Full Length Research Paper

Rhizosphere microflora of noni (*Morinda citrifolia*) as influenced by organic manures and drip irrigation

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An experiment was conducted at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam to elucidate the effect of different irrigation regimes and organic manures on rhizosphere microbial population of noni (*Morinda citrifolia*). The trial was carried out in split plot design with irrigation regimes on main plot (four levels) and organic manures on sub plot (eight levels) with two replications. Among the different treatment combinations, M$_2$S$_2$ (100% WRc through drip irrigation + 50% farmyard manure + 50% vermicompost) registered the highest rhizosphere bacteria, fungi, actinomycetes, *Azotobacter*, *Azospirillum* and phosphobacteria population. The same treatment also recorded the highest score for organic matter decomposition. The rhizosphere microflora activity and organic matter decomposition was found to be the lowest in M$_4$S$_7$ (check basin method of irrigation + 100% recommended dose of NPK through inorganic fertilizers).

Key words: *Morinda citrifolia*, drip irrigation, farmyard manure, vermicompost, inorganic fertilizers, microbial population, organic matter decomposition.

INTRODUCTION

Over the past few years as natural products have become increasingly popular, the field of natural herbal remedies have flourished. The day to day demand for plant based natural raw materials for pharmaceuticals is increasing tremendously. Most of the world's population depends on traditional medicine to meet their daily health requirements, especially within the developing countries, where plants are the main source of medicine. One upcoming botanical name, the fruit of *Morinda citrifolia* very popularly known as NONI belongs to the Rubiaceae family. The roots, stems, bark, leaves, flowers and fruits of the noni plants are all involved in various combinations in almost 40 known and recorded herbal remedies. Noni is the biggest pharmaceutical unit in the universe because it has more than 160 nutraceuticals, vitamins, minerals, micro and macro nutrients that help the body in various ways from cellular level to organ level (Rethinam and Sivaraman, 2007). Noni fruit contains a number of enzymes and alkaloids that are believed to play a pivotal role in maintaining a good health. The fruit juice is in high demand in alternative medicine for different kinds of illnesses such as arthritis, diabetes, high blood pressure, muscle aches, pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcers, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problems and drug addiction (Wang et al., 2002).

The purpose of this medicinal herb will be fulfilled only if it is free from toxic residual effects due to chemical farming. Otherwise these herbs will become harmful than of medicinal value. Moreover, the medicinal plants have several active biochemical ingredients, which may get altered and deteriorated quality wise, when grown with the use of inorganic fertilizers and toxic pesticides. Rhizosphere microflora plays an important role in the maintenance of soil fertility because of their ability to

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carry out biochemical transformations (Thampan, 1995). Repeated and excessive application of inorganic fertilizers affected microorganisms which were essential for maintaining biological health of soil. The fertility of soil depends not only on its chemical components but also on the qualitative and quantitative nature of microorganisms inhabiting it. Soil microorganisms in the rhizosphere influence the plant growth in so many ways. Most of them play a role in carbon, nitrogen, phosphorus and sulphur cycle and availability of trace elements as reported by Karuthamani (2010). Soil rhizosphere microbes with specific functions can have significant effects on plant growth. These effects may be exerted directly on the host plant or indirectly through some effect on other microorganisms in the rhizosphere. For example, some microorganisms are pathogen antagonists, auxin producers, nitrogen fixers or phosphate solubilizers (Linderman, 1986). Hence, it is apparent that drip irrigation and organic manure application may have the greatest potential for plant growth enhancement.

Organic manures greatly influence the available soil microbial populations which are capable of regulating the supply of nutrients to higher plants. Therefore, it can be considered for better maintenance of soil organic matter (Gill and Cole, 1981). Organically grown products are in demand at present due to the awareness on health consciousness. This is true particularly in medicinal plants, wherein whole plant product is used in ayurvedic preparations (Maheswarappa et al., 1999). Because of the growing concern over the ill effects of inorganic fertilizers alternate sources of nutrients have been sought for and biofertilizers are an effective alternative or a supplement especially in the recent context of organic farming. A judicious and continuous use of one or more organic sources like animal manures, green manures, industrial wastes, oil cakes, crop residues and biofertilizers such as Azospirillum, phosphobacteria, VAM etc., could improve the soil fertility levels on a long term basis. The availability of irrigation water is dwindling day-by-day. Adoption of conventional methods of irrigation to crops leads to an acute scarcity of water and results in reduced production and productivity of crops. Therefore, it becomes highly imperative to go for alternate water saving methods for more crop and income for every drop of water. Drip irrigation can be used to improve the irrigation efficiency of horticultural crops by reducing evaporation and drainage losses by creating and maintaining soil moisture conditions that are favourable to crop growth. Drip irrigation can be considered as an efficient irrigation system, since it causes wetting of the soil only and maintain optimum moisture content in the root zone. It also offers several water management advantages like timely application of water and water supply.

Micro irrigation provides many unique agronomic, water and energy conservation benefits that address many of the challenges facing irrigated agriculture, now and in the future (Selvarani, 2009).

