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Effect of Organic Fertilizers on the Soil Properties and Bacterial Communities of Marigold Rhizosphere Soil

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Authors' contributions

This work was carried out in collaboration among all authors. Author KT' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author SM guided author KT all throughout the research programme and author MCT had major contribution in the soil analyses processes and interpretation of data. All authors read and approved the final manuscript.

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ABSTRACT

The investigation was undertaken to study the impact of different organic inputs on the soil properties of African marigold rhizosphere soil. Analysis of variance (ANOVA) for RBD with three replications was carried out using OPSTAT. The present investigation was conducted in experimental farm, Department of Horticulture, Assam Agricultural University, Jorhat, Assam, between 2020-2021. The African marigold variety 'Seracole' was chosen for the experiment consisting of 7 treatment combinations with one treatment comprising of the recommended dose of fertilizers and the other six treatment comprising of various organic manures, rock phosphate with a consortium of *Azotobacter*, *Azospirillum* and PSB. It was observed that T_7 {Enriched compost (5 t/ha)} exhibited the highest values for soil pH, soil moisture content, organic Carbon, available Nitrogen, Phosphorus, Potassium, Microbial Biomass Carbon and various soil enzymes followed by T_3 {Vermicompost (5t/ha) + Rock phosphate (100 kg/ha) + Microbial consortium}**.** Considering the positive effect on growth, yield, quality and soil health, T_3 and T_7 both can be considered best for adopting at the field level to reap good economic yield accompanied by better quality and sustainable soil health.

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1. INTRODUCTION

Marigold is a flower that grows wild throughout Central and South America, particularly in Mexico. During the early 16th century, it expanded from Mexico to various regions of the world. Tagetes was named after Tages, a deity who was noted for his beauty. Genus *Tagetes* belongs to subfamily Asteroideae of family Asteraceae*. Tagetes* is a flowering plant genus with 56 species, 27 of which are annual and 29 perennials, that is widely dispersed throughout the world and is one of the most extensively exploited tropical and subtropical flower crops*.* The plants are stout and branched. The leaves are segmented, pinnate and fern like which are green in colour and are strongly scented. Flowers are usually found in 3 varying colours from yellow and golden to orange, red and mahogany. Four annual species *Tagetes patula*, *Tagetes lunulata*, *Tagetes erecta* and *Tagetes tenuifolia* are commonly cultivated throughout the world for ornamental purposes. The taller and large flowered *Tagetes erecta* is known as African marigold while the smaller *Tagetes patula* is called French marigold. *Tagetes erecta*, popularly known as 'Mexican marigold' or 'Aztec Marigold,' is one of the genus Tagetes' most important species. The chromosome number of African marigold is 2n=24. This plant reaches a height of 50-100 cm. It has a tendency to flower for a short period, resulting in marketable flowers with a wide range of appealing colours, shapes, and sizes, as well as good keeping qualities. Marigold was introduced by Portuguese in India during 16th century [1].

Bioactive extracts from numerous Tagetes plant sections have been found to have nematocidal, fungicidal, and insecticidal action. Thienyls are the nematocidal components of essential oils from flowers and leaves, while terpenoids are the biocidal components of essential oils from flowers and leaves. Tagetes carotenoid pigments can also be utilized in food coloring [2]. The finding of [3] indicated that higher intake of carotenoids such as lutein and zeaxanthin reduced the risk of age-related macular degeneration in the retina of eye which has been documented by various workers [4] and [5]. Carotenoids (Xanthophyll) are abundant in marigold flower petals, which have a significantly higher concentration of this pigment than other plant components [6]. Since some of the artificial color additives have cancerous effects, the natural color additives are preferred for improving egg yolk pigmentation and hence marigold petal can be used as alternative sources of natural carotenoids as pigmenting agents for egg yolk.

It is found in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogues, egg products, fats and oils, frozen dairy desserts and mixes, gravies and sauces, soft and hard candy, infant and toddler foods, milk products, processed fruits and fruit juices, soups and soup mixes at levels ranging from 2 to 330 mg/kg [7]. So there is scope of using organic marigold petals as a source of natural dye in food and poultry industry.

To meet the rising demand, both quantity and quality production are critical. Higher yields can be obtained by applying inorganic fertilizers, but continued use of agrochemicals degrades soil health and causes environmental imbalance by polluting the air, water, and soil. Chemical fertilizer use has a negative impact on soil texture and structure, as well as organic content and microbial activity [8].

