddy and Saravanan 2020). Foliar application of organic fertilizers has increased nutrient concentrations in marigolds. Furthermore,

integrated nutrient management, which combines inorganic nutrients, vermicompost, poultry manure, and biofertilizers, has also been tried on other varieties of *T. erecta* but not on Sirakole (Kaur et al. 2022). However, there is no report on intensive integrated nutrient management involving inorganic nutrients (nitrogen, phosphorus, and potassium), Vermicompost (VC), Poultry Manure (PM), and biofertilizers to evaluate the plant performance and the consistency in yield of *T. erecta* cv. Sirakole. The *T. erecta* cv. Sirakole is India's most preferred marigold variety for all-season cultivation. In addition, OJIP fluorescence and photosynthetic parameters have not yet been explored for evaluating the physiological efficiency and yield of horticultural crops under organic practice. On the other hand, in various crop species, OJIP fluorescence has been proven to be a valuable parameter for evaluating plant performance. Plant efficiency, particularly photosystem (PS) II behavior, is more accurately evaluated by prompt chlorophyll fluorescence (Strasser et al. 1995). The OJIP test and direct measurement of photosynthesis and other physiological processes give detailed information on the plants' physiological efficiency under different nutrient regimes (Chhotaray et al. 2014; Shasmita et al. 2022). This study reports the cultivation performance, growth, and physiological responses of *T. erecta* cv. Sirakole under different nutrient management and the results of this study provide recommendations for an ideal integrated nutrient combination for effective augmentation of flower quality and yield.

MATERIALS AND METHODS

Study area and treatment plot design

The experiment spanned a year and consisted of three crop phases – Kharif (July-October), Rabi (November-February), and Summer (March-June) plantations, which were initiated in the first week of July, November, and March, respectively. The experimental plots were chosen from Krishi Vigyan Kendra, Jajpur of Odisha University of Agriculture and Technology, Bhubaneswar, India. These plots had been under nursery use since 2007 and followed conventional cultivation practices. Different plots from the same field were chosen for each crop phase to ensure statistical rigor, with each plot treated as a replicate.

Before the commencement of the experiments, the plots were tilled, divided into 2.1 m x 1.8 m subplots having a 0.6 m gap between two adjacent plots. The subplots were allotted randomly, in triplicates, for each nutrient treatment. Well-decomposed farm yard manure (FYM @9.5 $kgplot^{-1}$ corresponding to 25 tha^{-1}) was uniformly spread on the plots, lightly irrigation, and soils were mixed thoroughly. The soil (0-15 cm on top) was analyzed for N, P, and K composition before transplantation. The N, P, and K contents before amendments were 251.8, 25.13, and 282.4 $kgha^{-1}$, respectively.

Six different treatment combinations of fertilizers and manures were made as per the proportions in Table 1. A common dose of K_2O (100 $kgha^{-1}$) was added to all the experimental plots except T1 three weeks before transplantation. This was followed by adding organic manures (VC and PM) in plots as per the proportions given in Table 1. Half the nitrogen, the full dose of phosphorus, and the remaining potash were added to plots at the time of transplantation. Biofertilizer preparation contains *Azospirillum* and PSB in a 1:1 ratio (400 gha^{-1}) in T5 and T6 at the time of transplantation. The other chemical fertilizer amendments were made per the proportion and schedule in Tables 1 and 2, respectively.

Table 1. The scheme of fertilizer and manure amendments of the experimental plots

Combination	Nutrient amendment
T1	No external nutrient addition
T2	The recommended dose of fertilizers [450 kg urea (209.79 kg nitrogen), 200 kg single super phosphate (13.97 kg phosphorus), and 200 kg mureate of potash (101.23 kg potassium] per hectare
T3	VC equivalent to 50% of RDN + PM equivalent to 50% of RDN
T4	50% RDNP + VC equivalent to 25% of RDN + PM equivalent to 25% of RDN
T5	VC equivalent to 50% of RDN + PM equivalent to 50% of RDN + Biofertilizers (*Azospirillum* and PSB in equal proportion) @ 400 gha^{-1}
T6	50% RDNP + VC equivalent to 25% of RDN + PM equivalent to 25% of RDN + Biofertilizers as in T5

Note: PM: Poultry Manure, RDN: Recommended Dose of Nitrogen, RDNP: Recommended Dose of Nitrogen and Phosphorus, VC: Vermicompost

Table 2. The scheme of amendments of manures and fertilizers in the plots during 120 days of the experiment

Schedule	Amendment
21 days before transplantation	FYM (25 tha^{-1}) in all plots + mureate of potash (100 $kgha^{-1}$) (except T1) + VC and PM
At the time of transplantation	Urea (225 $hgha^{-1}$) + mureate of potash (100 $kgha^{-1}$) + single super phosphate (200 $kgha^{-1}$)
30 days after transplantation	Urea (225 $kgha^{-1}$)

Note: N, P, and K were applied as urea, single super phosphate, and mureate of potash, respectively. The amendments were made in proportion mentioned in Table 1

Each plot was prepared by transplanting 20 seedlings (21 days old; raised from stem cutting) during the evening hours of 4.00 PM to 6.00 PM. The newly transplanted seedlings were irrigated immediately and subsequently watered regularly. Thirty Days After Transplantation (DAT), the first earthing-up operation was performed, and the remaining half dose of nitrogen was added. Therefore, pinching of the apical meristem of primary branches was initiated 30 days after transplantation to stimulate secondary branching. Subsequent pinching operations were carried out weekly until the first flower bud was observed. Furthermore, earthing-up operations were performed every 15 days until 90 DAT to ensure optimal plant growth and development.

Experimental procedure

The growth parameters were recorded during 30-120 days of observation at 30 days intervals. Plant height (cm), stem girth (cm) at 5 cm above the soil level, the number of primary and secondary branches and leaves per plant, canopy diameter (cm), and canopy cover (%) were recorded as the morphological parameters. The yield parameters viz., duration of 50% flower bud initiation (days), first flowering (days) and flowering period of the crop (days), fresh and dry weight of flowers (g), flower diameter (cm), number of flowers per plant, flower yield ($kgha^{-1}$), petal meal yield (gkg^{-1} flower) and xanthophyll yield (gkg^{-1} flower) were recorded during the cropping period following the methods described by Mohanty (2014). The Xanthophyll content of the flower was estimated by extraction with a combination extract (hexane: acetone: methanol: toluene: 10:7:6:7).

