



ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(12): 135-143
© 2020 TPI
www.thepharmajournal.com
Received: 14-10-2020
Accepted: 21-11-2020

Yogendra Singh
Department of Fruit Science,
College of Horticulture and
Forestry, Jhalawar, Rajasthan,
India

Prerak Bhatnagar
Department of Fruit Science,
College of Horticulture and
Forestry, Jhalawar, Rajasthan,
India

Jitendra Singh
Department of Fruit Science,
College of Horticulture and
Forestry, Jhalawar, Rajasthan,
India

CK Arya
Department of SWE, College of
Horticulture and Forestry,
Jhalawar, Rajasthan, India

MK Sharma
Department of Soil Science, ARS
Kota, Rajasthan, India

IB Maurya
Department of Vegetable
Science, College of Horticulture
and Forestry, Jhalawar,
Rajasthan, India

Yogendra Kumar Sharma
Department of Fruit Science,
College of Horticulture and
Forestry, Jhalawar, Rajasthan,
India

Corresponding Author:
Yogendra Singh
Department of Fruit Science,
College of Horticulture and
Forestry, Jhalawar, Rajasthan,
India

Studies on consortium of *Azospirillum brasilense* and Vermicompost application on plant growth attributes, soil physico-chemical characteristics and microbial population in custard apple cv. Raydurg

Yogendra Singh, Prerak Bhatnagar, Jitendra Singh, CK Arya, MK Sharma, IB Maurya and Yogendra Kumar Sharma

Abstract

A field experiment entitled “Studies on consortium of *Azospirillum brasilense* and Vermicompost application on plant growth attributes, soil physico-chemical characteristics and microbial population in Custard apple cv. Raydurg” were conducted for two successive seasons (July 2018 to November 2018) and (July 2019 to November 2019) at the Fruit Instructional Farm, Department of Fruit Science, College of Horticulture and Forestry, Jhalawar district under Rajasthan state of India. The experiment consisted of different treatments of *Azospirillum brasilense* (AZS) and Vermicompost (V.C.) was laid out in Randomized Block Design with three replications. The sixteen different treatment combinations of *Azospirillum brasilense* and Vermicompost were applied to soil canopy of custard apple cv. Raydurg plants during first week of July 2018 and July 2019 in canopy rhizosphere of black vertisols in custard apple plants. Among various treatment combinations, T₁₀ treatment containing (AZS @ 50g + V.C. @ 10 kg/plant) was found comparatively better over all other treatments with respect to furthest of the plant growth parameters, including soil physico-biochemical properties and microbial population density in soil availability in canopy of custard apple cv. Raydurg plants. The pooled consequences of two years demonstrated proportional improved plant growth parameters viz. rootstock girth (64.60 mm), scion girth (56.87 mm), plant height attribute (2.90 m), number of nodes per shoot (11.00), number of nodes per branch (64.17), number of shoots per branch (32.00), number of shoots per plant (147.83) have shown augmenting response in improvement of plant growth parameters under T₁₀ treatment (AZS @ 50g + V.C. @ 10 kg /plant) in custard apple cv. Raydurg plants. However, important soil parameters regarding bulk density (1.28 g/cm³), soil pH (7.38) and electrical conductivity (0.46 dSm⁻¹) lower down significantly under T₁₀ treatment over other treatments. The soil physico-chemical properties especially change in porosity (50.22%), soil texture, organic carbon (0.55%), available nitrogen content (344.30 Kg/ha), available phosphorous content (42.44 Kg ha⁻¹) and available potassium content (317.00 Kg ha⁻¹) were recorded better improve under T₁₀ treatment (AZS @ 50g + V.C. @ 10 kg /plant). In T₁₀ treatment (AZS @ 50g + V.C. @ 10 kg /plant) the values of bacterial population (27.33 cfug⁻¹), fungi population (14.67 cfug⁻¹) and actinomycetes population (10.50 cfug⁻¹) have shown rise. In view of overall effect and equivalence of treatments T₁₀ treatment was found comparatively better in plant growth, soil physico-chemical and microbial population. This treatment may be considered worth application in order to obtain better plant growth, soil physico-chemical and microbial population in custard apple cv. Raydurg plants.

Keywords: custard apple, soil health, raydurg plants, fungi population, phosphorous

Introduction

Custard apple (*Annona squamosa* L.) belongs to family Annonaceae of the order Magnoliales. Custard apple (*Annona squamosa*) is most important energizing and delicious fruit crops of arid and semi-arid regions. It is originated from tropical America and being acclimatized in India since last five decades. Custard apple is known as numerous local names such as sweet sop, sugar apple, Sitaphal and Sharifa in different part of India. It is grow well in tropical and warmer sub-tropical parts of India under sub humid and humid environmental regions. Custard apple occupies very an important place among underutilized fruit crops grown in India. Custard apple is a hardy and potential fruit crop for profitable growing in marginal soils and degraded lands, as well as waterless and salt affected soils. Though, the natural fertility of soils is infrequently adequate to give economic yields. In sand culture grown custard apple saplings nitrogen deficiency was characterized by restricted growth of plants with pale green to

yellowish leaves. Phosphorus deficiency indications to growth reduction, appearance of brown necrotic bands at the tips and margin of leaves, while potassium deficiency produces marginal scorching of leaves (Sadhu and Ghosh, 1976) [13]. Mandal and Chattopadhyay (1993) [8] reported application of fertilizers at 240 g N, 240 g P and 240 g K per plant per year produced quality fruits in custard apple. The area under custard apple is augmenting in India on profitable scale and no work has been done with respect to fertilization of custard apple cv. 'Raydurg' in the Vertisols of Rajasthan. Hence, the present study was carried out to find out the requirement of NPK levels for commercial cultivation of custard apple cv. 'Raydurg'. In Rajasthan state, custard apple is in profusion as natural gene sanctuary of sub-mountainous tracts in Aravalli hills. The commercial cultivation of custard apple is gaining momentum in Chittorgarh and Jhalawar districts of Rajasthan state. In nature, custard apple thrives on shallow gravely soils in forest areas with green micro-climate under the cover of big forest tree species.