2. MATERIALS AND METHODS

2.1 Experimental Site

The experimental area was located at 26º47'N and 91º12'E longitude at an elevation of 86.8m above mean sea level and under Upper Brahmaputra Valley Agro Climatic Zone of Assam. The field experiment was conducted during 2020-2021 at the Experimental Farm, Department of Horticulture, Assam Agricultural University, Jorhat, Assam.

The experiment was laid out in Randomized Block Design (RBD) with three replications. There were 7 treatments which were applied as T_1 {RDF (10:10:10 g/m^2 NPK) + FYM @ 4 kg/m^2}, T_2 {Vermicompost (2.5t/ha) + Rock Phosphate (100 kg/ha) + Microbial consortium}, T_3 {Vermicompost (5t/ha) + Rock phosphate (100 kg/ha) + Microbial consortium}, T_4 {compost (2.5t/ha) + Rock phosphate (100 kg/ha) + Microbial consortium}, T_5 {Compost (5t/ha) + Rock phosphate (100kg/ha) + Microbial consortium}, T_6 {Enriched compost (2.5 t/ha)}

and T_7 {Enriched compost (5 t/ha)}. Microbial consortium slurry was prepared with water and the root dip treatment of seedlings was done an hour prior to transplanting. Consortium used in the treatment was a mixture of *Azospirillum*, *Azotobacter* and Phosphate solubilizing bacteria (PSB). The inputs like vermicompost, compost and enriched compost used in the experiment was procured from Assam Agricultural University, Jorhat and applied during the land preparation. The cuttings used were about 5-7 cm height when planted and were of a uniform height. The cuttings were planted in a prepared plot at a spacing of 30 cm x 30 cm.

2.2 Microbial Consortium

A microbial consortium is two or more microbial groups living symbiotically. Consortium used in the experiment was the mixture of *Azospirillum*, *Azotobacter* and Phosphate solubilizing bacteria.

2.2.1 Azotobacter

They are aerobic, free-living soil bacteria that bind atmospheric nitrogen and play a vital part in the nitrogen cycle in nature. *Azotobacter* species are nitrogen-fixing bacteria that do not require symbiotic relationships with plants to fix molecular nitrogen from the atmosphere. *Azotobacter* also produces biologically active substances such as auxins, gibberellins, cytokinins, and antibiotics that suppress and control plant pathogens (fungi, bacteria, and viruses), promoting plant growth and aiding in the mineralization of plant nutrients and the proliferation of beneficial microorganisms. *Azotobacter* fixes 20 kg of nitrogen per year [9]

2.2.2 Azospirillum

Azospirillum is the most well-studied genus of rhizobacteria that promote plant development. Under microaerophilic circumstances, *Azospirillum* colonises the root mass and fixes a significant amount of nitrogen. *Azospirillum* fixes nitrogen from 10 to 40 kg/ha [10]

2.2.3 Phosphate solubilizing bacteria

The rhizophere's Phosphate Solubilizing Bacteria (PSB) is known to increase the solubility of insoluble phosphorus by producing aliphatic and aromatic acids, as well as phytase and phospholipase [11] Some bacteria like *Bacillus megatharium*, *Bacillus polymyxa*, *Pseudomonas striata* etc. can solubilize 20-30% insoluble phosphate. The production of organic acid in the micro environment *viz.* citric acid, fumaric acid, salicyclic acid, humic acid and benzoic acid around the roots considered as the most important cause of phosphorus solubilisation.

2.3 Soil Analysis

2.3.1 Collection and preparation of soil samples

Soil samples were collected from each plot after the harvest of the crop were air dried, ground and sieved through 2mm diameter and stored in butter paper bags with proper tagging and used for various analyses pertinent to the experiment.

2.3.2 Soil pH

Soil pH was determined before and at harvest by glass electrode method [12]. For the purpose, soil water suspension was prepared at the ratio of 1:2.5 and the pH of the suspension were determined with pH meters with a glass electrode.