The physiological efficiency of the crop was measured between 8.00 AM-10 AM at 90 DAT from the fourth pair of fully opened leaves from the apex. The chlorophyll content of these leaves (rel units) was estimated by a SPAD meter (SPAD-502, Spectrum Technology Inc., USA). Photosynthesis (P_N), transpiration (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured in the field condition with the help of an Infrared Gas Analyzer (IRGA; CIRAS-2, PP-Systems) under ambient temperature and CO_2 concentration following the procedure described by Chhotaray et al. (2014). The range of leaf surface temperature, relative humidity, and ambient irradiance at the time of measurement during the year was 24.3-36.8ºC, 50.4-74.5%, and 785-1140 µE $m^{-2}s^{-1}$, respectively. For measuring respiration, the leaves were darkly adopted for 10 min in the cuvette (auto PLC 6, PP Systems), after which respiratory CO_2 evolution was measured in the dark using IRGA.

The OJIP fluorescence rise was measured using a plant efficiency analyzer (Handy PEA, Hansatech Instruments, UK) following the procedure described by Chhotaray et al. (2014). The fluorescence rise of O, J, I, and P peaks were taken after 20 µs (F_{20} µs), 2 ms (F_2ms), 20 ms (F_{20}ms), and at t_{FM} (F_M), respectively. The fluorescence parameters, viz., 2 ms relative variable fluorescence (V_J), the net rate of PS II closure (M_0), the quantum yield of primary photochemistry (φP_0), rate of trapped exciton movement beyond Q_A (Ψ_0), the quantum yield of electron transport (φE_0), the quantum yield of energy dissipation (φD_0), the performance index of primary photochemistry (PI_φ), performance index of exciton movement beyond Q_A (PI_Ψ), performance index on absorption basis (PI_{ABS}) and performance index of reduction of end electron acceptor of PS I (PI_{TOTAL}) were calculated using the fluorescence equations of Stirbet and Govindjee (2011).

The plant samples for uptake of N, P, and K were made by collecting the sample at 120 DAT. The whole plant was uprooted, washed thoroughly, and dried in a hot air oven for 48 h at 70ºC. The dried plant parts were ground to a fine powder in a 'Willey Mill.' This fine powder was again dried in an oven at 60ºC for 24 h before estimating total N, P, and K. Total nitrogen was measured by digesting the samples with concentrated H_2SO_4 and digestion mixture in Microkjeldhal assembly. For phosphorus and potassium determination, powdered plant samples were digested with a diacid mixture (HNO_3 + $HClO_4$ in a 3:2 ratio) after predigesting with concentrated nitric acid.

Moreover, a soil sample from the top 15 cm of each experimental plot was collected using a sterile steel corer, aseptically sealed in a polyethylene bag for the enzyme and microbial analysis. The samples were homogenized aseptically in the laboratory and were used for analysis. The microbial populations were estimated by the dilution plate method. The inoculated plates were incubated for 24 h at 37ºC for bacteria and at 25ºC for 72 h in the case of fungi, after which the colony-forming unit (CFU g^{-1} dry soil) was counted. Nutrient agar and potato dextrose agar media were used to grow bacteria and fungi. The activities of amylase, invertase, and cellulase were measured spectrophotometrically (Systronic, India) by taking starch, sucrose, and carboxymethyl cellulose as substrates, respectively. The absorbance values were quantified into enzyme activity as glucose produced ($mgg^{-1}soilh^{-1}$) using the regression equation of a glucose standard. The activities of protease and dehydrogenase were measured using sodium caseinate and trichloro-Triphenyl Tetrazolium Chloride (TTC) as substrates, and tyrosine and triphenyl formazan as standards, respectively, as described by Chhotaray et al. (2014). Phosphatase was extracted and quantified spectrofluorimetrically (excitation at 323 nm and emission at 452 nm) with the help of a Varian spectrofluorometer (Cary-eclipse, Varian) (Chhotaray et al. 2014).

Data analysis

Samples were collected from 5 plots of each amendment. Samples of in vivo observations were collected from 10 plot plants and averaged. Further, the data of five season plots were pooled together to serve as a replicate. The experiments were conducted in three crops grown in Kharif, Rabi, and Summer seasons in an equal number of plots and were repeated for two years. The values presented as tables and figures are thus pooled by six replicates (three seasons x two years). That means each parameter was separated using Duncan's Multiple Range Test (DMRT) tests at P=0.05.

RESULTS AND DISCUSSION

Growth and yield attributes

The morphological attributes of *T. erecta*, grown in different experiment plots, were measured for 120 d at 30 d intervals. No significant difference in plant height, stem girth, and number of branches was reported among T2 through T6 after 30 d of transplantation, though in T1, these attributes were significantly low. After 60 d of transplantation, however, all the mentioned morphological parameters were the highest in T6 and the lowest in T1. Minimum variation among the treatments was observed in the number of leaves per plant, whereas maximum variation was observed concerning canopy cover on all observation days (Table 3). Canopy diameter and cover density showed the most remarkable variation among the treatments indicating that the amendments appreciably encouraged the growth of plants. A comparison of T2 with T5 and T6 showed that T5 and T6 (biofertilizer amended) had better growth performance during 60 days than T2 (with RDNP). Consistently T6 showed the highest performance of all selected morphological attributes, followed by T5, T4, T2>T3>T1. Significant variation among the treatments was observed in the prolongation of the crop to > 90 d (Table 3). The morphological parameters followed the pattern T6>T5>T4>T2>T3>T1. T5 and T6 showed significantly high values of the morphological attributes during 120 days of observations compared to T2.