Biofertilizers play helpful role along with organic manure in augmenting availability present in rhizosphere of Raydurg plant nutrients but also increases nutrient uptake through proton efflux under friendly situations of moisture, temperature and humidity surrounding plant environment variables with more obtainability of macro - micronutrients, superior synthesis of plant growth promoters, antifungal substances also enriching the soil fertility and sustainability in natural ecosystem. In recent years, biofertilizers have arisen as a significant supportive component of integrated nutrient source chain management. Several research findings showed huge potentialities and likely options via biofertilizers inoculants in adding inorganic fertilizers. *Azospirillum brasilense* are utilized widely to additional nitrogenous fertilizers in nutrient management programmes in horticultural fruit crops. *Azospirillum* are surface dominated rhizobacteria. Research carried out over the past various years on different other crops have showed that the plants inoculated with *Azospirillum* derive positive advantage in terms of augmented yield, plant biomass and N uptake are attributed to small augmentation in N from biological nitrogen fixation, development and branching of roots, augmentation of root volume, production of plant growth hormones, augmentation in uptake of NO_3^- , NH_4^+ , H_2PO_4^- , K^+ and Fe^{2+} , enhanced water status of plants, augmented nitrate reductase activity in plants and production of antibacterial and antifungal compounds as reported by Wani (1990) [22]. *Azospirillum brasilense* are highly mobile in soil and increases the root surface available for nutrients. Besides, *Azospirillum brasilense* have also been found to stimulate synthesis of growth promoting substances like auxins, gibberellins, cytokinins and antibiotic metabolites which in turn enhanced resistance against biotic and abiotic stress. The role of *Azospirillum* is considered as important in revival of soil nutrient cycle through symbiotic biological fixation. The supplementation of organic manures with mineral fertilizers can have helpful effect on the amelioration of physical, microbiological and chemical properties of soil, which are indirectly responsible for supporting growth and development of plants as reported by Adak *et al.* (2012) [1]. Excessive and indiscriminate use of chemical fertilizer has led to harmful effects both on soil and environments. The leftover of residues by the synthetic fertilizers got entry into food chain thereby causing environmental pollutions. The inorganic fertilizers generate problems of environmental pollution along

with powerful hazards to flora, fauna counting human beings. The chemical fertilizers being used as expensive input and have a relevant harmful effect for developing country like India for last four decades. Rapid increase in costs of inorganic fertilizers and depleting soil health required organic farming. Depleting structure of soil health demands restoration of soil through incorporation. Large scale use of chemical fertilizers has caused soil imbalance in terms of NPK and C: N ratio. Organic farming using vermicompost which were only source for supplying nutrients to crops in past, has regained its due importance keeping in view of soil health and balanced nutrition. The soil quality plays in important role in determining the sustainability and growth as well as productivity of any agro ecosystem as noticed by Dwivedi and Dwivedi (2007) [4]. Vermicompost is an eco-friendly supportive organic additive derived from biodegradable organic wastes by earthworms and microorganisms especially *Eisenia foetida*. Vermicompost is devoid from chemical inputs and rich in supporting beneficial micro - flora such as N fixers, P - solubilizers, cellulose decomposing micro - flora etc. It contains earthworm cocoons which increases the population and enhances activity of earthworm in the soil through degradation of organic matter. Vermicompost contains valuable vitamins, enzymes and hormones like auxins, gibberellins etc, that enhances the decomposition of organic matter in soil, improvement soil structure, texture, aeration and improves better water holding capacity and thus prevents soil erosion. It also improves mineral nutrient content of soil in terms of macro and micronutrients, hitherto, because of its added organic matter content it promotes better rhizosphere development, root volume area and mineral nutrient absorption. Vermicompost has very high porosity, aeration, drainage and water holding capacity as reported by Sinha (2009) [16]. Nutrient profile available in applied Vermicompost was: N (1.6%), P (0.7%), K (0.8%), Ca (0.5%), Mg (0.2%), Fe (175 ppm), Mn (96.5 ppm), Zn (24.5 ppm), Cu (5.0 ppm), C: N ratio 15.5. Vermicompost have many tremendous biological properties. It is rich in bacteria, actinomycetes, fungi as reported by Edwards (1983) [2, 5]; Tomati *et al.* (1987) [5, 19, 20]; Werner and Cuevas (1996) [23] and beneficial cellulose-degrading bacteria (Werner and Cuevas, 1996) [23]. In addition, Tomati *et al.* (1983) [5, 19, 20] reported that earthworm castings, obtained after process of sludge digestion were rich in microorganisms especially bacteria. In Vermicompost, the content of Nitrogen (N), Phosphorus (P) and Potassium (K) generally depends upon the substrate used. Red worm (*Eisenia foetida*) is mainly utilized in India for urban and peri-urban areas for sustenance of livelihood by progressive farmers raising Vermicompost production in Indian scenario. The present study was undertaken on custard apple cv. Raydurg during 2018 and 2019 to assess the response of *Azospirillum brasilense* and Vermicompost in black vertisols with humid agro-ecological conditions.

Material and Methods

Site description

The experiment was carried out at the Fruit Instructional Farm, Department of Fruit Science, College of Horticulture and Forestry, Jhalawar during the two consecutive seasons of 2018-19 and 2019-20. The Jhalawar district is located at 23°4 and 24°52 N-Latitude and 75°29 to 76°56 E-Longitude in South Eastern Rajasthan. Agro-climatically, the district falls in Zone V, known as Humid South Eastern Plain. About

84.22 per cent population of the district is rural whose main occupation is agriculture. Average precipitation in the region is 954.7 mm. The precipitation pattern is continuous with heavy downpour of rains during July and August months and continues till first week of September. High humidity prevails during rainy season i.e. up to 98 per cent and weather remains mostly cloudy during July-August month. Overall high humidity prevails during entire year except April to June months of the year in Jhalawar district of Rajasthan state. Maximum temperature range in the summer is 43-48° C and minimum 1-2.6 °C during winter. Agriculture and forest lands occupy 73.5 per cent area, respectively in the district. Major fruit crops of the district are Nagpur Mandarin and Guava. While in kharif season, the major cash crops are soybean, maize and pulses and during Rabi - wheat, mustard, coriander and garlic are main crops.

Experimental details

The field experiment was laid out in Randomized Block Design (RBD) with three replications. The factors of experimentation comprising of sixteen treatments combinations of *Azospirillum* and Vermicompost on soil canopy of custard apple cv. Raydurg plants. Eight years old custard apple cv. Raydurg plants of uniform size growth and vigour were selected at Fruit research farm, Department of Fruit Science, College of Horticulture and Forestry, Jhalrapatan, Jhalawar for experimentation. For this experiment, a total of 48 plants were selected from the established custard apple block for two consecutive research trials from July 2018 to November 2019 and July 2019 to November 2019, respectively. T₀ (control), T₁ (AZS @ 25 g/plant), T₂ (AZS @ 50 g/plant), T₃ (AZS @ 75 g/plant), T₄ (V.C. @ 10 kg/plant), T₅ (V.C. @ 15 kg/plant), T₆ (V.C. @ 20 kg/plant), T₇ (AZS @ 25 g + V.C. @ 10 kg/plant), T₈ (AZS @ 25 g + V.C. @ 15 kg/plant), T₉ (AZS @ 25 g + V.C. @ 20 kg/plant), T₁₀ (AZS @ 50 g + V.C. @ 10 kg/plant), T₁₁ (AZS @ 50 g + V.C. @ 15 kg/plant), T₁₂ (AZS @ 50 g + V.C. @ 20 kg/plant), T₁₃ (AZS @ 75g + V.C. @ 10 kg/plant), T₁₄ (AZS @ 75g + V.C. @ 15 kg/plant), T₁₅ (AZS @ 75g + V.C. @ 20 kg/plant).