**MATERIALS AND METHODS**

This study was conducted at Horticultural College and Research Institute, TNAU, Periyakulum, Tamil Nadu, India which is situated at 77°E longitude, 10°N latitude and at an altitude of 300 m above mean sea level. The nature of soil of the experimental plot is sandy loam. The details of the initial soil chemical and physico-chemical characteristics of the experimental field were furnished in Table 1. The methods used where as follows:

a. Statistical design: Split plot design
b. Factors: 2
c. Replications: 2
d. Spacing: 3.6 × 3.6 m
e. Number of plants per replication: 5

**Treatment details**

**Main plot (irrigation)**

- M₁ - 75% WRc (Computed water requirement through drip irrigation)
- M₂ - 100% WRc (Computed water requirement through drip irrigation)
- M₃ - 125% WRc (Computed water requirement through drip irrigation)
- M₄ - Check basin method of irrigation (5 cm depth)

**Sub plot (organic manures)**

- S₁ - 100% farmyard manure (FYM)
- S₂ - 100% vermicompost (VC)
- S₃ - 100% Coir Pith Compost (CPC)
- S₄ - 50% FYM + 50% VC
- S₅ - 50% FYM + 50% CPC
- S₆ - 50% VC + 50% CPC
- S₇ - 100% recommended dose (RD) of NPK through inorganic fertilizers (60:30:30 g NPK plant⁻¹)
- S₈ - control (no manures and no fertilizers)

All organic manures were applied on equivalent weight of recommended dose of nitrogen (60 g plant⁻¹) on N equivalent basis. The treatments S₁ to S₈ are applied in addition with Azospirillum (10 g plant⁻¹) + phosphobacteria (10 g plant⁻¹) + VAM (20 g plant⁻¹). Nutrient content of organic manures were given in Table 2.

**Computed water requirement**

Computed water requirement of noni was calculated by using the following formula:

\[
WRc = CPE \times K_0 \times K_c \times A \times Wp \; \text{lit plant}^{-1}
\]

Where WRc is computed water requirement (lit plant⁻¹), CPE is cumulative pan evaporation for two days (mm), Kᵣ is pan coefficient (0.75), K₀ is crop factor (0.90 for vegetative stage, 0.95 for flowering and harvesting stage) (Allen et al., 1998), A is area occupied by the noni tree (3.6 × 3.6 m), Wp is wetting percentage (40). The quantity of water applied during the study period (June 2011 to March 2013) is enclosed in Table 3.

**Observations**

**Enumeration of rhizosphere soil microbial population**

The rhizosphere soil sample from noni was analysed for bacteria,
Table 1. Initial soil chemical and physico-chemical characteristics of the experimental field.

| Properties                   | Details          |
|------------------------------|------------------|
| Chemical properties          |                  |
| Available nitrogen           | 173 kg ha\(^{-1}\) |
| Available phosphorus         | 24 kg ha\(^{-1}\) |
| Available potassium          | 340 kg ha\(^{-1}\) |
| Physico-chemical properties  |                  |
| EC                           | 0.32 dSm\(^{-1}\) |
| pH                           | 7.93             |

Table 2. Nutrient content of organic manures.

| Organic manure  | Nutrient content (%) |   |
|-----------------|----------------------|---|
|                 | N        | P        | K   |
| FYM             | 0.75     | 0.37     | 0.71|
| Vermicompost    | 1.67     | 1.51     | 0.80|
| Coir pith compost | 1.06    | 0.87     | 1.20|

Table 3. Total water used during the study period.

| Treatments | Water applied (mm) | Effective rainfall (mm) | Total water used (mm) |
|------------|--------------------|-------------------------|-----------------------|
| MiS\(_1\)  | 619.85             | 400.5                   | 1020.35               |
| MiS\(_2\)  | 619.85             | 400.5                   | 1020.35               |
| MiS\(_3\)  | 619.85             | 400.5                   | 1020.35               |
| MiS\(_4\)  | 619.85             | 400.5                   | 1020.35               |
| MiS\(_5\)  | 619.85             | 400.5                   | 1020.35               |
| MiS\(_6\)  | 619.85             | 400.5                   | 1020.35               |
| MiS\(_7\)  | 619.85             | 400.5                   | 1020.35               |
| MiS\(_8\)  | 619.85             | 400.5                   | 1020.35               |
| M\(_4\)S\(_1\) | 2450.0      | 565.4                   | 3015.4                |
| M\(_4\)S\(_2\) | 2450.0      | 565.4                   | 3015.4                |
| M\(_4\)S\(_3\) | 2450.0      | 565.4                   | 3015.4                |
| M\(_4\)S\(_4\) | 2450.0      | 565.4                   | 3015.4                |
| M\(_4\)S\(_5\) | 2450.0      | 565.4                   | 3015.4                |
| M\(_4\)S\(_6\) | 2450.0      | 565.4                   | 3015.4                |
| M\(_4\)S\(_7\) | 2450.0      | 565.4                   | 3015.4                |
| M\(_4\)S\(_8\) | 2450.0      | 565.4                   | 3015.4                |
fungi, actinomycetes, *Azotobacter*, phosphobacteria and *Azospirillum*.

**Serial dilution of soil sample**

Ten grams of rhizosphere soil sample was transferred to 90 ml of sterile distilled water to get $10^{-1}$ dilution. After thoroughly mixing it, 1 ml of this dilution was transferred to 9 ml water blank to get $10^{-2}$ dilution. Likewise, sample was diluted serially with 9 ml water blanks till appropriate dilution was obtained (Parkinson et al., 1971).

**Bacteria**

The total bacterial population was enumerated by plating 1 ml of $10^{-3}$ dilution in sterile petriplates using nutrient agar medium. The bacterial colonies appearing on the plates after 48 h of incubation at 30°C were counted and expressed per gram of dry weight of the soil.

**Fungi**

For the enumeration of fungal population, 1 ml of $10^{-3}$ dilution of the soil sample was plated in sterile plate with Rose Bengal agar medium. After 72 h of incubation, the fungal colonies were counted and expressed per gram of dry weight of soil.

**Actinomycetes**

The total actinomycetes population was enumerated by plating 1 ml of $10^{-3}$ dilution with Kenknights agar medium. The powdery colonies of actinomycetes appearing after 5 days were counted and expressed per gram of dry weight of soil.