The yield characteristics of the crop were measured after 120 d of transplantation. No significant variation from T1 through T5 was observed concerning the initiation of flowering. In contrast, in T6, the duration for initiation of flowering (50% bud initiation and first flowering) was slightly prolonged compared to other treatments. However, significant variations among treatments were observed concerning flowering duration (d), flower diameter (cm), fresh wt and dry wt of flowers (g), number of flowers per plant, flower yield ($kgha^{-1}$), petal yield (gkg^{-1} flower) and xanthophylls yield (gkg^{-1} flower) (Table 4). All these parameters were found to be the highest in T6, whereas the lowest was recorded in T1, as expected. The flowering duration continuously increased from T1 through T6 and could be almost doubled in T6 compared to T1. Consequently, T5 and T6 had significantly bigger flowers, more flowers per plant, and higher yields than T1 and other nutrient combinations. However, the petal meal yield ($g\ kg^{-1}$ flower) did not vary significantly among treatments, whereas the xanthophyll yield (gkg^{-1} flower) of T1 was significantly lower than any of the nutrient combinations.

Table 3. The morphological attributes of *T. erecta* grown in different experimental plots at mentioned Days After Transplantation (DAT) during 120 d of cropping

DAT	Parameters	T1	T2	T3	T4	T5	T6
30	Plant height (cm)	12.83^{b}	27.30^{a}	28.36^{a}	28.11^{a}	26.91^{a}	27.53^{a}
	Stem girth (cm)	0.63^{b}	1.09^{a}	1.08^{a}	0.96^{a}	0.99^{a}	0.98^{a}
	Primary branches (no. $plant^{-1}$)	3.46^{c}	8.42^{a}	8.36^{a}	6.76^{b}	7.83^{a}	8.03^{a}
	Secondary branches (no. $plant^{-1}$)	10.43^{b}	17.33^{a}	17.73^{a}	16.06^{a}	16.93^{a}	17.82^{a}
	Leaves (no. $plant^{-1}$)	27^{a}	71^{a}	70^{a}	70^{a}	69^{a}	73^{a}
	Canopy diameter (cm)	8.67^{c}	23.15^{a}	21.72^{a}	18.85^{b}	22.24^{a}	22.63^{a}
	Canopy cover (%)	3.12^{c}	22.28^{a}	19.61^{a}	14.77^{b}	20.56^{a}	21.29^{a}
60	Plant height (cm)	25.56^{d}	43.06^{c}	43.9^{c}	45.54^{b}	44.83^{b}	48.84^{a}
	Stem girth (cm)	0.86^{c}	1.05^{b}	1.26^{a}	1.25^{a}	1.28^{a}	1.32^{a}
	Primary branches (no. $plant^{-1}$)	5.96^{c}	10.66^{b}	13.76^{a}	12.91^{a}	12.23^{a}	12.76^{a}
	Secondary branches (no. $plant^{-1}$)	16.36^{c}	31.43^{b}	35.16^{a}	35.32^{a}	34.43^{a}	33.96^{a}
	Leaves (no. $plant^{-1}$)	106^{b}	161^{a}	159^{a}	164^{a}	163^{a}	167^{a}
	Canopy diameter (cm)	12.84^{d}	29.37^{bc}	28.51^{c}	30.69^{ab}	29.48^{bc}	31.8^{a}
	Canopy cover (%)	6.85^{d}	26.13^{c}	31.28^{b}	39.16^{ab}	36.13^{b}	42.04^{a}
90	Plant height (cm)	34.36^{e}	48.36^{c}	44.13^{d}	51.43^{b}	53.66^{b}	57.6^{a}
	Stem girth (cm)	0.99^{e}	1.29^{c}	1.13^{d}	1.31^{c}	1.51^{b}	1.72^{a}
	Primary branches (no. $plant^{-1}$)	8.9^{e}	14.27^{c}	13.03^{d}	15.01^{c}	16.93^{b}	19.21^{a}
	Secondary branches (no. $plant^{-1}$)	24.03^{e}	39.22^{c}	36.06^{d}	41.26^{c}	45.13^{b}	50.16^{a}
	Leaves (no. $plant^{-1}$)	180^{e}	215^{c}	204^{d}	219^{c}	237^{b}	259^{a}
	Canopy diameter (cm)	16.55^{f}	33.94^{de}	32.47^{e}	34.58^{cd}	38.18^{b}	43.17^{a}
	Canopy cover (%)	11.39^{f}	42.41^{d}	29.13^{e}	49.71^{c}	60.60^{b}	77.48^{a}
120	Plant height (cm)	38.83^{e}	53.73^{c}	49.4^{d}	54.57^{c}	59.03^{b}	63.47^{a}
	Stem girth (cm)	1.05^{e}	1.32^{cd}	1.21^{d}	1.35^{c}	1.59^{b}	1.83^{a}
	Primary branches (no. $plant^{-1}$)	10.42^{e}	15.87^{cd}	14.73^{d}	16.75^{c}	18.77^{b}	21.37^{a}
	Secondary branches (no. $plant^{-1}$)	25.87^{e}	41.3^{c}	37.43^{d}	42.74^{c}	47.79^{b}	52.77^{a}
	Leaves (no. $plant^{-1}$)	203^{e}	240^{c}	226^{d}	246^{c}	265^{b}	281^{a}
	Canopy diameter (cm)	19.17^{e}	36.75^{c}	33.5^{d}	36.52^{c}	40.82^{b}	45.45^{a}
	Canopy cover (%)	15.28^{f}	47.35^{d}	33.77^{e}	55.45^{c}	69.27^{b}	85.88^{a}

Note: The means indicated by the same letter(s) in the superscript in a row are not significantly different from each other at P = 0.05 and n = 36. DMRT made the mean comparison

Photosynthesis and transpiration

A significant change in the chlorophyll content of *T. erecta* leaves, measured at 90 DAT, was observed with various treatments. The leaf chlorophyll content (in SPAD units) of T5 and T6 differed significantly from other amended and unamended plots (Table 5). Correspondingly, there was a great variation in P_N among treatments, which increased continuously from T1 through T6. Nevertheless, the plants in plots only with organic amendment (T3) had lower photosynthetic performance than in T2. However, E and g_s were not significantly influenced by the nutrient or organic amendments though there was a marginal increase in water loss from T1 through T6. The value of C_i was lower in T3 through T6 than in T1 and T2, though a corresponding pattern of change in g_s was not observed.

