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INFLUENCE OF BIOFERTILIZERS AND IRRIGATION SYSTEMS FOR THE GROWTH AND YIELD OF MULBERRY PLANTS

D.Nithya, S.M. Poornima, R.Pazhani murugan, V.Gopikrishnan, M.Radhakrishnan[1], Dhagira Bhivi[2] and R.Balagurunathan[*]

Department of Microbiology, Periyar University, Salem-636011, Tamil Nadu.

[1]Head Department of Microbiology, Sankara Arts & Science College, Kanchipuram-631561

[2]Regional Sericultural Research Station, Salem

[*]Corresponding Author: E. mail: rbalaguru@yahoo.com

ABSTRACT : The present study emphasizes the application of various irrigation systems and the effects of chemical and biofertilizers on rhizosphere microorganisms of mulberry plants. The highest total bacterial population was $580x10^3$cfu/gm and fungal population was $39x10^4$cfu/gm. Among the bacterial and fungal population *Azotobacter* and *Aspergillus* spp were found predominantly in drip irrigation with green manure (T4) method. Among the four irrigation systems T4 method effectively increases the microbial population in the rhizosphere of mulberry plants. The initial pH of the mulberry garden soil was 8.2 but after the application of chemical (control) and biofertilizer (test), the chemical fertilizer treated soil retained the pH as 8.2 and biofertilizer treated soil pH was found to be 6.8. The rhizosphere microbial population (both bacteria and fungi) was least in the control sample (chemical fertilizer) but in test (biofertilizer) sample, the bacterial and fungal population was gradually increased from 1st day to 60th day. Typically, phosphate solubilzing bacteria and *Aspergillus* spp were present in the biofertilizer treated soil and effectively increasing the growth of mulberry plant's height, branches and leaves.

Key words: *Morus indica*, Mulberry plant, Rhizosphere, Biofertilizer, Drip irrigation.

INTRODUCTION

Mulberry (*Morus* spp.) is a fast growing, deciduous, woody and perennial plant. It has a deep root system. The leaves are simple, alternate, stipulate, petiolate and lobed. The ideal range of temperature varies from 24-28ºC, humidity in the range of 65-80% and the optimum pH is 6.5 to 6.8. There are about 68 species of the genus *Morus*, the majority of them occurred in Asia, especially in China (24 species) and Japan (19 species). In India, there are many species of *Morus* of which *Morus alba*, *M.Indica, M.serrata* and *M.caevigata* are grow wildly in Himalayas and most states have taken up sericulture as an important agro industry with an excellent results. The total acre of mulberry in India is around 2,82,244 hectare and total area of mulberry cultivation in Tamilnadu is around 9,491 hectare (Vijayan,2009).

Mulberry can grow both in the tropics and in the temperate regions. It can be cultivated in different soil types. It can be raised both in rain-fed and irrigated conditions. It is comparatively resistant to environmental fluctuations and is relatively free from pests and diseases. The nutritive value of leaf changes according to the photosynthetic and respiratory activities accordingly. Leaves of mulberry have great medicinal value. Mulberry leaves have been found effective in lowering the blood sugar level and arterial pressure (Shailesh Soni *et al*.,2009).

Mulberry is an economically important plant being cultivated for fruits and leaves, though in sericulture the emphasis is on leaves to feed the silkworm (*Bombyx mori* L.). The production of mulberry leaf and cocoon crop is entirely depend on the maintenance of the soil fertility of mulberry garden, through the periodical application of organic manures, green manures and fertilizers in required quantity.

The mulberry garden should be irrigated at regular intervals of 8 – 14 days depending on the type of soil. About one and half to two hectare inches of water is required for irrigation. More efficient and mechanized methods are used for irrigating the mulberry garden such as, drip irrigation, flood irrigation and micro irrigation. Since the cost of mulberry leaf production was estimated to be more than 60% of the total cost of silkworm cocoon production (Das and Krishnaswami, 1965), efforts are being taken to develop new varieties and agronomic practices to increase the leaf productivity to sustain profitability in sericulture. The general requirement of urea for mulberry plantation in India was estimated as 330 kg/ha year. This excessive use of chemical fertilizers has been found deleterious to the silkworm growth and developments in addition to the soil degradation. Therefore, emphasis has been recently shifted to replace chemical fertilizers with biological materials (Sudhakar *et al*., 2000). Beneficial effect of application of *Azotobactor* in mulberry leaf production was established and demonstrated by Das *et al*. (1990, 1994 and 1996), Gangwar and Thengavelu (1992). Advantages of foliar application of bacterial biofertlizer on mulberry leaf production were also reported by Sudhakar *et al*. (2000). With this view, the present investigation focused on the effects of chemical and biofertilizers and various irrigation systems on the growth of mulberry plants and its components.