2.3.3 Soil moisture content

Soil moisture content on dry weight basis was determined by gravimetric method from the time of planting to maturity at periodic intervals. The soil samples were taken from soil depth 0-15 cm with the help of auger. Soil moisture content (%) was determined by drying the soil samples in hot air oven at 105ºC until the entire moisture was driven off and a constant weight was obtained. The loss in soil moisture content as percentage of oven dry soil by the following formula:

$$\text{Per cent moisture} = \frac{\text{loss in weight}}{\text{oven dry weight}} \text{x} 100$$

2.3.4 Organic carbon

Organic carbon in the soil (0.2g) was oxidized with a mixture of $K_2Cr_2O_7$ (1N), conc. H_2SO_4 (Sulphuric acid) and conc. H_2PO_4 (Orthophosphoric acid) for reduction of $K_2Cr_2O_7$ (Potassium dichromate) by organic compounds as per the method described by [13]. The unused $K_2Cr_2O_7$ was back titrated with Ferrous ammonium sulphate (FAS) $[(NH_4)_2SO_4FeSO_46H_2O]$ (0.5M) using Diphenylamine indicator till the colour changes from violet blue to green. Blank contained no soil but all reagents treated similarly for calculation. Oxidizable Organic Carbon and total organic

carbon (TOC) were calculated using the following formula.

Oxidizable OC (w/w) = $\frac{(V_b - V_s)\text{x}0.3\text{x}\text{M}}{\text{Wt}}$

TOC (w/w) = 1.334 x Oxidizable Organic Carbon

Where M = Molarity of ferrous ammonium sulphate (0.5M)

V_b= Volume of FAS for blank (ml)

V_s= Volume of FAS for sample (ml)

Wt= Weight of soil (g)

0.3 = 3 × 10^{-3} ×100 where 3 is equivalent weight of C

2.3.5 Available nitrogen

Available N of the soil sample was estimated by modified Kjeldahl's method as described by [12] and the available nitrogen present in the sample was expressed as kg ha^{-1}.

2.3.6 Available phosphorus

Available P in soil sample was extracted by Bray's method as outlined by [12]. The Phosphorus was determined calorimetrically and expressed as available P_2O_5 (kg ha^{-1})

2.3.7 Available potassium

Available K content of the soil sample was extracted with neutral normal ammonium acetate as outlined by [12]. The Potassium content was determined with the help of Flame Photometer and expressed as available K_2O (kg ha^{-1})

2.4 Soil Microbial Analysis

2.4.1 Microbial biomass carbon

Microbial biomass carbon was determined by chloroform fumigation extraction technique following the method of [14]. Moist samples (5g soil) in 50 ml glass beakers are placed in a desiccator and a vial of soda lime. A beaker containing 50ml ethanol free $CHCl_3$ was boiled vigorously for 2 min. The desiccator was then incubated in dark at 25ºC for 24 hr. After fumigation, $CHCl_3$ was removed by repeated evacuation, the soil was then extracted with 25ml 0.5 M K_2SO_4 (Pottasium sulphate) (5:1) for 30 min by oscillating shaking at 200 rpm and then filtered through a Whatman no. 42 filter paper. Controls were prepared by extracting soils without fumigation. OC content in the extracts was measured with dichromate (66.7 mM) and 15 ml of the digestion mixture (2:1 conc. H_2SO_4: H_3PO_4 (v/v) was added. The mixture was gently refluxed for 30 min, allowed to cool and diluted with 20ml distilled water. The excess $K_2Cr_2O_7$ was measured by back titration with FAS (40.0 mM) using 1.10-phenanthrolineferroussulphate complex (25mM) solution as an indicator. MBC was calculated from the differences in extractable OC between the fumigated and non- fumigated compost and expressed as (µg g-1) on a dry basis as:

MBC (µg g-1) = Ec/kEC

Where Ec = [(OC extracted from fumigated soil) – (OC extracted from non-fumigated soil)] and kEC = 0.38 (Vance *et al*., 1987)

2.4.2 Dehydrogenase activity

DH activity was determined by the reduction of Triphenyl tetrazolium chloride (TTC) to Triphenylformazan (TPF) as described by [15] with modifications. Moist soil (1g) was treated with 1ml of 3% TTC and then incubated at 28ºC for 24hr in a screw cap test tube (30 ml). After the incubation period, the soil was extracted by addition of extractant (methanol) following incubation in dark with agitation for 1hr. After the extraction, 1.2ml of extractant with soil mixture was transferred to a 1.5ml microcentrifuge tube and removed the soil by centrifugation at 10,000 rpm for 10 min. The absorbance of the supernatant was measured in the Nanodrop 1000 spectrophotometer at 485nm. To account for any abiotic TTC reductions, sterile controls consisted of autoclaved soil (121ºC, 20 min foe three consecutive days) were used. Spectrophotometer blanks for both autoclaved and not autoclaved treatment consisted of compost and TTC replaced with Millipore water. Controls and blanks treated like samples. A calibration curve was constructed by determining OD 485nm values for working standard of TPF (20,40,80,120,200,300 and 500µg ml-1). The OD 485nm was compared to that of TPF standards. DH activity was expressed on dry weight as µg TPF g-1 24 h-1 on a dry weight basis as:

DH activity (µg TPF g-1 24 h-1) = [(TPFc)] [(TPFs)] ×11/Edw

Where, TPFs = TPF conc. (µg ml-1) in the sample

TPFc = TPF conc. (µg ml-1) in the sterile control; Edw is the equivalents dry wt. of 1g soil;

11 is the volume of solution added in the assay (TTC + Extractant)

2.4.3 Acid phosphatase

The soil acid phosphatase activity was determined by rapid enzyme assay based on the use of p-nitrophenyl phosphate (pNPP) as artificial substrate as described by [16] and measuring the colour intensity colorimetrically.

2.5 Statistical Analysis

The data of the respective field experiment were subjected to appropriate statistical analysis. Analysis of variance (ANOVA) for simple Randomized Block Design (RBD) with three replications was carried out using OPSTAT and the comparison was done by calculating critical difference (CD) at a 5% probability level.

3. RESULTS AND DISCUSSION

3.1 Effects of Organic Amendments on the Soil Physicochemical Properties

Data pertaining to application of organic inputs on the soil properties are presented in Tables 1-2. Perusal of data in Table 1 and 2 clearly showed that the various soil properties were significantly influenced by the organic inputs. Application of Enriched compost @5t/ha) i.e T_7 exhibited the highest values for soil pH (5.31), soil moisture content (45.18%), organic Carbon (8.8 g kg^{-1}), available Nitrogen (284.59 kg/ha), Phosphorus (60.13 kg/ha), Potassium (60.13 kg/ha), Microbial Biomass Carbon (291.22 µg g^{-1} soil 24 $hour^{-1}$), Dehydrogenase activity (126.03 µg TPF g^{-1} h^{-1}) and Acid phosphatase (72.52 µg pnitrophenol g^{-1} h^{-1}). However, appreciable response of vermicompost was also observed and T_3 was seen to have performed exceptionally well.

3.1.1 Soil pH

Soil pH is a manifestation of H^+ and OH^- activity by dissociation of water molecules. Higher pH in the organic treatments might be because the deactivation of Al^{3+} and concomitant release of basic cations due to incorporation of organic matter [17]. However, application of different organic sources did not affect the soil pH much perhaps due to great buffering action of organic matter found in organic manures [18]. These results were in agreement with the findings of [19] and [20]. Also higher pH might be due to the rise in microbial action in the root zone which decomposes organic manures and also fix unavailable form of mineral nutrients into available forms in soil thereby substantiates crop requirement and improve organic carbon level and stabilize soil pH. Similar findings were also reported by [21-23] in cauliflower and [24]. Soil organic carbon serves as a nutrient sink and supply for the microbial community, which regulates nutrient availability through microbial transformation. However, soil pH 5.14 and organic carbon 6.8 g/kg were measured before the experiment.

3.1.2 Available nitrogen

Available form of nitrogen is always in a state of dynamic change and hence its content in soil is highly variable. Treatment T_7 (Enriched compost @5t/ha) recorded the highest available N content (284.59 kg/ha). The presence of *Azospirillum* and *Azotobacter* to fix atmospheric N in the rhizosphere during the cropping period could account for such a buildup of accessible N. Similar results have been reported by [25]. Application of *Azotobacter* helps in acceleration of phytohormones like Indole-3-acetic acid production, nitrogen fixation, obviation of various stressors, pesticides and oil globules degradation, etc. [26]. *Azotobacter* alone is capable of fixing N equivalent to 25-30kg ha^{-1} as reported by [27]. This buildup might be due to the fact that pH value rises as a result of organic sources and thus lowered the oxidation-reduction process. Organic acid and microbial product of decomposition from organic sources solubilizes the insoluble compounds by interacting with their specific bindings cations and clay minerals. Therefore, it was seen that application of organic sources was found to be good in enhancing the nitrogen availability in soil. The lowest nitrogen content of 256.89 kg/ha observed in T_1 (RDF i.e., 10:10:10 NPK g/m^2 + FYM @ 4kg/m^2). This could be because chemical fertilizers have more leaching and other losses than organic manures [28] and [29].