**Azobacter**

*Azobacter* population was enumerated by plating 1 ml of $10^{-3}$ dilution of rhizosphere soil sample with Waksman medium No. 77. *Azobacter* cells grow as raised and slimy colonies on agar surface. The colonies were counted and expressed per gram of dry weight of soil.

**Azospirillum**

*Azospirillum* population was enumerated by plating 1 ml of $10^{-5}$ dilution of rhizosphere soil sample with N-free semisolid malic acid medium. At the end of the incubation time, the media colour change from yellowish green to blue colour and white sub surface pellicle like colonies appear at 5 days of incubation period. The colonies were counted and expressed per gram of dry weight of soil.

**Phosphobacteria**

Phosphobacteria population was enumerated by plating 1 ml of $10^{-5}$ dilution of rhizosphere soil sample with Katznelson and Bose medium using soil extract from the rhizosphere region. After incubation formation of transparent and clear zones around the bacterial colonies indicates the extent of phosphate solubilization. The colonies were counted and expressed per gram of dry weight of soil.

**Organic matter decomposition**

The organic matter decomposition was estimated using the method described by Nagarajan and Ramalakshmi (2010). In a 500 ml conical flask, 100 g of respective treatment soils were taken and 10 ml of 1 M NaOH was taken in penicillin vial. The NaOH containing vial was hanged over in the conical flask with the help of thread. To make the flasks air tight, wax coating was given to the area of mouth of the conical flask and rubber cork with paraffin wax then incubated for 7 days. Then, penicillin vial was taken without disturbing NaOH and 1 ml of BaCl$_2$ was added then transferred to conical flask and titrated against 1 N HCl with phenolphthalein indicator. The end point is disappearance of pink colour. The organic matter decomposition is expressed in terms of mg CO$_2$ per 100 g of soil.

**Statistical analysis**

The statistical analysis of data was done by adopting the standard procedures of Panse and Sukhatme (1985). The AGRES software (version 3.01) was used for analysis of data.

**RESULTS**

**Bacteria**

Among the irrigation regimes, $M_2$ (100% WRc through drip irrigation) recorded the highest rhizosphere bacterial population of $108.55 \times 10^6$ cfu g$^{-1}$ of soil in vegetative, flowering and harvesting stages respectively (Table 4 and Figure 1). The rhizosphere bacterial population was found to be the lowest (72.62, 91.66 and $104.61 \times 10^5$ cfu g$^{-1}$ of soil) in the check basin method of irrigation ($M_4$) during different crop growth stages. Among the sub plot treatments, $S_8$ (50% FYM + 50% VC) resulted in increased rhizosphere bacterial population in vegetative ($118.94 \times 10^5$ cfu g$^{-1}$ of soil), flowering ($155.66 \times 10^6$ cfu g$^{-1}$ of soil) and harvesting ($178.87 \times 10^6$ cfu g$^{-1}$ of soil) stages. The rhizosphere bacterial population was found to be the lowest (40.88, 49.79 and $58.79 \times 10^5$ cfu g$^{-1}$ of soil) in the treatment plots receiving 100% RD of NPK through inorganic fertilizers ($S_7$) in various stages of crop growth. The treatment $S_9$ (no manure and no fertilizers) registered the rhizosphere bacterial population of 63.19, 79.59 and $92.86 \times 10^6$ cfu g$^{-1}$ of soil in vegetative, flowering and harvesting stages, respectively. Between the interactions, the experimental plots receiving 100% WRc through drip irrigation + 50% FYM + 50% VC ($M_4S_4$) recorded the highest rhizosphere bacterial population in vegetative ($142.28 \times 10^6$ cfu g$^{-1}$ of soil), flowering ($188.26 \times 10^6$ cfu g$^{-1}$ of soil) and harvesting ($216.48 \times 10^6$ cfu g$^{-1}$ of soil) stages.

The rhizosphere bacterial population was found to be the lowest in $M_3S_7$ (check basin method of irrigation + 100% RD of NPK through inorganic fertilizers) with 35.26, 41.25 and $48.96 \times 10^6$ cfu g$^{-1}$ of soil in vegetative, flowering and harvesting stages, respectively. The rhizosphere bacterial population of 58.25, 72.27 and
83.68 × 10^6 cfu g⁻¹ of soil was observed from M₂S₈ (check basin method of irrigation + no manure and no fertilizers).

**Fungi**

Concerning the main plot treatments, M₂ (100% WRC through drip irrigation) recorded the highest rhizosphere fungal population of 32.08, 35.89 and 39.51 × 10^3 cfu g⁻¹ of soil in vegetative, flowering and harvesting stages, respectively (Table 5 and Figure 2). The rhizosphere fungal population was found to be the lowest (11.72, 11.99 and 12.32 × 10^3 cfu g⁻¹ of soil) in the treatment plots receiving 100% RD of NPK through inorganic fertilizers (S₇) in various stages of crop growth. The treatment S₈ (no manure and no fertilizers) registered the rhizosphere fungal population of 18.12, 19.08 and 20.07 × 10^3 cfu g⁻¹ of soil in vegetative, flowering and harvesting stages, respectively. In the combined effect of treatments, the treatment combination comprising 100% WRC through drip irrigation + 50% FYM + 50% VC (M₂S₇) recorded the highest counts for rhizosphere fungal population in vegetative (43.50 × 10^3 cfu g⁻¹ of soil), flowering (51.28 × 10^3 cfu g⁻¹ of soil) and harvesting (58.65 × 10^3 cfu g⁻¹ of soil) stages.