Consequently, the E/P_N showed a significant decreasing trend from T1 through T6. On the other hand, P_N/Chl showed an increasing trend, which was significant when compared to T1 but insignificant among T2 through T4 and between T5 and T6. Because of a continuous improvement of P_N, the WUE showed an increasing trend. In addition, T6 showed the highest WUE, 63% and 20% higher than T1 and T2, respectively. Similarly, the respiration rate of all amended plots was high, with T5 and T6 showing significantly higher respiration than other treatments.

The crop's biomass's total nutrient uptake (kgha^{-1}) during cropping time varied significantly among treatments (Table 6). Higher plant growth and improved crop yield resulted in a significantly high nutrient removal rate in the plots amended with manures or biofertilizers. The removal of N, P, and K was the highest in T6 and the lowest in T1. The nutrient accumulation by the plant biomass followed the pattern T6>T5>T4=T3>T2>T1.

Table 4. The yield characteristics of *T. erecta* grown in different experimental plots during 120 d of cultivation

Yield parameters	T1	T2	T3	T4	T5	T6
50% flower bud initiation (d)	50.77^{b}	49.63^{b}	50.53^{b}	48.93^{b}	51.47^{ab}	53.13^{a}
First flowering (d)	60.97^{ab}	59.14^{b}	59.73^{b}	58.92^{b}	60.27^{ab}	63.31^{a}
Flowering duration (d)	36.63^{e}	57.35^{c}	47.97^{d}	57.38^{c}	63.93^{b}	69.71^{a}
Flower diameter (cm)	3.52^{e}	4.77^{c}	4.46^{d}	4.85^{c}	5.32^{b}	5.76^{a}
Flower fresh weight (g)	4.13^{e}	5.43^{c}	5.12^{d}	5.52^{c}	5.95^{b}	6.37^{a}
Flower dry weight (g)	0.202^{e}	0.282^{c}	0.258^{d}	0.282^{c}	0.332^{b}	0.361^{a}
Flowers per plant (no.)	49.16^{e}	67.17^{c}	64.44^{d}	68.27^{c}	72.73^{b}	77.63^{a}
Flower yield (kgha^{-1})	9901^{e}	16001^{c}	14501^{d}	16400^{c}	17301^{b}	18201^{a}
Petal meal (g kg^{-1} flower)	78.97^{b}	79.51^{ab}	78.63^{b}	80.15^{a}	79.34^{ab}	80.67^{a}
Xanthophyll yield (g kg^{-1} flower)	1.873^{b}	2.188^{a}	2.126^{a}	2.217^{a}	2.211^{a}	2.266^{a}

Note: Same as for Table 3

Table 5. The photosynthetic and physiological performance of *T. erecta* grown in different experimental plots was measured at 90 DAT

Parameter	T1	T2	T3	T4	T5	T6
Chl	31.35^{d}	40.57^{b}	38.21^{c}	41.11^{b}	44.77^{a}	46.26^{a}
P_N	9.09^{d}	12.35^{b}	11.19^{c}	12.26^{b}	14.52^{a}	15.47^{a}
E	6.74^{a}	6.78^{a}	6.79^{a}	6.82^{a}	6.95^{a}	7.06^{a}
g_s	569^{a}	576^{a}	572^{a}	581^{a}	588^{a}	584^{a}
C_i	369^{a}	327^{b}	357^{a}	319^{b}	316^{b}	323^{b}
E/P_N	741^{a}	543^{c}	607^{b}	556^{c}	485^{d}	458^{d}
P_N/Chl	0.290^{c}	0.304^{b}	0.293^{bc}	0.298^{bc}	0.320^{a}	0.334^{a}
WUE	0.899^{e}	1.214^{c}	1.099^{d}	1.198^{c}	1.393^{b}	1.461^{a}
R	3.483^{e}	3.947^{c}	3.788^{d}	3.895^{c}	4.245^{b}	4.369^{a}

Note: Chl: Chlorophyll content (SPAD units); P_N: Net photosynthesis (μmol CO_2 $m^{-2}s^{-1}$); E: Transpiration (mmol H_2O $m^{-2}s^{-1}$); g_s: Stomatal conductance (mmol H_2O $m^{-2}s^{-1}$); C_i: Intercellular CO_2 concentration (μmol m^{-2} s^{-1}); WUE: Water Use Efficiency (gCkg^{-1} H_2O); R: Respiration (μmol CO_2 $m^{-2}s^{-1}$)

Table 6. The total nutrient uptake (kgha-1) by *T. erecta* grown in different experimental plots during the cropping period

Nutrient	T1	T2	T3	T4	T5	T6
Nitrogen	35.87^{e}	47.63^{c}	41.24^{d}	48.56^{c}	56.28^{b}	64.09^{a}
Phosphorus	8.25^{e}	12.59^{c}	10.34^{d}	12.74^{c}	14.98^{b}	17.56^{a}
Potassium	36.92^{e}	48.44^{c}	41.01^{d}	49.52^{c}	55.28^{b}	61.32^{a}

The pigment fluorescence

The OJIP fluorescence transient of *T. erecta*, measured at 90 DAT, showed significant variation among treatments. With the amendments from T1 through T6, the fluorescence showed a continuous and significant increase at the peak and intermediate inflections. Such an increase in any of the amendments was significantly higher than in T1. The variations were more in I and P levels of the transient, though only T1 showed a very low level of P fluorescence as compared to other amendments (Figure 1.A). Nevertheless, P rise increased with the amendments from T2 through T6, and the difference in F_M among treatment plots was significant except between T3 and T4. There was also a continuous increase of F_v from T1 through T6 but no appreciable variation of tF_M was noted (tF_M ranged between 240-260 ms). The highest P rise was observed in T6, followed by T5, and the variations were insignificant between the two treatments. The variable fluorescence spectra, calculated by normalizing the F_0 (to zero) and F_M (to one), showed a variation among treatments at J inflection. As expected, the highest J rise was seen in the T1 plot, followed by T2 (Figure 1.B). The difference among the fluorescence rise at J inflection in other treatment plots was insignificant. To measure the rate of change in the fluorescence at intermediate inflections and verify the hidden peaks in the fluorescence rise, the differential variable fluorescence spectra were determined by normalizing the variable fluorescence spectra with T6 (Figure 1.C). It was observed that T1 had significantly high fluorescence (12.84%) at J (2 ms). However, there was no indication of any fluorescence change at ≤ 2 ms, but in T1, a higher rise was seen at 5 ms.