Soil sample collection and analysis

In order to assess the physico-chemical properties of the experimental soil, the soil samples were drawn randomly from different location in the field at a depth of 0-30, 30-60 and 60-90 cm before the commencement of the experiment. A representative sample was prepared and subjected to mechanical, physical and chemical analysis.

Methodology used for observation

The various methods used for studying plant growth attributes of custard apple cv. Raydurg are given under suitable headings:

Plant growth attributes

Rootstock girth (mm)

For measuring the girth of rootstock, the custard apple cv. Raydurg plants were marked at a fix point. Initial girth of rootstock was measured separately under allotted treatment combinations and final rootstock girth was measured after completion of experiment by using digital Vernier Calipers. The observations pertaining to rootstock girth were recorded for two consecutive seasons i.e. during July 2018 and November 2018 for first season and during July 2019 and

November 2019 for second season.

Scion girth (mm)

For recording the scion girth, plants of custard apple cv. Raydurg were marked at a fix point. Initial scion girth was measured before initiation of experiment the allotted treatment combinations of plants and final scion girth was recorded after completion of experiment with the help of digital Vernier Calipers. The observations related to scion girth were measured for two successive seasons i.e. during July 2018 and November 2018 for first research trial and during July 2019 and November 2019 for second research trial.

Plant height (m)

The plant height attribute was measured from the base of the soil surface to the highest apex of the plant with the help of measuring tape. Initial plant height was recorded before initiation of experiment on plants with allotted treatment combinations and final plant height was measured after completion of experiment for two consecutive seasons i.e. during July 2018 and November 2018 for first research duration and subsequently during July 2019 and November 2019 for second duration respectively.

Number of nodes/shoot

The numbers of nodes per shoot on related five shoots were counted manually at initiation and completion of experiment from related tagged shoots during July 2018 and November 2018 as well as during July 2019 and November 2019 successively.

Number of nodes/branch

The numbers of nodes per branch on related five branches were counted manually at starting and termination of experiment on selected tagged branches during July 2018 and November 2018 and subsequently during July 2019 and November 2019, respectively.

Number of shoots/branch

The numbers of shoots per branch were recorded manually at initiation and completion of experiment on selected five branches and average was marked out. Observations pertaining to number of shoots per branch were recorded for two consecutive seasons i.e. during July 2018 and November 2018 and subsequently during July 2019 with final observation during November 2019.

Number of shoots per plant

The numbers of shoots per plant were counted manually on allotted treatment combinations of custard apple plants during initiation and completion of experiment. The data concerning number of shoot per plant was recorded for two successive research periods i.e. during July 2018 and November 2018 and subsequently during July 2019 and final observation at November 2019, respectively.

Soil parameters

Bulk density (g/cm³) (Piper, 1950) [11]

Bulk density of soil of 0-15 cm depth was determined. The core sampler was pressed in the soil for enough depth to fill the core. Carefully removed the sampler and trimmed the soil extending out of the core with a sharp knife. Soil was oven dried at 105 °C to a constant weight, cooled and weighed. Soil

volume was taken equal to inner volume of core sampler. Bulk density was calculated using the following formula and expressed as g/cm^3 .

$$\text{Bulk density} = \frac{\text{Mass of oven dry soil}}{\text{Volume of soil including pore space}}$$

Porosity (%)

The porosity was calculated at end of experiment as per the formulae

$$\text{Porosity (\%)} = 1 - \frac{\text{Bulk density}}{\text{Particle density}} \times 100$$

pH: (Jackson, 1973) [7]

Soil pH was determined by using 1:2 soil water suspensions by glass electrode pH meter. 1:2 soil water suspension was prepared by taking 20 g of soil and 40 ml distilled water in 100 ml beaker.

Electrical conductivity (dSm^{-1}): (Jackson, 1973) [7]

Electrical conductivity of soil was determined in 1:2 soil water suspension by using standard precision conductivity bridge. Digital conductivity meter 1:2 soil water suspension was prepared by taking 20 g of soil and 40 ml distilled water in 100 ml beaker.

Organic carbon (%)

The determination of soil organic carbon is based on the Walkley Black's (1934) [21] chromic acid wet oxidation method. Oxidisable matter in the soil is oxidized by 1 N $K_2Cr_2O_7$ solution. The reaction is assisted by the heat generated when two volumes of H_2SO_4 are mixed with one volume of the dichromate. The remaining dichromate is titrated with ferrous sulphate. The titre is inversely related to the amount of C present in the soil sample. Following reagents was used to determined organic carbon percentage in soil.

- Standard N, Potassium Dichromate ($K_2Cr_2O_7$) solution: Exactly 49.04 g of $K_2Cr_2O_7$ is dissolved in water and diluted to one litre.
- 0.5 Ferrous ammonium sulphate solution: Exactly 196.1 g $Fe(NH_4)(SO_4)_2 \cdot 6H_2O$ is dissolved in 800 ml of distilled water containing 20 ml of concentrated H_2SO_4 and diluted to one litre.
- Diphenyl amine indicator: 0.5 g of diphenyl amine is dissolved in 20 ml of water and 100 ml of concentrated H_2SO_4 .
- Orthophosphoric acid (85%)
- Concentrated sulphuric acid

Calculation

$$\text{Organic Carbon (\%)} = \frac{10(B-T)}{B} \times \frac{0.003 \times 100}{\text{Wt. of soil (g)}}$$

Where,

B = Volume (ml.) of ferrous ammonium sulphate solution required for blank titration.

T = Volume of ferrous ammonium sulphate solution needed for titration of soil sample.

Available nitrogen (kg/ha)

Available nitrogen was determined by using alkaline potassium permanganate method as suggested by Subbiah and Asija (1956).

Reagents

- 0.32% $KMnO_4$: Dissolve 3.2 g pure $KMnO_4$ in distilled water and dilute to one litre.
- 2.5% NaOH Solution: Dissolve 25 g NaOH in distilled water and dilute to one litre.
- 0.02% H_2SO_4 : Dissolve 0.56 ml of concentrated H_2SO_4 and make the volume to one litre and then standardize with 0.02 N NaOH using phenolphthalein indicators.
- 0.02 N NaOH: Dissolve 0.8 g NaOH in distilled water and diluted to one litre.
- Methyl red indicator: Dissolve 0.07 g methyl red in 100 ml of 95% ethanol.

$$\text{Calculation - Available N (kg/ha)} = R \times 31.36$$

Where R = Volume of 0.02 N H_2SO_4 required for titration

Available phosphorus in soil (Kg/ha)

Available phosphorus was determined with extraction by 0.5 M $NaHCO_3$ solution adjusted at pH 8.5 as suggested by Olsen *et al.*, (1954) [10].