The fungal population was found to be the lowest in the treatment combination M₂S₇ (check basin method of irrigation + 100% RD of NPK through inorganic fertilizers) with 10.20, 10.48 and 10.66 × 10^3 cfu g⁻¹ of soil during various stages of crop growth. The treatment combination M₂S₈ (check basin method of irrigation + no manure and no fertilizers) showed the rhizosphere fungal population of 17.01, 18.06 and 18.96 × 10^3 cfu g⁻¹ of soil.

**Actinomycetes**

Among the main plots, application of 100% WRC through drip irrigation (M₂) registered the high actinomycetes population of 22.24, 25.26 and 27.24 × 10^3 cfu g⁻¹ of soil in vegetative, flowering and harvesting stages respectively (Table 6).

In the main plot, the actinomycetes population was found to be the lowest in the treatment comprising check basin method of irrigation (M₄)
Table 5. Effect of different water regimes and organic manures on rhizosphere fungal population (× 10^3 cfu g^-1 of soil).

| Treatments | Vegetative stage | Flowering stage | Harvesting stage |
|------------|------------------|-----------------|------------------|
|            | M1 | M2 | M3 | M4 | Mean | M1 | M2 | M3 | M4 | Mean | M1 | M2 | M3 | M4 | Mean |
| S1         | 29.65 | 36.17 | 36.09 | 23.53 | 31.36 | 32.58 | 40.02 | 40.19 | 25.09 | 34.47 | 34.69 | 44.12 | 44.30 | 26.66 | 37.44 |
| S2         | 31.29 | 38.06 | 38.15 | 24.87 | 33.09 | 34.26 | 43.76 | 44.07 | 26.56 | 37.16 | 36.98 | 48.06 | 48.67 | 28.72 | 40.61 |
| S3         | 29.19 | 35.10 | 34.22 | 23.05 | 30.39 | 32.06 | 38.92 | 38.05 | 24.67 | 33.43 | 33.79 | 42.80 | 42.16 | 26.10 | 36.21 |
| S4         | 31.85 | 43.50 | 39.28 | 25.09 | 34.93 | 34.92 | 51.28 | 44.81 | 27.18 | 39.55 | 37.62 | 58.65 | 49.62 | 29.27 | 43.79 |
| S5         | 29.37 | 35.92 | 35.18 | 23.12 | 30.90 | 32.40 | 39.66 | 39.60 | 24.46 | 34.03 | 34.07 | 44.02 | 42.98 | 25.88 | 36.74 |
| S6         | 31.02 | 36.42 | 36.65 | 24.61 | 32.18 | 33.95 | 40.72 | 40.87 | 26.42 | 35.49 | 36.80 | 44.68 | 44.52 | 29.02 | 38.76 |
| S7         | 11.86 | 12.61 | 12.19 | 10.20 | 11.72 | 12.03 | 12.97 | 12.48 | 10.48 | 11.99 | 12.49 | 13.21 | 12.93 | 10.66 | 12.32 |
| S8         | 18.22 | 18.84 | 18.42 | 17.01 | 18.12 | 19.12 | 19.78 | 19.36 | 18.06 | 19.08 | 20.18 | 20.50 | 20.63 | 18.96 | 20.07 |
| Mean       | 26.56 | 32.08 | 31.27 | 21.44 | 27.84 | 28.92 | 35.89 | 34.93 | 22.87 | 30.65 | 30.83 | 39.51 | 38.23 | 24.41 | 33.24 |
| SE(d)      | 0.1321 | 0.2127 | 0.4193 | 0.4254 | 0.1457 | 0.2364 | 0.4656 | 0.4728 | 0.1588 | 0.2580 | 0.5082 | 0.5161 |
| CD at 5%   | 0.4205 | 0.4358 | 0.9062 | 0.8715 | 0.4638 | 0.4842 | 1.0056 | 0.9865 | 0.5054 | 0.5286 | 1.0973 | 1.0572 |
| CD at 1%   | 0.7719 | 0.5879 | 1.2871 | 1.1758 | 0.8514 | 0.6533 | 1.4273 | 1.3066 | 0.9278 | 0.7131 | 1.5573 | 1.4263 |

Table 6. Effect of different water regimes and organic manures on rhizosphere actinomycetes population (× 10^4 cfu g^-1 of soil).

| Treatments | Vegetative stage | Flowering stage | Harvesting stage |
|------------|------------------|-----------------|------------------|
|            | M1 | M2 | M3 | M4 | Mean | M1 | M2 | M3 | M4 | Mean | M1 | M2 | M3 | M4 | Mean |
| S1         | 20.70 | 26.53 | 26.18 | 16.28 | 22.42 | 22.59 | 29.76 | 29.45 | 17.69 | 24.87 | 24.46 | 32.04 | 31.65 | 19.26 | 26.85 |
| S2         | 21.34 | 27.25 | 27.60 | 17.04 | 23.31 | 23.26 | 31.32 | 31.86 | 18.40 | 26.21 | 25.15 | 33.65 | 34.20 | 20.06 | 28.27 |
| S3         | 20.79 | 23.24 | 23.78 | 16.10 | 20.98 | 22.70 | 26.15 | 27.02 | 17.57 | 23.36 | 24.55 | 28.35 | 29.16 | 19.08 | 25.29 |
| S4         | 21.86 | 29.85 | 28.05 | 17.62 | 24.35 | 24.08 | 34.80 | 32.49 | 19.16 | 27.63 | 26.12 | 37.65 | 34.88 | 20.68 | 29.83 |
| S5         | 20.18 | 24.39 | 24.66 | 16.52 | 21.44 | 22.15 | 27.63 | 28.12 | 18.02 | 23.98 | 23.95 | 29.84 | 30.36 | 19.64 | 25.95 |
| S6         | 21.69 | 27.05 | 26.84 | 17.16 | 23.19 | 23.82 | 31.12 | 30.35 | 18.68 | 25.99 | 25.78 | 33.36 | 32.58 | 20.21 | 27.98 |
| S7         | 6.68 | 7.50 | 7.12 | 5.10 | 6.60 | 7.20 | 8.12 | 7.68 | 5.56 | 7.14 | 7.66 | 8.75 | 8.12 | 5.84 | 7.59 |
| S8         | 11.59 | 12.09 | 12.46 | 10.54 | 11.67 | 12.55 | 13.17 | 13.58 | 11.42 | 12.68 | 13.64 | 14.29 | 14.81 | 12.38 | 13.78 |
| Mean       | 18.10 | 22.24 | 22.09 | 14.55 | 19.24 | 19.79 | 25.26 | 25.07 | 15.81 | 21.48 | 21.41 | 27.24 | 26.97 | 17.14 | 23.19 |
| SE(d)      | 0.0919 | 0.1488 | 0.2932 | 0.2977 | 0.1035 | 0.1672 | 0.3294 | 0.3343 | 0.1115 | 0.1804 | 0.3554 | 0.3608 |
| CD at 5%   | 0.2925 | 0.3049 | 0.6333 | 0.6098 | 0.3295 | 0.3424 | 0.7117 | 0.6848 | 0.3549 | 0.3695 | 0.7678 | 0.7391 |
| CD at 1%   | 0.5368 | 0.4113 | 0.8990 | 0.8227 | 0.6048 | 0.4619 | 1.0105 | 0.9239 | 0.6514 | 0.4986 | 1.0900 | 0.9971 |
Table 7. Effect of different water regimes and organic manures on rhizosphere *Azotobacter* population (×10^3 cfu g^-1 of soil).