Q_A reoxidation and the rate of electron movement beyond Q_A were determined by calculating φP_0, φE_0, and ψ_0. φP_0 ranged between 0.712 to 0.768 in the selected six treatments, with a variation of only 8% among the treatments (Figure 2). However, there was significant variation in φE_0 and ψ_0, which continuously and significantly increased from T1 through T6, showing a difference of 37% and 28%, respectively. Consequently, energy dissipation (φD_0) showed the reverse trend among the treatments from T1 through T6. There was nearly 24% higher dissipation in T1 when compared to that of T6. Similarly, the M_0 of T1 was significantly higher than any other treatment, and a continuous increase of M_0 from T1 to T6 was also recorded. The highest variation was observed concerning V_J, about 51% of the total absorbed energy in T1, and decreased to 38% in T6. The performance indicating bioenergetic parameters, viz., PI_φ, PI_ψ, $PI_{ABS,}$ and PI_{TOTAL}, showed a similar pattern of increase with the amendments (Figure 3). While T1 showed the minimum values of these indices, the maximum values were reported in T6. The increase of the photosynthetic performance was continuous from T1 through T6, though the differences were not always significant. Nevertheless, there was a significant correlation between the performance indices and the organic amendments, alone or in combination with the fertilizers (R^2 = 0.89, 0.92 0.87, and 0.83 for PI_φ, PI_ψ, $PI_{ABS,}$ and PI_{TOTAL}, respectively).

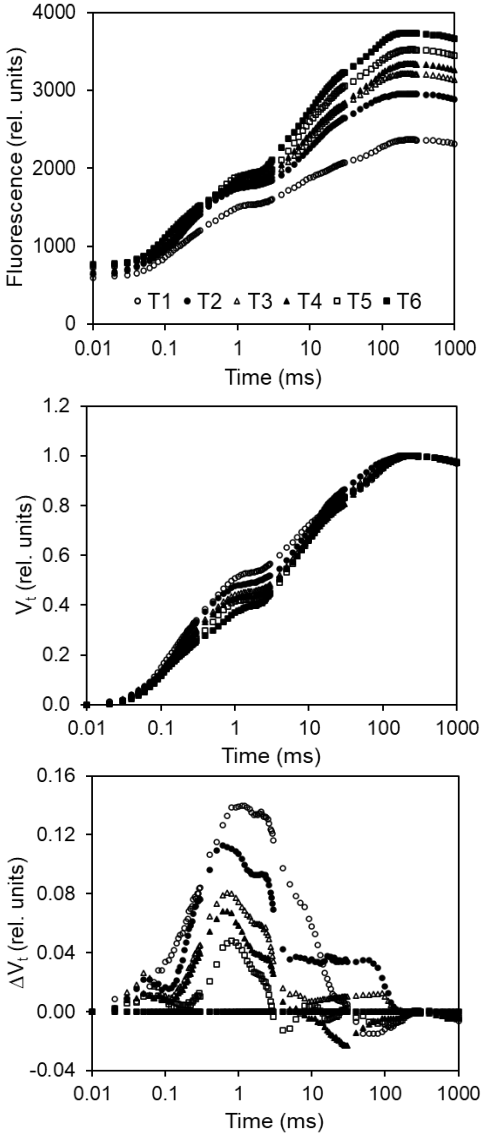

Figure 1. A. The OJIP, B. Variable (V_t), C. Relative variable (ΔV_t) fluorescence transients of *T. erecta* grown in plots with different amendments. The transients were measured at 90 DAT. Legends: o- T_1; •-φT_2; Δ-T_3; ▲-T_4; □-T_5; ■-T_6

The soil enzyme activities

The microbiological analyses of soil were made to determine the dynamics of change in the soil properties of the plots at 90 DAT. The unamended and the fertilizer (RDNP) amended plots showed the minimum levels of bacterial and fungal population counts, even though T2 had a comparable (and, in some cases, superior) performance concerning the crop's growth and yield attributes. All organically amended plots (T3 through T6) showed significantly high microbial count and soil enzyme activities. Furthermore, T5 showed the highest population count of bacteria and fungi, significantly higher than any other nutrient and fertilizer combinations (Figure 4). Correspondingly these plots also showed the highest rate of soil enzyme activities, which was significantly higher than of T6 concerning carbohydrate enzymes and phosphatases, but insignificantly higher in the case of protease and dehydrogenases (Table 7).

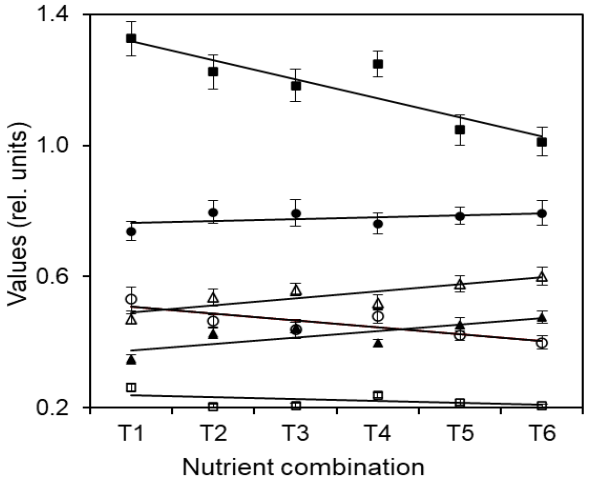

Figure 2. The derived parameters of OJIP fluorescence transients *T. erecta* were measured at 90 DAT. Legends: o- V_J; Δ-Ψ_0; □-φD_0; •-φP_0; ▲-φE_0; ■-M_0