MATERIALS AND METHODS

Description of study area

The present study was carried out at Regional Sericultural Research Station, Salem (Latitude 11º 39' N and Longitude 78º12' E) Tamilnadu. The study was conducted during January-March 2011. The maximum temperature was found to 33ºC and the minimum temperature was 21ºC and relative humidity was fluctuates between 72% and minimum 29%.

Preparation of mulberry garden

Two years old, paired V-1 variety of mulberry garden was selected to find out the effects of various irrigation systems and impact of chemical and biofertilizer on rhizosphere microflora. The nature of mulberry garden soil was deep black and the pH of the soil sample was analysed by using pH meter before and after the treatment of fertilizers. The mulberry garden was pruned at 10-20 cm above the ground level. The land was country ploughed at two to three times repeatedly and it was maintained under weed free conditions and the irrigation was carried out for 7 days intervals.

Effect of irrigation types on the rhizosphere microflora

Flood irrigation with green manure (Daincha) (T1)

The water was flooded to the mulberry plant field by plastic pipe and the water is allowed to gently pass through rhizosphere of mulberry garden. The irrigation was given for 7 days of interval up to 0 day to 60th days. After flood irrigation, 7 kg of green manure daincha was applied on the flood irrigated soil on 5th day. After 10 days interval, the flood irrigated with green manure applied rhizosphere soil was collected aseptically for enumeration of the rhizosphere miroflora and its characterization.

Flood irrigation (T2)

In this method, the water was flooded to the mulberry garden field by plastic pipe and simply flows over the ground through the rhizosphere of mulberry garden. The flood irrigation was given at 7days of intervals upto 0 day to 60th day. Rhizosphere microflora was enumerated as described above.

Drip irrigation (T3)

Drip irrigation method was carried out in mulberry garden by using a dripper to supply the water in to the mulberry garden .The water directly reached to the root surface of the plant and the irrigation was given at 7 days of interval upto 0 day to 60th day (Ananthakrishna *et al.,*1995**)**.

Drip irrigation with green manure (Daincha) (T4)

Drip irrigation method was carried out in mulberry garden by using dripper to supply the water in to the mulberry garden. The water directly reached to the root surface of plant and after the irrigation, 7 kg of the green manure daincha was applied on the drip irrigated soil on 5th day.

Effects of chemical and bio fertilizer

Control plot

The mulberry garden was treated with 5 tonnes of farm yard manure (FYM), 27 kg of ammonium sulphate, 14 kg of super phosphate and 4 kg of potassium. Mulberry plant was implanted on control plot and irrigation was carried out for 7 days intervals. On 5th day, the mulberry plant was treated with farm yard manure (FYM) and following that on 25th day mulberry plant applied with chemical fertilizers such as ammonium sulphate, super phosphate and potassium in respective amount (Baqual *et al.,*2006).

Test plot

The mulberry garden was treated with 5 tonnes of farm yard manure (FYM) and 2 kg each of *Azospirillum* and *Phosphobacter*. The mulberry plant was implanted on test plot and irrigation was carried out for 7 days of intervals. On 5th day the mulberry plant was treated with farm yard manure (FYM) and following that on 15th day mulberry plant applied with Biofertilizers (Baqual *et al.,*2006).

Isolation and enumeration of microorganisms

Irrigated soil sample

Four different types of irrigated soil samples (T1, T2, T3 and T4), were collected on 10 days interval of 60 days and 1g of each soil sample was serially diluted and about 0.1 ml was plated on sterile nutrient agar plates for bacteria and potato dextrose agar plates for fungi by using spread plate method and incubated at room temperature for 24 hours to 78 hours for bacteria and 5 days for fungi (Vijayan *et al*.,2007)

Biofertilizer and chemical fertilizer treated soil sample

Control soil sample

In the control sample (treated with chemical fertilizer) ten different plants were randomly selected, and the one gram of each soil sample was collected from rhizosphere root system in different days viz., 0, 10, 20, 30, 40, 50 and 60th day and serially diluted and about 0.1 ml of diluted sample was plated on nutrient agar plates for bacteria and potato dextrose agar plates for fungi by using spread plate method and incubated at room temperature for 24-72 hours for bacteria and 5 days for fungi.

Test soil sample

The test sample (treated with biofertilizer), ten different plants were randomly selected, and 1 gram of soil sample was collected from rhizosphere root system in different days viz., 0, 10, 20, 30, 40, 50 and 60th day and serially diluted and about 0.1 ml of diluted sample was plated on nutrient agar plates for bacteria and potato dextrose agar plates for fungi by using spread plate method and incubated at room temperature about 24-72 hours for bacteria and 5 days for fungi.