| Treatments | Vegetative stage | Flowering stage | Harvesting stage |
|------------|------------------|----------------|----------------|
|            | M1  | M2  | M3  | M4  | Mean | M1  | M2  | M3  | M4  | Mean | M1  | M2  | M3  | M4  | Mean |
| S1         | 14.20 | 17.12 | 16.95 | 11.54 | 14.95 | 15.87 | 19.10 | 18.79 | 12.42 | 16.55 | 17.14 | 20.46 | 20.21 | 13.58 | 17.85 |
| S2         | 15.25 | 18.73 | 18.82 | 12.05 | 16.21 | 16.80 | 21.06 | 21.19 | 13.02 | 18.02 | 18.06 | 22.48 | 22.69 | 14.13 | 19.34 |
| S3         | 14.10 | 16.32 | 16.47 | 11.35 | 14.56 | 15.65 | 18.50 | 18.42 | 12.18 | 16.19 | 17.01 | 19.89 | 19.80 | 13.35 | 17.51 |
| S4         | 15.39 | 19.85 | 19.20 | 12.22 | 16.67 | 16.92 | 22.60 | 21.82 | 13.31 | 18.66 | 18.20 | 24.25 | 23.36 | 14.42 | 20.06 |
| S5         | 14.26 | 17.36 | 17.30 | 11.86 | 15.20 | 16.04 | 19.63 | 19.22 | 12.75 | 16.91 | 17.40 | 21.13 | 20.62 | 13.80 | 18.24 |
| S6         | 15.02 | 17.84 | 18.06 | 12.14 | 15.77 | 16.65 | 19.78 | 20.15 | 13.22 | 17.45 | 17.87 | 21.25 | 21.67 | 14.27 | 18.77 |
| S7         | 6.45  | 6.95  | 6.80  | 5.85  | 6.51  | 6.88  | 7.35  | 7.28  | 6.15  | 6.92  | 7.35  | 7.89  | 7.76  | 6.51  | 7.38  |
| S8         | 8.38  | 8.60  | 8.79  | 7.73  | 8.38  | 9.14  | 9.36  | 9.61  | 8.34  | 9.11  | 9.92  | 10.25 | 10.54 | 9.06  | 9.94  |
| Mean       | 12.88 | 15.35 | 15.30 | 10.59 | 13.53 | 14.24 | 17.17 | 17.06 | 11.42 | 14.98 | 15.37 | 18.45 | 18.33 | 12.39 | 16.14 |

| M | S     | M at S | S at M | M | S     | M at S | S at M | M | S     | M at S | S at M | M | S     | M at S | S at M |
|---|-------|--------|--------|---|-------|--------|--------|---|-------|--------|--------|---|-------|--------|--------|
| SE(d) | 0.0648 | 0.1028 | 0.2030 | 0.2057 | 0.0720 | 0.1143 | 0.2257 | 0.2287 | 0.0777 | 0.1231 | 0.2430 | 0.2461 |
| CD at 5% | 0.2063 | 0.2106 | 0.4393 | 0.4213 | 0.2290 | 0.2342 | 0.4883 | 0.4684 | 0.2474 | 0.2521 | 0.5259 | 0.5041 |
| CD at 1% | 0.3787 | 0.2842 | 0.6248 | 0.5684 | 0.4204 | 0.3160 | 0.6944 | 0.6320 | 0.4541 | 0.3401 | 0.7480 | 0.6801 |

in vegetative (14.55 × 10^4 cfu g^-1 of soil), flowering (15.81 × 10^4 cfu g^-1 of soil) and harvesting (17.14 × 10^4 cfu g^-1 of soil) stages, respectively. Pertaining to the sub plot, application of 50% FYM + 50% VC (S4) recorded the highest actinomycetes population of 24.35, 27.63 and 29.83 × 10^4 cfu g^-1 of soil in vegetative, flowering and harvesting stages, respectively. The actinomycetes population was found to be the lowest (6.60, 7.14 and 7.59 × 10^4 cfu g^-1 of soil) in the treatment S7 (100% RD of NPK through inorganic fertilizers). The treatment S6 (no manure and no fertilizers) registered the rhizosphere actinomycetes population counts of 11.67, 12.68 and 13.78 × 10^4 cfu g^-1 of soil in vegetative, flowering and harvesting stages, respectively. The treatment combination comprising 100% WRc through drip irrigation + 50% FYM + 50% VC (M2S4) recorded the highest scores for rhizosphere actinomycetes population in vegetative (29.85 × 10^4 cfu g^-1 of soil), flowering (34.80 × 10^4 cfu g^-1 of soil) and harvesting (37.65 × 10^4 cfu g^-1 of soil) stages, respectively.