3.1.3 Available phosphorus

The highest available soil phosphorus status 60.13 kg/ha was observed in T_7 (Enriched compost @5t/ha). This build-up of available P might be due to attributed improvement of soil condition by application of compost and phosphate solubilizing and mineralizing ability of microbes [30]. Similar findings have been

reported by [31] and [32]. Microbial culture plays a vital role in the release of phosphorus sources due to the production of phosphate solubilizing enzymes. It has been established that the application of phosphate solubilizing bacteria increased the available phosphorus status in soil, which could be attributed to the production of organic acids, which behave as chelating agents and form fixed complexes with Fe and Al, which are available in acid soil, releasing phosphorus from Fe and Al's clutches into the solution [29]. This was in line with the view of [33] who opined that phosphorus mineralization is closely related to the analogous transformation of nitrogen.

3.1.4 Available potassium

In case of residual potassium, T_7 (Enriched compost @5t/ha) showed higher potassium content 186.25 kg/ha. This might be due to release of potassium from these organic amendments and also due to solubilization of mineral based potassium or native potassium. The positive influence of organic manure on the available potassium was earlier reported by [34]. Besides, it could be also due to the prevention of leaching loss due to retention of more potassium by organic components while inorganic fertilizers could have released potassium at a faster rate. These result confirms the findings of [35]; [29]; [36] and [37]. Organic manures had a good effect on reducing potassium fixation by interacting with potassium clay to release potassium from the non-exchangeable fraction into the accessible pool [38].

3.2 Effects of Organic Amendments on Soil Biological Properties

Organic amendments have been shown to improve soil aggregation, structure, and fertility while also enhancing microbial variety and populations, improving soil moisture holding capacity, and increasing crop yields [39]. Study on biological properties may give a valid platform for analysis of organic advantage.

Table 1. Effects of organic amendments on the soil physicochemical properties

Treatments	Soil pH	Soil Moisture Content (%)	Soil organic carbon (g kg^{-1})	Available N(kg/ha)	Available P_2O_5 (kg/ha)	Available K_2O(kg/ha)
T_1	5.23	38.52	7.2	256.89	44.29	125.48
T_2	5.25	43.29	7.8	276.02	55.20	176.66
T_3	5.27	45.11	8.4	283.65	59.80	184.59
T_4	5.18	41.90	7.7	275.35	45.06	136.18
T_5	5.24	42.88	8.0	280.13	48.35	142.26
T_6	5.26	44.07	8.3	278.31	54.43	175.94
T_7	5.31	45.18	8.8	284.59	60.13	186.25
S.Ed (±)	0.20	2.20	0.3	2.45	1.29	2.92
$CD_{(0.05)}$	NS	4.59	0.5	5.12	2.70	6.44

[1]*NS*

Table 2. Effects of organic amendments on soil biological properties

Treatments	Microbial Biomass Carbon (µg g^{-1} soil 24 $hour^{-1}$)	Dehydrogenase activity (µg TPF g^{-1} h^{-1})	Acid phosphatase (µg pnitrophenol g^{-1} h^{-1})
T_1	256.39	92.52	41.23
T_2	281.27	110.55	61.15
T_3	287.04	122.85	70.76
T_4	268.14	96.66	60.71
T_5	273.73	102.01	61.23
T_6	284.37	117.83	65.17
T_7	291.22	126.03	72.52
S.Ed (±)	1.53	2.84	1.49
$CD_{(0.05)}$	3.21	6.25	3.28