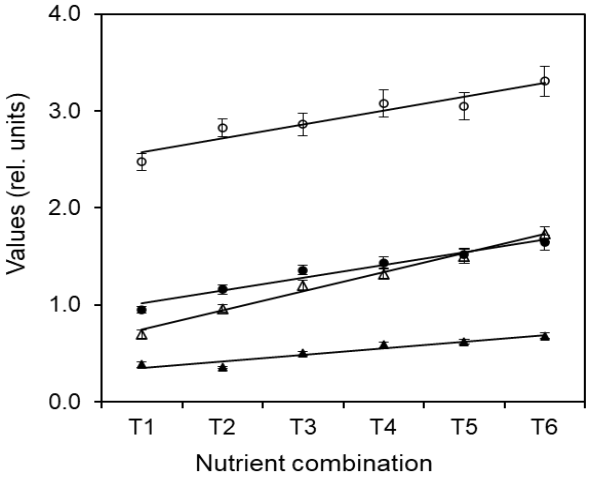

Figure 3. The performance indices (PI) of photosynthetic activity in *T. erecta* were measured at 90 DAT. The PIs were derived from the OJIP fluorescence transients. Legends: o- PI_φ; •-PI_Ψ; Δ-PI_{ABS}; ▲-PI_{TOTAL}

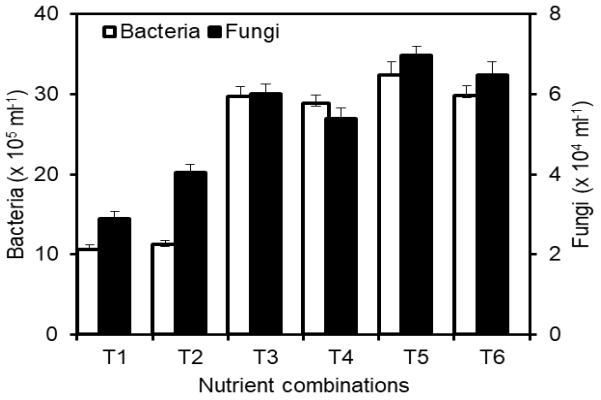

Figure 4. The density of bacteria and fungi in the top 15 cm soil of the experimental plots was measured after 90 days of transplantation

Discussion

Marigold is one of the most common loose flowers covering the flower market in every state of India and is in demand throughout the year. In this study, most of the morphological attributes of *T. erecta* did not significantly vary up to 60 DAT, but after that, remarkable differences among the treatments were observed. As expected, plants of unamended plots showed the slowest growth rate than those of other amended plots. However, plots with only organic amendments had lower growth attributes than plots with fertilizer, manure, or biofertilizer amendments. This indicated that only organic amendment failed to keep the plants photosynthetically active and supply nutrients required for plant growth, like RDNP and the combination treatments. Further, it was observed that a combination of fertilizers (NPK), manures (VC+PM), and biofertilizers (*Azospirillum* and PSB) significantly aided the growth of the plants, which was the highest among the treatments tried (Table 3).

The fertilizer, manure, and biofertilizer amendments highly influenced the yield characteristics. Irrespective of the amendment, the plants have a higher yield, bigger flowers, greater flower diameter, and more flower dry weight than the unamended field. When T2 (RDNP) and T4 (VC+PM) were compared, there was no significant difference between the two treatments concerning the yield parameters, which indicated that the organic amendments with the VC+PM corresponding to the recommended dose of nitrogen made in the present experiment, was enough to satisfy the nutrient requirement of the crop and to maintain the crop yield, even though there were lower values of morphological attributes than in T2. However, Alvarez (2021) observed that plots amended only with organic manures create a yield gap, as observed in the present case between T2 and T3. On the other hand, Mockeviciene et al. (2021) reported stabilization of the production potential of soil in long-term organic practice. Moreover, T5 (VC+PM+biofertilizers) and T6 (fertilizers and manures along with the biofertilizer application) have shown the best performance concerning the flower quality, quantity, pigment yield, and flowering duration. Thus, the biofertilizer amendment, in this case, caused better nutrient mobilization and supported plant growth, translating into a longer flowering period and better yield. Choudhury et al. (2020) have reported that a combination of RDF+VC and biofertilizer (*Azotobacter* + *Azospirillum* + PSB@ 4 $kgha^{-1}$) enhanced plant growth and significantly improved flower yield of *T. erecta* cv. Pusa. It has been proposed that biofertilizer application with a healthy organic base in soil stimulated growth promoting substance in soil (IAA and Gibberellins), resulting in more vigorous plant growth, which is essential for higher crop yield (Dalawai and Naik 2014) and integration of organic manure with inorganic fertilizers builds a robust Soil Organic Carbon (SOC) base for consistent nutrient mobilization (Gao et al. 2022).

Similarly, by testing eight different biofertilizers separately and in combination, Reddy and Saravanan (2020) observed enhanced plant growth and qualitative and quantitative improvement of flowers. The authors have recommended the combination of *Pseudomonas*

fluorescence + *Trichoderma viride* as the best to improve yield, though; however, these do not have any role in nutrient mobilization. A combination of 75% recommended dose of NPK per hectare + 25% of RDN of VC and AM + *Azotobacter* has been reported to have increased flowering and flower diameter in *T. erecta* cv. Poornima yellow (Sivasankar et al. 2020).

It has already been reported that P_N is adversely affected by nutrient stress due to poor nutrient mobilization or nutrient deficiency in soil (Geider et al. 1998; Chhotaray et al. 2014; Shi et al. 2020). This study observed that Chl content and P_N were consistently higher in combination amendments than in separate amendments with RDNP or manure (VC+PM). The best rate of P_N and pigmentation was observed in T5 and T6, which were amended with biofertilizers. Further, these combinations also had higher P_N/Chl ratios indicating a high photosynthetic rate on a pigment basis compared to other amended plots. However, E and g_s remained unchanged as the plants were adequately irrigated and were not water stressed, but C_i decreased significantly in T5 and T6, which was thus of non-stomatal type and was due to efficient assimilation of CO_2 for photosynthesis with these treatments. This is supported by higher P_N and P_N/Chl in T5 and T6 compared to other treatments and has been reported by earlier workers (Mohapatra et al. 2010; Chhotaray et al. 2014; Shi et al. 2020).