Reagents

- 0.5 Sodium bicarbonate: Exactly 42 g of $NaHCO_3$ is dissolved in water and volume is making up to one litre. The pH with 1 N NaOH.
- Activated charcoal (Phosphorus free)
- Ammonium molybdate: (1.5%) Dissolve 15 g of ammonium molybdate in 300 ml of warm distilled water (about 50 °C). Filter the mixture and allow it to cool. Then 342 ml of concentrated HCL is added gradually by stirring it. It was to one litre by distilled water.
- Stannous chloride (Stock solution): Exactly log of $SnCl_2$ is dissolved in 25 ml concentrated HCL, and a piece of tin is added followed by heating, till it becomes clear. The solution is stored in amber coloured bottle.
- Working solution of stannous chloride: 0.5 ml stock solution of $SnCl_2$ is taken, and volume is made up to 66 ml with distilled water.
- Standard solution of phosphorus: Exactly 0.43 g of analytical reagent KH_2PO_4 (oven dried at 40 °C) is taken and it is dissolved in about half a litre of distilled water. Then 25 ml of 7 N H_2SO_4 is added and volume is made up to 1 litre with distilled water. This gives 100 ppm P.

Standard curve

2.5 ppm P solution was prepared by taking 2.5 ml of 100 ppm solution in 100 ml volumetric flask and its volume was made up by distilled water. The standard curve was prepared by taking different concentration of P, i.e. 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0 ppm P. These readings were taken on calorimeter.

$$\text{Available P (kg/ha)} = \frac{R \times \text{volume of extract}}{\text{Volume of aliquot}} \times \frac{2.24 \times 10^6}{\text{Wt. (g) of soil} \times 10^6}$$

$$\text{Where R = ppm in the aliquot} = \frac{R \times 100}{5} \times \frac{2.24}{5}$$

(Obtained from standard curve) = (ppm P) R \times 8.96 \times 2.29

Available potassium (Kg/ha)

Available potassium was determined by extracting the soil by

shaking with N neutral ammonium acetate solution by flame photometer as suggested by Metson (1956) [9].

- N neutral ammonium acetate solution: The 700 ml of distilled water is taken in a one liter volumetric flask. Then, 57 ml glacial acetic acid (95.5%) along with 69 ml of concentrated ammonium hydroxide is added. It is then diluted to volume of 900 ml and pH is adjusted to 7.0 by the addition of 3N NH₄OH, and volume is make up to 1 litre.
- Standard solution: Exactly 1.908 g of AR grade KCl was dissolved in distilled water and volume is make up to 1 litre. This solution contains 1 mg K/ml i.e. 1000 ppm K.
- Working solution of K: Taking 10 of 1000 ppm K solution in 100 ml volume flask and making, its volume, this gives 100 ppm K.

Standard curve

From the 100 ppm solution 10, 15, 20, 25, 30 and 40 ml of K were taken in each 100 ml volumetric flask and volume was made up. The curve was obtained by plotting the reading of flame photometer against the various concentrations of K.

Calculation

$$\text{Available K (Kg/ha)} = \frac{R \times \text{Vol. of extract} \times 2.24 \times 10^6}{\text{Wt. of soil taken} \times 10^6}$$

Where, R = ppm of K in the extract (obtained from the standard curve) = ppm K × 11.2

The soil of experimental site was clay loam in texture (Black cotton soil), normal in reaction and fair in fertility status with respect to nitrogen, phosphorus, and potassium.

Microbial population (cfug⁻¹)

Fourty-eight soil samples under different treatment combinations of *Azospirillum brasilense* and Vermicompost application were collected from the custard apple cv. Raydurg plants rhizosphere from 1.5³ area volume and were brought to laboratory in polythene bags and screened through 2 mm sieve. The samples were pooled and thoroughly mixed to make a representative sample. The serial dilution technique was employed for isolation and identification of viable Bacteria, Fungi and Actinomycetes count. Prepared the media for desired microflora. Pour the autoclaved and cooled (45°C) medium into sterile Petri plates and then allowed the medium to solidify. Add one gram of sieved (2mm) soil to 9 ml sterile water and shake it for 15-20 minutes. Prepare serial dilution 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, 10⁻⁷ and 10⁻⁸. Add 1 ml of aliquots of various dilutions over cooled and solidified medium in Petri plates. The plates were rotated for uniform distribution of spores. Incubate the plates at 28 °C for 3-4 days. Observe the plates for appearance of colonies onto the surface of medium. Population count of Bacteria, Fungi and Actinomycetes were measured using dilution plate technique by employing Martin Rose Bengal agar media and Ken Knight's agar media, respectively as suggested by Rangaswamy (1966) [12].

Nutrient agar (Bacteria)

Beef extract: 10.0 g
Peptone: 0.5 g
NaCl: 0.1 g
Agar-agar: 15.0 g

Distilled water: 1000 ml

Martin's rose bengal medium for (Fungi)

Glucose: 1.0 g
Peptone: 0.5 g
K₂HPO₄: 1.0 g
MgSO₄.7H₂O: 0.5 g
Rose Bengal: 0.33 g
Streptomycin sulphate (10%) sol.: 3.0 ml
Agar-agar: 15.0 g
Distilled water: 1000 ml

Kenknight and Munaires medium for (Actinomycetes)

K₂HPO₄: 1.0 g
NaNO₃: 0.1 g
KCl: 0.1 g
MgSO₄: 0.1 g
Glucose: 1.0 g
Agar-agar: 15-20 g
Distilled water: 1000 ml

Statistical analysis

The data obtained during the experimentation were subjected to individual year analysis of the data during 2018 and 2019 in custard apple cv. Raydurg to followed by pooled statistical analysis of two years (2018 and 2019) using Fisher's (1950) [6] analysis of variance technique. The significance of the treatments was tested through F test at 5 per cent level of significance. The critical difference CD was calculated to assess the significance of difference among the different treatments.

Results and Discussion

Plant growth parameters

It is clear from the data presented in table 1 that the morphological growth attributes viz. rootstock girth (mm), scion girth (mm), plant height (m), number of nodes/shoot, number of nodes/branch, number of shoots/branch, number of shoots/plant augmented significantly with biofertilizers and vermicompost at different treatment combinations, the maximum rootstock girth (67.34 mm), scion girth (59.08 mm), plant height attribute (2.94 m), number of nodes per shoot (12.83), number of nodes per branch (68.17), number of shoots per branch (34.33), number of shoots per plant (150.67) were found better under T₁₄ treatment (AZS @ 75g + V.C. @ 15 kg /plant) and have shown amenable response in enhancement of plant growth parameters. However, considering the equivalence of treatments in obtaining plant growth parameters at par performance with T₁₀ treatment (AZS @ 50g + V.C. @ 10 kg/plant), it was observed as best treatment for holistic improvement of custard apple cv. Raydurg plants for two successive ontogenical seasons. In T₁₀ treatment, the equivalent values for plant growth parameters including rootstock girth (64.60 mm), scion girth (56.87 mm), plant height characteristic (2.90 m), number of nodes per shoot (11.00), number of nodes per branch (64.17), number of shoots per branch (32.00), number of shoots per plant (147.83) have shown at par response to T₁₄ treatment in enhancement of above mentioned plant growth parameters. The better plant growth parameters in T₁₀ treatment (AZS @ 50g + V.C. @ 15 kg/plant) might be due to increased root volume, augmented photosynthetic and photoreceptor pigments, enhanced proline levels, mobilization of enzymes, improvement of physical condition of soil, macro and micro-

nutrients availability, improved auxin synthesis, improved source-sink ratio and apportionment of multinutrients within the plant system.