[1] *NS- Non significant*

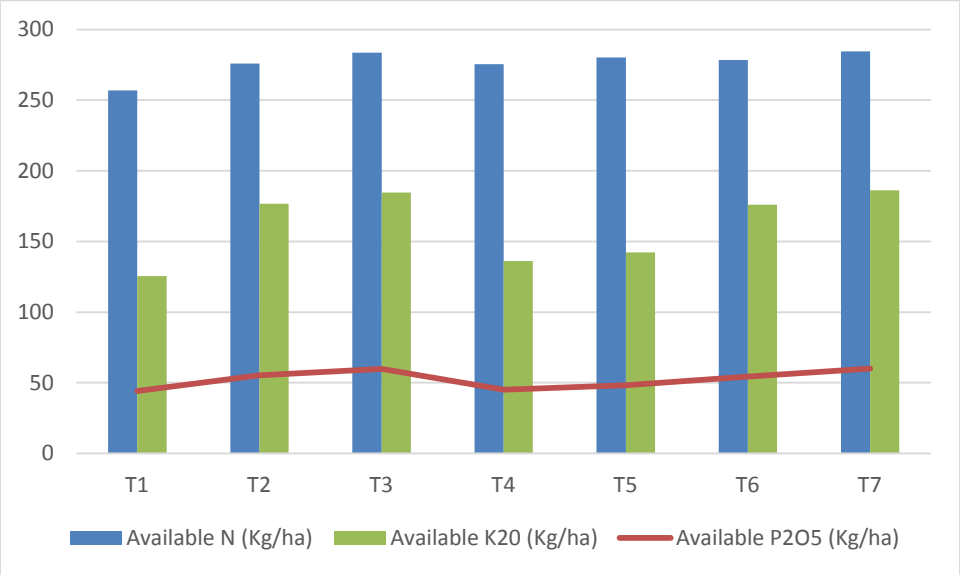

Fig. 1. Effects of organic inputs on available N, P and K

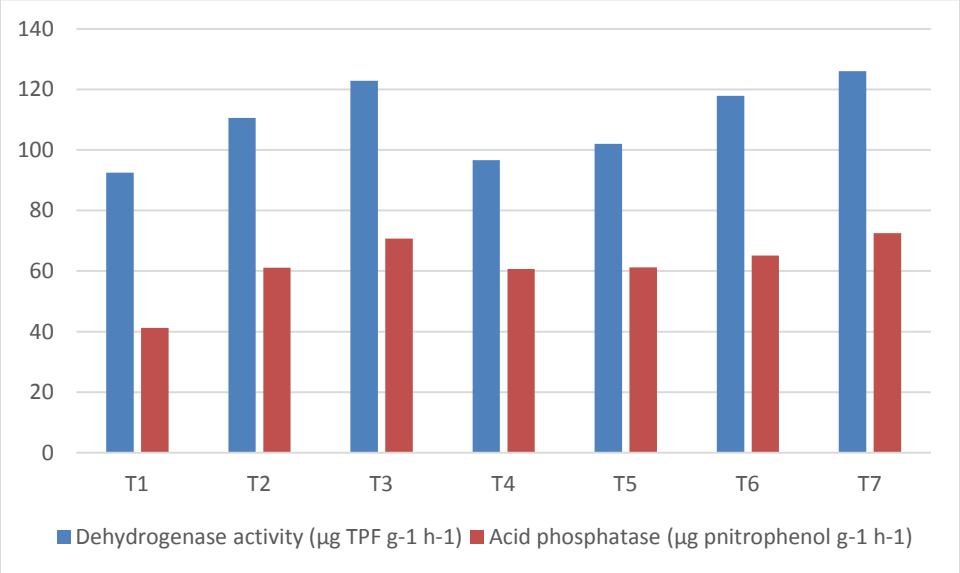

Fig. 2. Effects of organic inputs on soil enzyme activity

3.2.1 Microbial biomass carbon

Significant variation in MBC was observed in the present study. In the present study, application of T_7 (Enriched compost @5t/ha) resulted in the highest MBC 291.22 µg g^{-1} soil 24 $hour^{-1}$. This could be due to the use of an organic nutrition source, which boosts soil microbial and enzymatic activity [40]. The biological properties were higher in the soil under organic treatment. This might be due to the increase in organic carbon, total N and P content in the soil with the application of organic inputs specially enriched compost and vermicompost, which are directly related to the biological properties of the soil. This results were in agreement with the findings of [40] and [41].