Because the combination positively influenced metabolic efficiencies, the plants in plots with amendments supporting better growth also had higher rates of respiratory oxygen consumption. Elanchezhian and Panwar (1997) reported that *Azospirillum* treatment stimulated lateral roots' development and growth, improving the rhizosphere zone, nutrient utilization efficiency, and plant metabolic performance. This is supported by the present work's high nutrient utilization rate in biofertilizer-amended plots compared to corresponding plots without biofertilizer (T6 over T4; T5 over T3). It may be noted that N and P limitations cause a reduction in P_N, higher Ci, more NPQ, and low φPS II (Shi et al. 2020). The limited conditions reduce photosynthesis-irradiance response due to the low rate of reductant supply (or electron transport) via light reaction (Geider et al. 1998).

We observed no significant change in F_0 but a significant improvement in F_M, which correlated well with the crop's photosynthetic efficiency and yield characteristics. A higher J rise was reported from the variable fluorescence spectra of plants without nutrient amendment or RDNP amendment. The comparative J rise (as seen in the differential variable spectra; Figure 1C) of all other amendments was, however, insignificant ($\leq$ 7.38% higher than of T6). Nevertheless, the plants in T5 and T6 showed the minimum J variation (4.38%) between them and were proportionately lower than any other amendment. The mentioned two treatments have higher F_m and F_v, indicating a better PS II photochemical activity. No remarkable difference in the shape of the OJIP transient during the initial farming period indicated that the plants remained metabolically active during this period in all treatments, but at 90 DAT, the change in shape in T1 indicates nutrient deficiency. This is supported by the φP_0 level, which remains high in all treatments and indicates photosynthetic performance (Stirbet and Govindjee 2011).

Consequently, the plants grown in the treated plots also had low dissipation indicating that the plants were physiologically in a good state. However, significant variations in φE_0 and Ψ_0 among treatments showed that at 90 DAT, the metabolic state of the crop varied with the treatments (Figure 2). As the availability of basic nutrients determines the metabolic state (Chhotaray et al. 2014; Shi et al. 2020; Gao et al. 2022), the electron transport rate, photosynthesis, and the translation of the photosynthates to yield varied accordingly. A significant positive correlation between amendment (T1 through T6) and the magnitude of the photosynthetic efficiency of the crop was improved with the amendment, and the intensity of activity was amendment dependent.

There are reports that organic amendments significantly enhanced the density of bacteria and fungi in agricultural soil (Chhotaray et al. 2014; Cao et al. 2021; Wu et al. 2021). In the present case, all organically amended plots, with or without fertilizer application, had significantly higher levels of bacteria and fungi than the unamended or fertilizer-amended ones. Further, T5, which was amended with VC+PM+biofertilizer, showed the highest microbial density among the treatments indicating that applied biofertilizers could build a syntrophy to affect an increase in soil microbial density. Since biofertilizers are known to enhance rhizospheric surface (Elanchezhian and Panwar 1997; Dalawai and Naik 2014; Choudhury et al. 2020), the healthy microbial population in T5 was due to the availability of surface as well as substrates for microbial growth. This agrees with the findings in other cropping systems that organic amendments build a healthy soil organic carbon base required to grow and maintain soil microflora (Chhotaray et al. 2014; Cao et al. 2021; Gao et al. 2022). Mockeviciene et al. (2021) reported that long-term application of organic manure maintained favorable conditions for soil microbes and carbon sequestration.

The soil enzyme activities have been used as a measure of the quality of agricultural soil. We observed that all the organic amended plots (T3 through T6) had significantly high activities of all the selected soil enzymes, and the enzyme activities were in significant positive correlation with soil bacteria and fungi. Each soil enzyme activity was proportional to microbial density, and, as expected, biofertilizer application encouraged soil microbial population; these plots showed high soil enzyme activities. Soil enzymes are an early marker of soil SOC load and microbial richness. Significantly low enzyme activity and microbial count in T2 showed that even though good yield could be achieved, cultivation practice only with RDNP is unsustainable for continued production. On the other hand, in T6, soil nutrient level, microbial health, crop health, and yield were not only maximum, but the nutrient uptake by the crop was the highest, thus confirming efficient nutrient mobilization.

Table 7. The activity (mgg^{-1} soil h^{-1}) of key enzymes of the top 15 cm soil of experimental plots was measured at 90 DAT of the crop in the field

Enzyme	T1	T2	T3	T4	T5	T6
Amylase	1.344^{e}	1.685^{d}	2.586^{b}	2.073^{c}	2.878^{a}	2.608^{b}
Invertase	2.186^{c}	2.375^{c}	3.289^{ab}	2.868	3.485^{a}	3.219^{b}
Cellulase	8.218^{f}	9.684^{e}	14.57^{b}	13.43^{d}	15.88^{a}	14.19^{c}
Phosphatase	3.185^{e}	4.425^{d}	9.926^{b}	9.376^{c}	10.66^{a}	9.718^{b}
Protease	0.274^{c}	0.439^{c}	0.804^{ab}	0.685^{b}	0.886^{a}	0.829^{a}
Dehydrogenase	0.034^{d}	0.065^{c}	0.092^{a}	0.077^{b}	0.098^{a}	0.088^{ab}

In conclusion, in the present study, the morphological attributes did not significantly vary among treatments up to 60 DAT. Still, significant variations were noted after that, showing that the treatment variations made in this experiment significantly impact plant growth performances. The improved growth performances and yield attributes in T6 than in other treatments proved that the combination is suitable for enhancing yield and giving the farmers more profit. Lower growth performance and yield in T3, as compared to T2, showed that organic amendment alone is not suitable to satisfy the immediate nutrient demand of the crop, but there was significant growth and yield enhancement with biofertilizer addition in soil. All the organically amended plots, solo or combined with fertilizer (50% RDNP), had physiologically active plants, high photosynthetic activities, and plant performances. These plots had healthy microbial populations and activities, thus indicating sustainability. Further work and detailed investigation on fine-tuning the combinations of amendments may determine a more viable treatment for more profitable and sustainable crop yield.