It is clear from the data presented in table 1 and fig.1 depicts the reflective trend Vis a Vis different treatment combinations of *Azospirillum brasilense* and Vermicompost on performance of major growth characteristics in custard apple cv. Raydurg plants. The better effect of T₁₀ treatment over other treatment may also be elucidated in the light of the fact that balanced fertilization, appropriate uptake and better distribution of plant nutrients within the plant system might resulted with the combined dose of *Azospirillum brasilense* and Vermicompost. The augmented root surface area there by making it obtainable for more extinction of nutrient by *Azospirillum brasilense* under T₁₀ treatment might improve the nitrogen fixation and improved the plant growth

parameters. The better growth parameter under T₁₀ treatment (AZS @ 50g + V.C. @ 10 kg/plant) might be attributed to augmented biological nitrogen fixation and growth promoting phytohormones particularly auxin production along with stimulatory role through Vermicompost augmentation in canopy soils of custard apple cv. Raydurg. It could also be attributed with the fact that *Azospirillum brasilense* in combination with Vermicompost possibly augmented biological mechanisms which increased the obtainability of nutrients for better assimilation by custard apple plants. Similar explanations for development of plant growth parameters in horticulture crops under the influence of biofertilizer and vermicompost has been presented by Dutta *et al.* (2010) [3] in litchi and Singh *et al.* (2010) [14, 15] in strawberry.

Table 1: Effect of *Azospirillum brasilense* and Vermicompost on rootstock girth, scion girth, no. of nodes/shoot, no. of nodes/ranch, no. of shoots/branch and no. of shoots/plant of custard apple cv. Raydurg pooled via analysis for two consecutive seasons during (2018 and 2019)

Treatment combinations	Rootstock girth (mm)	Scion girth (mm)	Plant height (m)	No. of nodes/shoot	No. of nodes/branch	No. of shoots/branch	No. of shoots/plant
T ₀ (Control)	58.17	50.74	2.70	7.50	50.50	21.83	126.00
T ₁ (AZS @ 25 g/plant)	60.69	53.93	2.81	9.33	57.67	26.50	141.17
T ₂ (AZS @ 50 g/plant)	60.72	54.20	2.81	9.83	58.67	27.33	142.00
T ₃ (AZS @75 g/plant)	60.67	54.12	2.83	9.17	58.17	27.67	141.17
T ₄ (V.C.@ 10 kg/plant)	60.79	54.14	2.81	9.83	59.00	28.33	141.67
T ₅ (V.C. @ 15 kg/plant)	60.70	54.06	2.83	9.33	59.33	27.83	141.50
T ₆ (V.C. @ 20 kg/plant)	61.35	54.28	2.82	9.67	58.50	27.17	141.67
T ₇ (AZS @ 25 g +V.C. @ 10 kg/plant)	61.43	54.10	2.86	9.50	59.33	28.17	142.17
T ₈ (AZS @ 25 g + V.C. @ 15 kg/plant)	60.80	54.31	2.81	9.83	58.33	28.17	141.83
T ₉ (AZS @ 25g + V.C. @ 20 kg/plant)	61.73	54.24	2.82	9.67	61.00	28.17	142.33
T ₁₀ (AZS @ 50 g + V.C. @ 10 kg/plant)	64.60	56.87	2.90	11.00	64.17	32.00	147.83
T ₁₁ (AZS @ 50 g + V.C.@ 15 kg/plant)	64.80	57.40	2.90	11.50	64.83	32.17	148.17
T ₁₂ (AZS @ 50 g + V.C. @ 20 kg/plant)	64.81	57.37	2.90	11.17	64.00	32.17	147.67
T ₁₃ (AZS @ 75g + V.C. @ 10 kg/plant)	64.71	57.60	2.91	11.33	64.67	32.00	148.17
T ₁₄ (AZS @ 75 g + V.C. @ 15 kg/plant)	67.34	59.08	2.94	12.83	68.17	34.33	150.67
T ₁₅ (AZS @ 75 g + V.C. @ 20 kg/plant)	64.70	57.41	2.90	11.33	65.50	32.00	148.17
SEm (±)	1.02	0.42	0.01	0.78	1.38	1.22	1.35
CD. (5%)	3.17	4.01	0.06	2.59	5.53	3.96	4.89

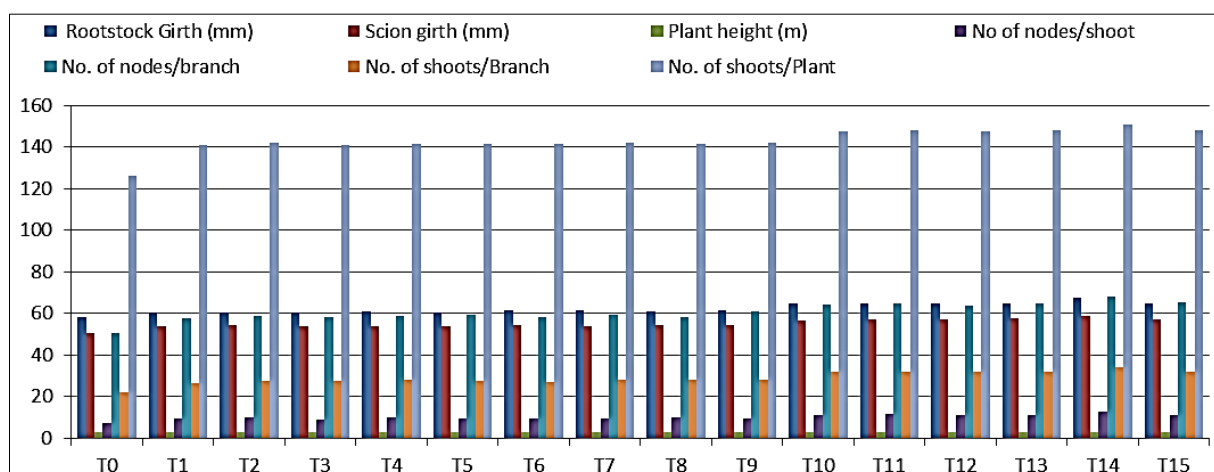


Fig 1: Effect of *Azospirillum brasilense* and vermicompost on rootstock girth, scion girth, no. of nodes/shoot, no. of nodes/ranch, no. of shoots/branch and no. of shoots/plant attributes of custard apple cv. Raydurg pooled representing analysis for two consecutive seasons during (2018 and 2019)