The actinomycetes population was found to be the lowest in the treatment combination M4S7 (check basin method of irrigation + 100% RD of NPK through inorganic fertilizers) with 5.10, 5.56 and 5.84 × 10^4 cfu g^-1 of soil during different stages of crop growth. The treatment combination M4S6 (check basin method of irrigation + no manure and no fertilizers) showed the rhizosphere actinomycetes population of 10.54, 11.42 and 12.38 × 10^4 cfu g^-1 of soil.

**Azotobacter**

Among the irrigation regimes, M2 (100% WRc through drip irrigation) recorded the highest rhizosphere *Azotobacter* population of 15.35, 17.17 and 18.45 × 10^3 cfu g^-1 of soil in vegetative, flowering and harvesting stages, respectively (Table 7). The rhizosphere *Azotobacter* population was found to be the lowest (10.59, 11.42 and 12.39 × 10^3 cfu g^-1 of soil) in the check basin method of irrigation (M4) during all the three crop growth stages. With reference to the sub plot treatments, S4 (50% FYM + 50% VC) resulted in increased rhizosphere *Azotobacter* population in vegetative (16.67 × 10^3 cfu g^-1 of soil), flowering (18.66 × 10^3 cfu g^-1 of soil) and harvesting (20.06 × 10^3 cfu g^-1 of soil) stages. The rhizosphere *Azotobacter* population was found to be the lowest (6.51, 6.92 and 7.38 × 10^3 cfu g^-1 of soil) in the treatment plots receiving 100% RD of NPK through inorganic fertilizers (S7) in various growth stages of the crop. The treatment S6 (no manure and no fertilizers) registered the rhizosphere *Azotobacter* population of 8.38, 9.11 and 9.94 × 10^3 cfu g^-1 of soil in vegetative, flowering and harvesting stages, respectively.
The rhizosphere phosphobacteria population was found to be the lowest in M₃S₇ (check basin method of irrigation + 100% RD of NPK through inorganic fertilizers) with 5.85, 6.15 and 6.51 × 10⁵ cfu g⁻¹ of soil in vegetative, flowering and harvesting stages, respectively. The rhizosphere Azotobacter population was found to be the lowest in M₄S₇ (check basin method of irrigation + no manure and no fertilizers).

Phosphobacteria

The rhizosphere phosphobacteria population increased from vegetative to harvesting stage (Table 8). Concerning the main plot treatments, M₂ (100% WRc through drip irrigation) recorded the highest rhizosphere phosphobacteria population of 26.63, 26.76 and 28.68 × 10⁵ cfu g⁻¹ of soil in vegetative, flowering and harvesting stages, respectively. The rhizosphere phosphobacteria population was found to be the lowest (16.45, 17.51 and 18.19 × 10⁵ cfu g⁻¹ of soil) in the check basin method of irrigation (M₁) during three different crop growth stages. Pertaining to the sub plot, application of 50% FYM + 50% VC (S₄) registered the highest rhizosphere phosphobacteria population of 26.51, 28.84 and 31.34 × 10⁵ cfu g⁻¹ of soil in vegetative, flowering and harvesting stages, respectively. The rhizosphere phosphobacteria population was found to be the lowest (8.13, 8.54 and 8.72 × 10⁵ cfu g⁻¹ of soil) in the treatment S₇ (100% RD of NPK through inorganic fertilizers). The treatment S₈ (no manure and no fertilizers) registered the rhizosphere phosphobacteria population of 13.93, 14.34 and 14.76 × 10⁵ cfu g⁻¹ of soil in vegetative, flowering and harvesting stages, respectively.

The treatment combination comprising 100% WRc through drip irrigation + 50% FYM + 50% VC (M₂S₄) recorded the highest scores for rhizosphere phosphobacteria population in vegetative (33.75 × 10⁵ cfu g⁻¹ of soil), flowering (37.44 × 10⁵ cfu g⁻¹ of soil) and harvesting (41.82 × 10⁵ cfu g⁻¹ of soil) stages, respectively. The rhizosphere phosphobacteria population was found to be the lowest in the treatment combination M₃S₇ (check basin method of irrigation + no manure and no fertilizers) showed the rhizosphere phosphobacteria population of 12.88, 13.21 and 13.68 × 10⁵ cfu g⁻¹ of soil.
Table 9. Effect of different water regimes and organic manures on rhizosphere *Azospirillum* population ($\times 10^5$ cfu g$^{-1}$ of soil).