3.2.2 Dehydrogenase activity

The activity of Dehydrogenase enzyme in the soil increased significantly due to the application of organic amendments. The DH enzyme plays an important role in the initial stages of the oxidation of soil organic matter and is one of the reliable criteria that signify microbial activity in a given

situation. The enzyme is considered to exist as an integral part of the intact cell but does not accumulate extracellularly in the soil. DH enzyme is known to oxidize soil organic matter and it gives the indication of soil fertility and soil health. Dehydrogenase is an oxidoreductase enzyme because it participates in oxidation-reduction processes that include the transfer of H+ to an acceptor other than O_2. The DH activity was higher in T_7 (Enriched compost @5t/ha) with 126.03 µg TPF g^{-1} soil 24 $hour^{-1}$. The effect of higher microbial activity and microbial biomass carbon resulted in higher Dehydrogenase activity. This result was in conformity with the findings of [42] and [43]. The application of organic minerals which contains crop residues, animal feces and their compost, etc. to soil usually increases the soil biomass and activities [44].

3.2.3 Acid phosphatase

The soil treated with T_7 (Enriched compost @5t/ha) resulted in the higher Acid phosphatase activity of 72.52 µg pnitrophenol g^{-1} soil h^{-1}. It's possible that this is due to the release of more organically bound phosphorus as a result of enzyme synthesis, which is aided by the presence of organic substrate [45]. These findings are in agreement with [46] and [47]

4. CONCLUSION

From the foregoing discussion, it can be concluded that the treatments T_3 (Vermicompost @5t/ha + Rock Phosphate @100kg/ha + Microbial consortium) followed by T_7 (Enriched compost @5t/ha) were found to be the most efficient treatments in terms of both yield and quality as well as for sustaining soil health. The Indian government has just designated the whole Northeastern region as an organic zone, with the majority of agricultural regions classified as naturally organic. Hence, these two treatments may be placed under multi location trails in farmer's field to judge the efficacy for the commercial organic cultivation of African marigold in different agro-climatic zones.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

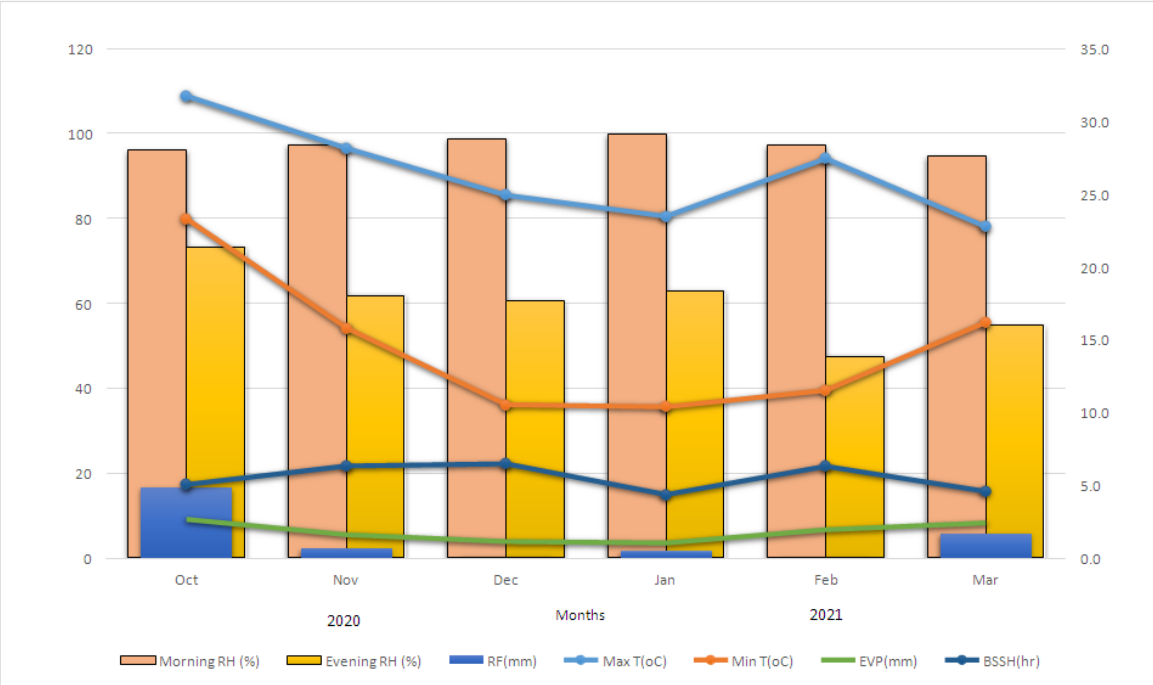

Fig:- Meteorological data observed during the experimental period (2020-2021)

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