Soil parameters

The T₁₄ treatment was found at par with T₁₀ treatment. Statistically T₁₀ treatment gave highest consequences at lower doses of *Azospirillum brasilense* and Vermicompost; therefore it was regarded as best treatment in improvement of overall soil health equilibrium. The data presented in table 2 and Fig.2 reflects declining trend with respect to bulk density, soil pH and electrical conductivity of soils with regard to

increase in doses of *Azospirillum brasilense* and Vermicompost and reached optimal levels at T₁₀ treatment and onwards remain stable up to T₁₄ treatment and performance of soil characteristics exhibited at par performance between T₁₀, T₁₁, T₁₂, T₁₃, T₁₄ and T₁₅ treatments. In T₁₀ treatment, the corresponding optimal values of bulk density (1.28 g/cm³), soil pH (7.38) and electrical conductivity (0.46 dSm⁻¹) recorded were found significantly

reduced under T₁₀ treatment as compared with other. The data presented in table 2, Fig. 2 and Fig. 3 reveals augmented mineral nutrient status of macro nutrients under different treatment combinations of *Azospirillum brasilense* and Vermicompost application in T₁₀ treatment. The soil physico-chemical properties like change in porosity (50.22%), organic carbon (0.55%), available nitrogen content (344.30 Kg/ha), available phosphorous content (42.44 Kg ha⁻¹) and available potassium content (317.00 Kg ha⁻¹) have shown better response in improvement of soil parameters under T₁₀ treatment (AZS @ 50g + V.C. @ 10 kg /plant) and were exhibited at par performance with T₁₄ treatment. The T₁₀ treatment was found at par with T₁₄ treatment, therefore T₁₀ treatment (AZS @ 50g + V.C. @ 10 kg/plant) was considered equivalent to T₁₄ treatment, since better plant growth response were recorded at lower doses of *Azospirillum brasilense* and Vermicompost application under T₁₀ treatment.

The data elucidated under table 2 enumerates that all soil parameters of custard apple cv. Raydurg exhibited better enhancement in treatment T₁₀ (AZS @ 50g + V.C. @ 10 kg/plant). The overall development with respect to sequential effect on bulk density, change in porosity, organic carbon (%) and available N, P, K content might be attributed to increased organic matter status of soil, better fixation of atmosphere nitrogen through symbiotic association with roots and better nutrient use efficiency through the combined application of

treatments.

Azospirillum and Vermicompost of (AZS @ 50g + V.C. @ 10kg/plant) under T₁₀ treatment. It could also be allocated to the fact that *Azospirillum* and Vermicompost combination under T₁₀ treatment might manifested helpful mutualistic interaction in tandem there by soil improved health through favourable membrane activities by tolerating abiotic stresses predominantly high precipitation during active growth season from July to October under present studies.

The reduction in soil pH and EC under T₁₄ treatment might be due to incorporation of organic matter in canopy soil of custard apple cv. Raydurg plants. This may be clarified in the light of the fact that incorporation of organic source through Vermicompost and bioinoculant through *Azospirillum brasilense* has simulative result in improvement through augmented mobilization of available nutrients by multiple mechanisms in canopy rhizosphere of custard apple cv. Raydurg soil fertility.

Similar type of observations was also described by Singh *et al.* (2010) [14, 15] who reported that increase in porosity was observed with the incorporation of organic source in apricot. Likewise, enhancement in soil quality in terms of physical properties was also reported by Singh (2007) [14, 15] who reported enhanced soil health through application of integrated nutrient management practice.

Table 2: Effect of *Azospirillum brasilense* and vermicompost on bulk density, soil pH, electrical conductivity, change porosity, soil organic carbon percentage content, available N₂, P₂O₅ and K₂O of canopy soils of custard apple cv. Raydurg through pooled analysis for two consecutive seasons during (2018 and 2019)

Treatment combinations	B.D. (g/cm ³)	Soil pH	EC (dSm ⁻¹)	Change in porosity (%)	O.C. (%)	N ₂ (kg ha ⁻¹)	P ₂ O ₅ (kg ha ⁻¹)	K ₂ O (kg ha ⁻¹)
T ₀ (Control)	1.40	7.82	0.65	43.75	0.42	326.32	34.83	306.10
T ₁ (AZS @ 25 g/plant)	1.30	7.57	0.61	46.60	0.43	332.76	36.55	308.83
T ₂ (AZS @ 50 g/plant)	1.37	7.55	0.59	46.42	0.44	335.43	36.44	309.23
T ₃ (AZS @ 75 g/plant)	1.36	7.54	0.59	46.85	0.44	334.65	36.77	310.74
T ₄ (V.C. @ 10 kg/plant)	1.35	7.52	0.57	47.46	0.48	335.99	37.83	311.73
T ₅ (V.C. @ 15 kg/plant)	1.33	7.50	0.56	47.48	0.47	336.61	38.04	312.07
T ₆ (V.C. @ 20 kg/plant)	1.32	7.47	0.54	47.48	0.48	337.45	38.37	312.77
T ₇ (AZS @ 25 g +V.C. @ 10 kg/plant)	1.32	7.46	0.51	47.58	0.48	339.24	38.96	313.34
T ₈ (AZS @ 25 g + V.C. @ 15 kg/plant)	1.31	7.43	0.49	47.70	0.50	341.99	39.22	313.87
T ₉ (AZS @ 25g + V.C. @ 20 kg/plant)	1.30	7.41	0.47	47.85	0.50	342.18	39.51	314.12
T ₁₀ (AZS @ 50 g + V.C. @ 10 kg/plant)	1.28	7.38	0.46	50.22	0.53	344.30	42.44	317.00
T ₁₁ (AZS @ 50 g + V.C. @ 15 kg/plant)	1.28	7.37	0.44	50.07	0.54	344.39	42.46	317.03
T ₁₂ (AZS @ 50 g + V.C. @ 20 kg/plant)	1.27	7.37	0.45	50.17	0.53	345.02	42.94	316.88
T ₁₃ (AZS @ 75g + V.C. @ 10 kg/plant)	1.27	7.36	0.44	50.13	0.54	345.07	42.54	317.19
T ₁₄ (AZS @ 75 g + V.C. @ 15 kg/plant)	1.26	7.34	0.43	51.58	0.57	346.92	44.34	319.72
T ₁₅ (AZS @ 75 g + V.C. @ 20 kg/plant)	1.28	7.36	0.44	50.08	0.54	345.32	42.73	317.75
SEm (±)	0.01	0.01	0.01	0.61	0.01	0.92	0.67	1.07
CD. (5%)	0.03	0.03	0.02	2.06	0.05	3.46	2.64	4.03