ACKNOWLEDGEMENTS

The authors thank the Head of the Department of Horticulture, OUAT, Bhubaneswar, and the Head of the Department of Botany, Ravenshaw University, Cuttack, India, for field and laboratory support, respectively. The financial assistance for infrastructure by DST, New Delhi, under the DST-FIST grant, is acknowledged. BBS thanks CSIR, New Delhi, for the award of JRF. The authors declare no conflict of interest.

REFERENCES

Acharya MM, Dashora LK. 2004. Response of graded levels of nitrogen and phosphorus on vegetative growth and flowering in African marigold (*Tagetes erecta* L.). J Ornam Hortic 7: 179-183.

Alvarez R. 2021. Comparing productivity of organic and conventional farming systems: A quantitative review. Arch Agron Soil Sci 6: 1-12. DOI: 10.1080/03650340.2021.1946040.

Attokaran M. 2017. Natural Food Flavors and Colorants. John Wiley & Sons Ltd, New York. DOI: 10.1002/9780470959152.

Borah N, Deka J, Deka NC, Barua IC, Saud BK, Das K, Deka K, Goswami K. 2020. Soil fertility status, nutrient uptake and productivity of marigold (*Tagetes erecta* Linn.) as influenced by fertilizer doses and plant growth regulators. Curr Appl Sci Technol 39 (24): 53-65. DOI: 10.9734/CJAST/2020/v39i2430873.

Cao Q, Li G, Yang F, Kong F, Cui Z, Jiang X, Lu Y, Zhang E. 2021. Eleven-year mulching and tillage practices alter the soil quality and bacterial community composition in Northeast China. Arch Agron Soil Sci 68 (9): 1274-1289. DOI: 10.1080/03650340.2021.1890719.

Chhotaray D, Chandrakala Y, Mishra CSK, Mohapatra PK. 2014. Farm practices influence the photosynthetic performance and plant efficiency of *Oryza sativa* L. Acta Physiol Plant 36: 1501-1511. DOI: 10.1007/s11738-014-1527-7.

Choudhury S, Sarangi D, Mishra K. 2020. Response of integrated nutrient management on plant growth, flower yield and shelf life of African marigold (*Tagetes erecta* L.) cv. Pusa Narangi Gainda. Intl J Chem Stud 8 (3): 2453-2456. DOI: 10.22271/chemi.2020.v8.i3ai.9577.

Dalawai B, Naik BH. 2014. Integrated nutrient management studies in carnation (*Dianthus caryophyllus* L.) cv. Soto under protected condition. Intl J Agric Vet Sci 2 (3): 19-24.

Elanchezhian R, Panwar JDS. 1997. Effects of 2,4-D and *Azospillum brasilense* on nitrogen fixation, photosynthesis and grain yield in wheat. J Agron Crop Sci 178: 129-133. DOI: 10.1111/j.1439-037X.1997.tb00480.x.

Gao Y, Lu Y, Liao Y, Nie J. 2022. Higher soil organic carbon accumulation in the subsoil layer by 37 years combined application of inorganic fertilizers with manure than with rice straw in a double-rice paddy soil. Arch Agron Soil Sci 68 (8): 1075-1088. DOI: 10.1080/03650340.2020.1869216.

Geider R, Macintyre, Graziano L, McKay RM. 1998. Responses of the photosynthetic apparatus of *Dunaliella tertiolecta* (Chlorophyceae) to nitrogen and phosphorus limitation. Eur J Phycol 33 (4): 315-332. DOI: 10.1080/09670269810001736813.

Kaur M, Bhat A, Sadik-Zada ER, Sharma R. 2022. Productivity analysis and employment effects of marigold cultivation in Jammu, India. Horticulturae 8 (3): 263. DOI: 10.3390/horticulturae8030263.

Mockeviciene I, Repsiene R, Amaleviciute-Volunge K, Karcauskiene D, Slepetiene A, Lepane V. 2021. Effect of long-term application of organic fertilizers on improving organic matter quality in acid soil. Arch Agron Soil Sci 68 (9): 1192-1204. DOI: 10.1080/03650340.2021.1875130.

Mohapatra PK, Khillar R, Hansdah B, Mohanty RC. 2010. Photosynthesis and fluorescence responses of *Solanum melangena* L. to field application of dimethoate. Ecotoxicol Environ Saf 73 (1): 78-83. DOI: 10.1016/j.ecoenv.2009.08.015.

Reddy KA, Saravanan SS. 2020. Effect of bio fertilizers on plant growth and flower yield of African marigold *Tagetes erecta*. Intl J Pharmacogn Phytochem 9: 1334-1338.

Shasmita, Swain BB, Mohapatra PK, Naik SK, Mukherjee AK. 2022. Biopriming for induction of disease resistance against pathogens in rice. Planta 255 (6): 113. DOI: 10.1007/s00425-022-03900-8.

Shi Q, Fu W, Zhang L, Wang F. 2020. Effects of stoichiometric traits of nitrogen and phosphorus in soil on photosynthetic characteristics of wheat. Commun Soil Sci Plant Anal 51 (9): 1204-1212. DOI: 10.1080/00103624.2020.1763392.

Sivasankar S, Ilakkiya P, Rajkumar M, Sureshkumar R, Sendhilnathan R. 2020. Effect of vam and *Azotobacter* on growth and yield characters of African marigold (*Tagetes erecta* linn.) cv. poornima yellow. Plant Arch 20: 1133-1136. DOI: 10.51470/plantarchives.2021.v21.s1.333.

Stirbet AA, Govindjee. 2011. On the relation between the Kautsky effect (chlorophylla fluorescence induction) and photosystem II: basics and applications of the OJIP fluorescence transient. J Photochem Photobiol 104: 236-257. DOI: 10.1016/j.jphotobiol.2010.12.010.

Strasser RJ, Srivastava A, Govindjee. 1995. Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. Photochem Photobiol 61 (1): 32-42. DOI: 10.1111/j.1751-1097.1995.tb09240.x.

Wu Q, Li S, Huang Z, Wang Q. 2021. Variations in soil bacterial communities and putative functions in a sugarcane soil following five years of chemical fertilization. Arch Agron Soil Sci 67 (6): 727-738. DOI: 10.1080/03650340.2020.1752916.