| Treatments | Vegetative stage | Flowering stage | Harvesting stage |
|------------|------------------|-----------------|-----------------|
|            | $M_1$ | $M_2$ | $M_3$ | $M_4$ | Mean | $M_1$ | $M_2$ | $M_3$ | $M_4$ | Mean | $M_1$ | $M_2$ | $M_3$ | $M_4$ | Mean |
| S₁         | 17.82  | 20.69  | 20.57  | 14.80  | 18.47  | 21.18  | 24.58  | 24.39  | 17.54  | 21.92  | 22.85  | 26.73  | 26.51  | 18.85  | 23.74 |
| S₂         | 19.10  | 21.80  | 21.87  | 15.57  | 19.59  | 22.44  | 25.83  | 25.94  | 18.40  | 23.15  | 24.16  | 27.89  | 28.12  | 19.79  | 24.99 |
| S₃         | 17.21  | 20.35  | 20.27  | 14.71  | 18.14  | 20.54  | 24.21  | 24.09  | 17.48  | 21.58  | 22.07  | 26.25  | 26.14  | 18.81  | 23.32 |
| S₄         | 19.20  | 23.65  | 22.09  | 15.62  | 20.14  | 22.64  | 27.86  | 26.18  | 18.47  | 23.79  | 24.37  | 30.28  | 28.41  | 19.92  | 25.75 |
| S₅         | 17.63  | 20.50  | 20.46  | 15.32  | 18.48  | 20.98  | 24.33  | 24.28  | 18.09  | 21.92  | 22.59  | 26.44  | 26.29  | 19.42  | 23.69 |
| S₆         | 19.14  | 21.44  | 21.56  | 15.39  | 19.38  | 22.49  | 25.44  | 25.62  | 18.25  | 22.95  | 24.30  | 27.60  | 27.81  | 19.71  | 24.86 |
| S₇         | 8.30   | 8.71   | 8.79   | 7.12   | 8.23   | 9.70   | 10.13  | 10.24  | 8.32   | 9.60   | 10.23  | 10.65  | 10.80  | 8.74   | 10.11 |
| S₈         | 12.03  | 12.58  | 12.66  | 11.24  | 12.13  | 13.87  | 14.60  | 14.73  | 13.02  | 14.06  | 14.68  | 15.52  | 15.67  | 13.78  | 14.91 |
| Mean       | 16.30  | 18.72  | 18.53  | 13.72  | 16.82  | 19.23  | 22.12  | 21.93  | 16.20  | 19.87  | 20.66  | 23.92  | 23.72  | 17.38  | 21.42 |

**Azospirillum**

Among the main plot treatments, $M_2$ (100% WRc through drip irrigation) recorded the highest rhizosphere *Azospirillum* population of 18.72, 22.12 and 23.92 $\times 10^5$ cfu g$^{-1}$ of soil in vegetative, flowering and harvesting stages respectively (Table 9). The rhizosphere *Azospirillum* population was found to be the lowest (13.72, 16.20 and 17.38 $\times 10^5$ cfu g$^{-1}$ of soil) in check basin method of irrigation ($M_4$) during the three different crop growth stages. Between the manure treatments, $S_4$ (50% FYM + 50% VC) recorded an increased rhizosphere *Azospirillum* population in vegetative ($20.14 \times 10^5$ cfu g$^{-1}$ of soil), flowering ($23.79 \times 10^5$ cfu g$^{-1}$ of soil) and harvesting ($25.75 \times 10^5$ cfu g$^{-1}$ of soil) stages. The rhizosphere *Azospirillum* population was found to be the lowest (8.23, 9.60 and 10.11 $\times 10^5$ cfu g$^{-1}$ of soil) in the treatment plots receiving 100% RD of NPK through inorganic fertilizers ($S_7$) in various stages of crop growth. The treatment $S_8$ (no manure and no fertilizers) showed the rhizosphere *Azospirillum* population of 11.24, 13.02 and 13.78 $\times 10^5$ cfu g$^{-1}$ of soil.

**Organic matter decomposition**

Among the main plots, application of 100% WRc through drip irrigation ($M_2$) registered the highest organic matter decomposition of 77.10, 91.42 and 103.56 mg CO$_2$ 100 g$^{-1}$ of soil in vegetative, flowering and harvesting stages, respectively (Table 10). The organic matter decomposition was found to be lowest in the treatment comprising check basin method of irrigation ($M_4$) in vegetative (50.24 mg CO$_2$ 100 g$^{-1}$ of soil), flowering (60.56 mg CO$_2$ 100 g$^{-1}$ of soil) and harvesting (69.15 mg CO$_2$ 100 g$^{-1}$ of soil) stages. Pertaining to the sub plot, application of 50% FYM + 50% VC ($S_4$) registered the highest organic matter decomposition
of 84.55, 100.61 and 114.39 mg CO$_2$ 100 g$^{-1}$ of soil in vegetative, flowering and harvesting stages, respectively. The organic matter decomposition was found to be the lowest (25.66, 30.85 and 37.72 mg CO$_2$ 100 g$^{-1}$ of soil) in the treatment S$_7$ (100% RD of NPK through inorganic fertilizers). The treatment S$_8$ (no manure and no fertilizers) registered the organic matter decomposition of 42.74, 50.09 and 57.23 mg CO$_2$ 100 g$^{-1}$ of soil in different stages, respectively. The treatment combination comprising of 100% WRC through drip irrigation + 50% FYM + 50% VC (M$_2$S$_4$) recorded the highest scores for organic matter decomposition during vegetative (104.92 mg CO$_2$ 100 g$^{-1}$ of soil), flowering (123.56 mg CO$_2$ 100 g$^{-1}$ of soil) and harvesting (140.86 mg CO$_2$ 100 g$^{-1}$ of soil) stages.

The organic matter decomposition was found to be the lowest in the treatment combination M$_4$S$_7$ (check basin method of irrigation + 100% RD of NPK through inorganic fertilizers) with 20.46, 25.38 and 30.76 mg CO$_2$ 100 g$^{-1}$ of soil during various stages of crop growth. The treatment combination M$_4$S$_8$ (check basin method of irrigation + no manure and no fertilizers) showed the organic matter decomposition of 39.43, 45.39 and 51.32 mg CO$_2$ 100 g$^{-1}$ of soil.