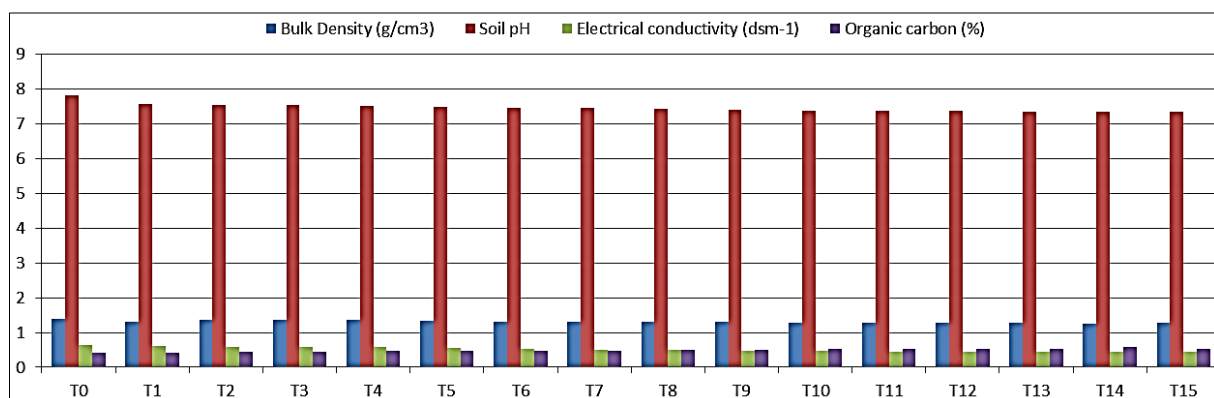


Fig 2: Effect of *Azospirillum brasilense* and vermicompost on bulk density, soil pH, electrical conductivity and soil organic carbon percentage content of canopy soils of custard apple cv. Raydurg representing pooled analysis for two consecutive seasons during (2018 and 2019)

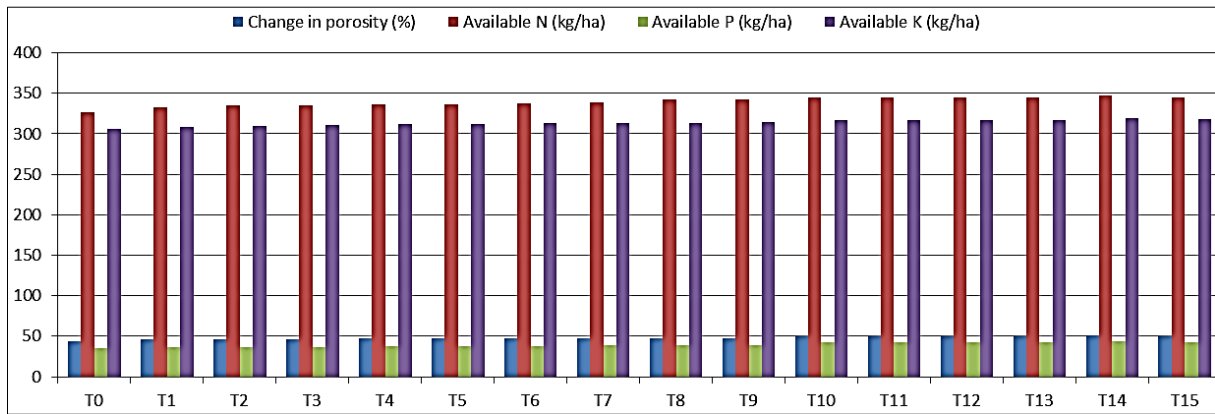


Fig 3: Effect of *Azospirillum brasilense* and vermicompost on change in porosity, available N₂, P₂O₅ and K₂O content of canopy soils of custard apple cv. Raydurg through pooled analysis for two consecutive seasons during (2018 and 2019)

Soil microbial populations

The data presented in table 3 and Fig. 4 reveals augmented mineral nutrient status of macro nutrients under different treatment combinations of *Azospirillum brasilense* and Vermicompost application in T₁₀ treatment. The T₁₄ treatment was found at par with T₁₀ treatment. In T₁₀ treatment, the corresponding values of bacterial population (27.33 cfug⁻¹), fungi population (14.67 cfug⁻¹) and actinomycetes population (10.50 cfug⁻¹) have shown better response in enhancement of soil parameters under T₁₀ treatment (AZS @ 50g + V.C. @ 10 kg /plant) and was found at par with T₁₄ treatment. The better

efficiency on augmentation of microbial population viz. bacteria, fungi and actinomycetes proliferation under T₁₀ treatment could be contributed to improved activity and buildup of overall soil micro- organisms which might utilized organic matter through production of extracellular enzymes in soil rhizosphere. The present results are in conformity with the findings as reported by Tejada and Gonzalez (2008) [18], Arancon *et al.* (2006) [2] who reported enhancement in enzymatic activities after incorporation of various type of Vermicompost in soil.

Table 3: Effect of *Azospirillum brasilense* and vermicompost on bacterial population, fungi population and actinomycetes population of canopy soils of custard apple cv. Raydurg representing pooled analysis for two consecutive seasons during (2018 and 2019)

Treatment combinations	Bacteria populations (cfug ⁻¹)	Fungi population (cfug ⁻¹)	Actinomycetes population (cfug ⁻¹)
T ₀ (Control)	20.50	9.33	7.50
T ₁ (AZS @ 25 g/plant)	23.67	10.33	8.50
T ₂ (AZS @ 50 g/plant)	24.00	11.00	9.50
T ₃ (AZS @75 g/plant)	24.00	10.67	8.50
T ₄ (V.C.@ 10 kg/plant)	24.33	11.33	8.83
T ₅ (V.C. @ 15 kg/plant)	24.00	12.00	8.50
T ₆ (V.C. @ 20 kg/plant)	22.67	12.33	8.83
T ₇ (AZS @ 25 g +V.C.@ 10 kg/plant)	24.00	11.50	8.83
T ₈ (AZS @ 25 g + V.C.@ 15 kg/plant)	23.67	12.33	9.17
T ₉ (AZS @ 25g + V.C.@ 20 kg/plant)	24.00	12.50	9.00
T ₁₀ (AZS @ 50 g + V.C. @ 10 kg/plant)	27.33	14.67	10.50
T ₁₁ (AZS @ 50 g + V.C.@ 15 kg/plant)	27.67	14.67	10.83
T ₁₂ (AZS @ 50 g + V.C.@ 20 kg/plant)	28.33	15.00	10.83
T ₁₃ (AZS @ 75g + V.C.@10 kg/plant)	28.00	15.00	11.83
T ₁₄ (AZS @ 75 g + V.C.@ 15 kg/plant)	31.17	15.83	12.83
T ₁₅ (AZS @ 75 g + V.C.@ 20 kg/plant)	28.67	14.33	11.17
SEm (±)	1.14	0.65	0.95
CD. (5%)	6.01	2.28	3.32