Identification and characterization of rhizosphere microflora

Cultural characterization of bacteria

The most frequently repeated different bacterial colonies were selected from various irrigated systems and fertilizer treated soil sample was selectively identified by using selective media such as Malic acid medium for *Azospirillum* and incubated at 28ºC for 3-5 days, Pikovskaya's medium for Phosphate solubilizing bacteria and incubated at 28ºC for 2 weeks, Jensen's medium for *Azotobacter* and incubated at 28ºC for 1 week and yeast extract mannitol agar medium for *Rhizobium* and incubated at 28º C for 7 days.

Microscopical identification and biochemical characterization

Selectively isolated colonies were identified by microscopical and biochemical methods such as Gram's staining, motility, catalase, oxidase tests etc as recommended by Bergey's Manual of Determinative Bacteriology (1992).

Identification of fungi

Morphologically different fungal colonies were selected and identified by lactophenol cotton blue staining method as recommended by Microbiology Laboratory Manual (Cappucino.2010).

Estimation of growth parameter

Randomly, five 60th days old mulberry plants of various irrigation systems, chemical and biofertilizer treated plants were selected for the growth measurement, leaves count and branches enumeration (Ram rao *et al.,* 2007).

RESULTS AND DISCUSSION

The results of the present study found that application of various irrigation systems (T1, T2, T3 and T4) influences the growth of mulberry plants. The highest total bacterial population was found as 580x10^{3}cfu/gm, among which the *Azotobacter* genus are predominant following the other genera like *Azospirillum* and *Rhizopium*. The fungal population was estimated as 39x10^{4}cfu/gm, among which, the majority of them are *Aspergillus* spp and *Penicillum* spp are distinctly presented in drip irrigation with green manure (T4) method. In the midst of four irrigation systems (Figure 1) T4 system effectively increases the microbial population in the rhizosphere of mulberry plants. Sinha (2001) reported that the application of green manure enriched with bacteria and fungi have proven to be great importance in improving the yield of mulberry plants but the present study indicated that drip irrigation along with green manure highly enrich the growth parameters of mulberry plants by favoring the rhizosphere microbial population. There is no report on the study of various irrigation systems in mulberry plants and its rhizosphere microflora effects on the roots system of mulberry plants.

This is the first report on the influence of irrigation systems on rhizosphere microflora and growth parameter of plants (*Morus indica*). Thus, this irrigation system for mulberry plant cultivation may be recommended to the farmers to conserve the water. Considering the impact of fertilizers on mulberry plant, one of the major growth factor is soil pH, Dassapa *et al*., (2006) reported that Cyanobacterial biofertilizer (CBB) treated mulberry plant soil pH was decreased to 8 from 8.4 after the one month treatment., (2007), reported on the soil pH was changed from 8.0 to 7.6 due to the biofertilizer treatment. In the present study, the initial pH of the mulberry garden soil was 8.2 but after the application of chemical (control) and biofertilizer (test), the chemical fertilizer retained the pH as 8.2 but the biofertilizer treated soil pH turned in to 6.8 this may be due to the biofertilizer interaction with rhizosphere of mulberry plant. The effect of fertilizers (chemical and biofertilizer) on microbial population of mulberry plants, the control sample (chemical fertilizer) belongs very least in the growth of microbial population from 0^{th} to 60^{th} day but in test (biofertilizer) soil, the bacterial and fungal population was gradually increased from 0^{th} day to 60^{th} day (Figure 2 and 3). Among the bacterial population of test sample, the Phosphate solubilizing bacteria are prevailed following that *Rhizobium* and *Azospirillum* spp. In fungal population, the majority of the fungi are *Aspergillus* spp and *Rhizopus* spp. The general requirement of urea for mulberry plantation in India was estimated as 330 kg/ha year, the excessive use of chemical fertilizer drastically reduces the soil fertility and its microflora. Advantages of foliar application of bacterial biofertlizer on mulberry leaf production were reported by Sudhakar *et al*. (2000). From this study, there is no report on the impact of microbial population on mulberry plant's rhizosphere after the treatment of chemical and biofertilizer. The biofertilizer treated plants significantly exhibit the better growth than chemical fertilizer treated plants components such as height, branches and plenty of healthy leaves. Sinha *et al.,*(2001) studied on Farm yard manure (FYM) and lowest level of NPK treated plants showed maximum of 149 cm in height, 15 branches and 273 number of leaves but the present study found that maximum of 189 cm in height, 13 branches and 40 leaves are presented in per stem (Figure 4).The present study, revealed that the drip irrigation with green manure system and biofertilizer effectively influencing the growth and yield of mulberry plants, and also it will be an economically valuable and also eco friendly.