**DISCUSSION**

The bacterial population in vegetative, flowering and harvesting stages was found to be higher in the treatment combination comprising 100% WRC through drip irrigation + 50% FYM + 50% VC (M$_2$S$_4$). The drip irrigation system provides continuous and uninterrupted supply of moisture for congenial microbial activity and proliferation. Similarly, under drip irrigation system, the soil moisture content did not too fluctuate between wet and dry extremes (Patil and Janawade, 1999) which favours the microbial growth and proliferation. The differences in bacterial population might also be due to the varied level of substrate availability and nutrient transformations taking place in the soil. The increased rhizosphere bacterial population in aforementioned best treatment was due to the prevalence of favourable environment for biological activity. This increase in bacterial count might be attributed to the consecutive addition of energy rich materials which increases the enzyme activities and ultimately the viable bacterial population. The least population was observed in M$_4$S$_7$ (check basin method of irrigation + 100% RD of NPK through inorganic fertilizers). The reduction of
Figure 1. Effect of different water regimes and organic manures on rhizosphere bacterial population \((x\ 10^3 \text{ cfu g}^{-1} \text{ of soil})\) at harvesting stage.

Figure 2. Effect of different water regimes and organic manures on rhizosphere fungal population \((x\ 10^3 \text{ cfu g}^{-1} \text{ of soil})\) at harvesting stage.
rhizosphere bacterial population in this treatment may be due to ill effect of inorganic fertilizers on bacterial population. The results are in line with the findings of Ravanachander (2009).

Manickam (1983) revealed that organic residues added to the soil underwent microbial decomposition and in that process it released organic acids and other products of decay which acted as strong binding agents in the formation of large stable aggregates which favours the growth of microbial population. The fungal population recorded was the highest in M2S4 (100% WRc through drip irrigation + 50% FYM + 50% VC). Since there was appreciable amount of N through applied organic manures in the soil and decomposition of it further encouraged multiplication of beneficial microorganisms. Moreover, Azospirilllum would have released growth regulators which also might have been favourable for microbial population. The results were also in line with the findings of Ravanachander (2009) in black pepper. The actinomycetes population was found to be the highest in plots supplied with 100% WRc through drip irrigation + 50% FYM + 50% VC (M2S3) as against the M2S2 (check basin method of irrigation + 100% RD of NPK through inorganic fertilizers). The drip irrigation provides optimum moisture level for microbial proliferation. The frequent application of irrigation through drip at optimum level maintained most of the rhizosphere soil as most conducive for microflora proliferation. The crops with conventional check basin method of irrigation affected by excess moisture which deleteriously affected the soil aeration. Similarly, the moisture availability is not uniform in check basin method of irrigation.

The excess water application under check basin method of irrigation tends to leach down the nutrients beyond the rhizosphere which creates nutrient deficient condition for microbial growth as a result, the microbial population was reduced. The highest actinomycetes population in M2S4 (100% WRc through drip irrigation + 50% FYM + 50% VC) might also be due to the higher availability of carbonaceous materials in those of the treatments with optimum N availability. Moreover, application of optimum quantity of organic amendments would have provided a conducive environment for the activity of actinomycetes. This observation was in line with the findings of Sutopo and Kuwatsuka (1992) who suggested that FYM application stimulated the microbial proliferation and the process related to N cycling in soil. The lowest population was noticed in experimental plot receiving check basin method of irrigation + 100% RD of NPK with inorganic fertilizers (M2S1) which indicates the ill effects of mineral fertilization on soil actinomycetes. Higher population of soil microbes under organic treatments acted as an index of soil fertility because it serves as temporary sink of nutrients flux as found by Hassink et al. (1991).

Added organic manure improves the water holding capacity and return the soil moisture status which would have supported the proliferation of beneficial rhizosphere microflora namely, Azotobacter, Azospirilllum and phosphobacteria. The improved beneficial microbial population may be due to optimum water and nutrient availability by the treatment combination M2S4 (100% WRc through drip irrigation + 50% FYM + 50% VC). This finding is in corroboration with the previous works by Thomas and Shantahram (1984) who indicated that application of organics helped the soil microbes to produce polysaccharides and thereby improving the soil structure. Chellamuthu et al. (1988) reported that the soil microbial population was increased due to addition of organic manure because they increase the proportion of labile carbon and nitrogen, directly stimulating the activity of the microorganism. Besides this, addition of organic manures would have resulted in increased secondary and micronutrients in the soil which might have helped to increase the load of beneficial microbial population. Combined use of organic manures with drip irrigation might have improved the microbial load of the soil, increasing the microbial population namely, bacteria, fungi and actinomycetes which conspicuously increased with application of different organic sources than the treatments with inorganic fertilizers and control plots as reported by Ravanachander (2009) in black pepper and Vanilarasu (2011) in banana.

The population dynamics of microorganism namely, bacteria, fungi, actinomycetes, Azotobacter, Azospirilllum and phosphobacteria showed a favourable trend. Wherever organic manures were applied along with drip irrigation, there was an increase in the population of soil microbes. The enhanced population of soil microflora under drip irrigated, organic manures and biofertilizers treated plots might be due to the synergistic effect of applied drip irrigation, organic manures and biofertilizers on the proliferation of existing native soil microflora. The organic matter decomposition was found to be comparatively higher in treatment combination comprising 100% WRc through drip irrigation + 50% FYM + 50% VC (M2S4). The drip irrigation provide conducive environment for microbial proliferation. Similarly, vermicompost having good water holding capacity, aeration, porosity and increased surface areas which facilitate more micro sites for microbial decomposing organism. As a result, microbial decomposition and CO2 generation was increased (Arancon and Edwards, 2005). The treatment combination M2S4 (100% WRc through drip irrigation + 50% FYM + 50% VC) exhibited superior performance rhizosphere microbial population and organic matter decomposition.

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