*c.f.u.g⁻¹(colony forming units per gram of soil)

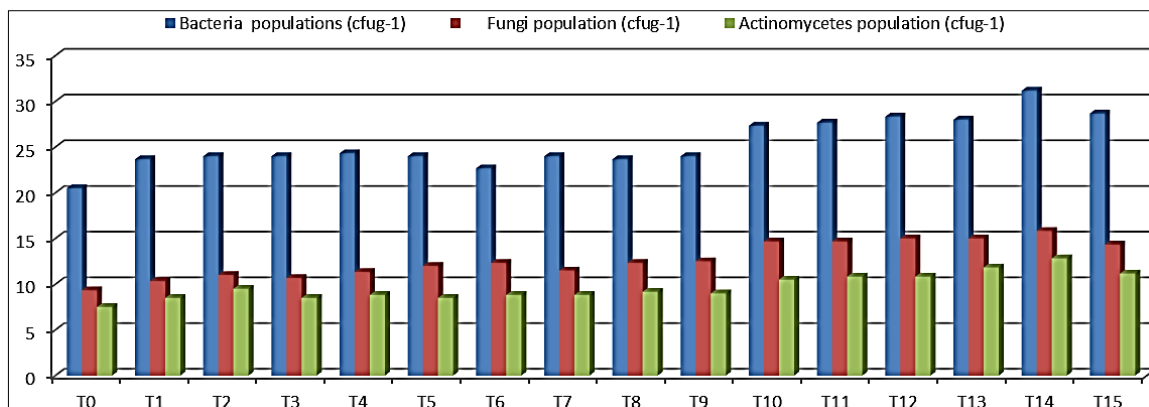


Fig 4: Effect of *Azospirillum brasilense* and vermicompost on bacterial population, fungi population and actinomycetes population of canopy soils of custard apple cv. Raydurg reflecting pooled analysis for two consecutive seasons during (2018 and 2019)

Conclusion

It may be concluded that application of *Azospirillum* and Vermicompost through T₁₀ treatment (AZS @ 50g + V.C. @ 10 kg/plant) was found relatively better considering at par performance with T₁₄ treatment at lower doses of *Azospirillum brasilense* for enhancement of plant growth characteristics as well as soil physicochemical properties and microbial populations under soil canopy soil of custard apple cv. Raydurg plants for two successive seasons (2018 and 2019) sub humid agro ecology of under Jhalawar conditions. Among the various treatments, T₁₀ treatment (AZS @ 50g + V.C. @ 10 kg/plant) was found moderately better over all other treatments with respect to most of the plant growth characteristics including soil physico-chemical properties and buildup of microbial population in soil canopy rhizosphere of custard apple cv. Raydurg plants. The consequences demonstrated helpful mutualistic interaction of *Azospirillum brasilense* and Vermicompost for harnessing the nutrients availability to get better growth parameters in custard apple cv. Raydurg plants coupled with increase of soil microbial population.

Reference

- Adak T, Kumar K, Singha A. Spatio temporal variations in soil moisture and soil temperature under high density guava orchard system. Proceedings of 5th Indian Horticulture Congress on Horticulture for Food and Environment Security. November 6 - 9, PAU, Ludhiana, India 2012, P397.
- Arancon NQ, Edwards CA, Bierman P. Influences of Vermicompost on field strawberries: Part 2. Effects on soil microbiological and chemical properties. Bio resource Technology 2006;97:831-840.
- Dutta P, Kundu S, Chatterjee S. Effect of Bio-Fertilizers on Homestead Fruit Production of Papaya Cv. Ranchi. Acta Horticulturae 2010;851:214-17.
- Dwivedi BS, Dwivedi V. Monitoring soil health for higher productivity. Indian Journal of Fertilizer 2007;3:1-23.
- Edwards CA. Utilization of earthworm composts as plant growth media. In: Tomati, U. International Symposium on Agricultural and Environmental Prospects in Earthworm. Rome, Italy 1983, P57-62.
- Fisher RA. Handbook of Agriculture statistics. Achal Prakashan Mandir 1950, P332-334.
- Jackson ML. Soil chemical analysis. Prentice Hall of India Pvt Ltd, New Delhi 1973.
- Mandal A, Chattopadhyay PK. Studies on nutrition of custard apple (*Annona squamosa* L.). J of Potassium Research 1993;9(4):375-379.
- Metson AJ. Methods of chemical analysis for soil survey samples. Dept Sci Md Res Soil Bur 1956, P12.
- Olsen SR, Cole CS, Wantable FS, Dean CA. Estimation of available phosphorus in soils by extraction with sodium bicarbonate USDA. Washington. D. C. Circular 1954;18:939.
- Piper CS. Soil and Plant Analysis. Academic Press, New York. Science 1950;37:29-38.
- Rangaswamy G. Agriculture microbiology. Asia Publishing House, New York 1966, P385.
- Sadhu MK, Ghosh SK. Effect of different levels of nitrogen, phosphorus and potassium on growth, flowering, fruiting and tissue composition of custard apple. Indian Agric 1976;20:297-301.
- Singh AK. Evaluation of soil quality under integrated nutrient management. Journal of the Indian Society of Soil Science 2007;55(1):58-61.
- Singh R, Sharma RR, Singh DB. Effect of Vermicompost on plant growth, fruit yield and quality of strawberries in irrigated arid region of northern plains. Indian Journal of Horticulture 2010;67(3):318-21.
- Sinha. Effect of Biofertilizers and bio regulators on growth, yield and nutrient status of Strawberry cv. Sweet Charlie. Indian Journal of Horticulture 2009;66:220-224.
- Suhasini SP, Hipparagi K, Biradar IB, Patil SN, Suma R, Awati M. Effect of integrated nutrient management on growth parameters of banana cv. Rajapuri. International Journal of Pure & Applied Bioscience 2018;6(1):1328-1334.
- Tejada M, González JL. Application of two Vermicompost on a Rice Crop: Effects on Soil Biological Properties and Rice Quality and Yield. Agronomy Journal 2008;10(2):336-344.
- Tomati U, Grappelli A, Galli E. Fertility factors in earthworm humus. Agriculture Environment Prospects in Earthworm Farming 1983, P49-56.
- Tomati U, Grappelli A, Galli E. The presence of growth regulators in earthworm worked wastes. In Bonvicini Paglioi, A. M. and P. Omodeo (eds) On Earthworms. Proceedings of International Symposium on Earthworms. Selected Symposia and Monographs, Unione Zoologica Italiana, 2, Mucchi, Modena 1987, P423-435.
- Walkley A, Black CA. An examination of digestion methods for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil 1934.
- Wani SP. Inoculation with associated nitrogen fixing bacteria: Role in cereal grain production improvement. Indian Journal of Microbiology 1990;30:366-393.
- Werner M, Cuevas R. Vermiculture in Cuba Bio cycle. Emmaus, PA. JG Press 1996;37:61-65.