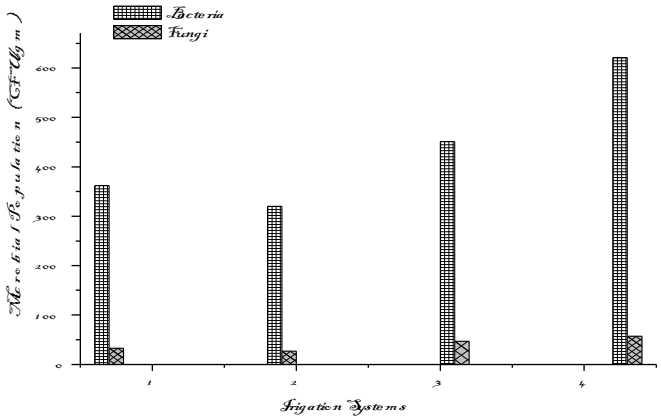

Figure-1. Bacterial and fungal population of various irrigation systems (T1, T2, T3 and T4) on rhizosphere of V-1 mulberry plant

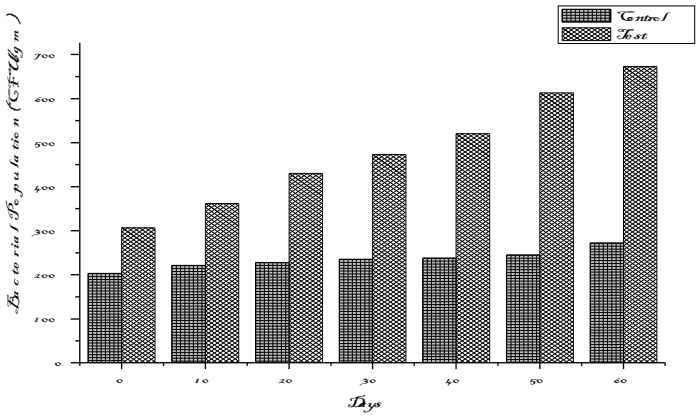

Figure-2. Bacterial population of control (chemical fertilizer) and test (biofertilizer) sample on rhizosphere of V-1 mulberry plant.

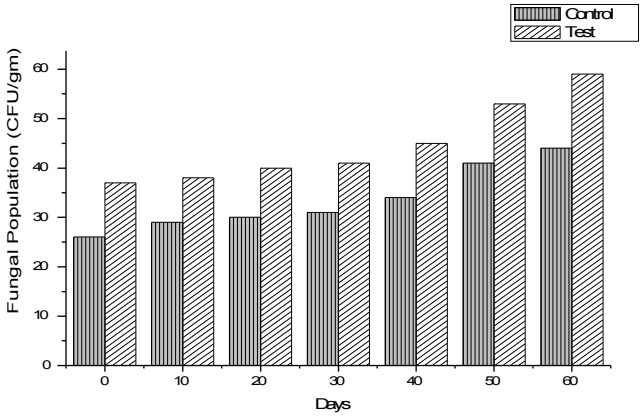

Figure-3. Fungal population of control (chemical fertilizer) and test (biofertilizer) sample on rhizosphere of V-1 mulberry plant.

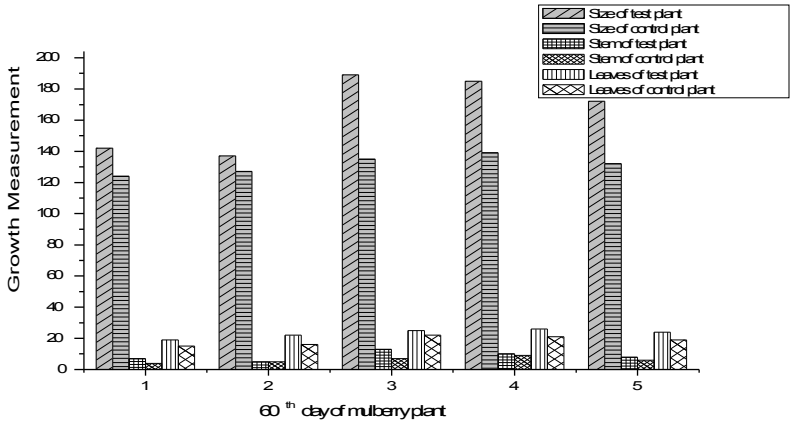

Figure- 4. Effects of Control (chemical fertilizer) and Test (biofertilizer) on V-1 mulberry plants growth parameters.

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