Experimental Procedure

3.0. EXPERIMENTAL PROCEDURE

The methodology adopted for the study "**Effect of Liquid biofertilizers, Chemical fertilizers and Vermicompost on the Growth and Yield of** *Hibiscus surattensis* (L.)" is given below.

3.1. Collection of biofertilizer and vermicompost

3.2. Soil preparation

3.3. Treatment of seeds

3.4. Layout treatments

3.5. Sowing of seeds and maintenance of crops

3.6. Harvest methodology

3.7. Biometric observations

3.8. Biochemical analysis

3.9. Antioxidant status

3.10. Soil analysis

3.11. Vermicompost analysis

3.12. Statistical analysis

3.1. Collection of biofertilizer and vermicompost

The liquid biofertilizers and Vermicompost were collected from Tamil Nadu Agricultural University, Coimbatore.

3.2. Soil preparation

All the pots used for the study were filled with 8kg of soil and treated with liquid biofertilizers such as *Azospirillum, Phosphobacteria, Azophos* and *Azophosmet.* Vermicompost was mixed at the rate of 5 tonnes ha^{-1}.

3.3. Treatment of seeds

Seeds of *Hibiscus surattensis* (L.) were collected from Super seeds Nursery, Coimbatore. Seeds were soaked in cold water, shade dried and treated with liquid biofertilizers using rice gruel as an adhesive agent.

3.4. Layout treatments

The experiment was laid out with three replications for each of the nine treatments in completely randomized block design.

The treatments were as follows:

C- *Azospirillum* + *Phosphobacteria* + 100% recommended dose of N and K

T_1- *Azospirillum* + 50% recommended dose of N and K

T_2 – *Phosphobacteria* + 50% recommended dose of N and K

T_3- *Azophos* + 50% recommended dose of N and K

T_4- *Azophosmet* + 50% recommended dose of N and K

T_5- *Azospirillum* + 25% recommended dose of N and K

T_6- *Phosphobacteria* + 25% recommended dose of N and K

T_7- *Azophos* + 25% recommended dose of N and K

T_8- *Azophosmet* + 25% recommended dose of N and K

The 100% recommended dose of N is 75 kg ha^{-1} and that of K is 25 kg ha^{-1}. The control group has the carrier based inoculum with the microbial population of about $18x10^6$ cells of *Azospirillum*/g and $15x10^5$ cells of *Phosphobacteria*/g. The treatment T_1 to T_8 consists of liquid biofertilizers with the microbial population of about $12x10^7$cells of *Azospirillum*/ml, $9x10^7$ cells of *Phosphobacteria*/ml and $3x10^6$ cells of *Azophosmet*/ml.

3.5. Sowing of seeds and maintenance of crops

About twenty seeds were sown in each pot and allowed to germinate. After germination 100% moisture condition was maintained throughout the study.

3.6. Harvest methodology

The plants were uprooted on the 30^{th} and 45^{th} day after transplantation without any damage. The adhering soil particles were removed by washing gently with water and the water droplets were removed by blotting with the filter paper. Then these plants were used for the biometric observations, biochemical analysis and antioxidant status.

3.7. Biometric observations

Biometric observations were recorded on the 30^{th} and 45^{th} day after transplantation of the young plantlets.

3.7.1. Root length

The root length was measured from the crown region of the plant to the top of the root and was expressed in cm $plant^{-1}$.

3.7.2. Shoot length

The shoot length was measured from the point of first cotyledonary node to the tip of the longest leaves and was expressed in cm plant $^{-1}$.

3.7.3. Number of leaves

The total number of leaves in each plant were counted on the 30th and 45th day after sowing and expressed as number of leaves plant^{-1}.

3.7.4. Fresh weight

The fresh plants were weighed and the weights were expressed in g plant^{-1}.

3.7.5. Dry weight

The plants after recording the fresh biomass were oven dried at 70°C for 36 hours and were weighed and expressed in g plant^{-1}

3.8. Biochemical analysis

The biochemical parameters like chlorophyll, carotenoid, protein, total phenol, reducing sugar and total free amino acid were analyzed in fresh leaves and phosphorus and iron were analyzed in plant dry sample on the 30th and 45th days after transplantation. The details of the methods and the parts used were given in Table I.

3.9. Antioxidant Status

The enzymic antioxidants like catalase, peroxidase, superoxide dismutase, glutathione- s- transferase and glutathione peroxidase were analysed in fresh leaves and non-enzymic antioxidants like ascorbic acid, tocopherol, and reduced glutathione were also analyzed in fresh leaves on the 30th and 45th days after transplantation. The details of the methods and the parts used were given in Table I.

3.10. Soil analysis

The enzymes like amylase, cellulase, dehydrogenase phosphatase and urease were analyzed in the post harvesting stage of soil samples and the method of analysis were given in the Table I. The soil samples were analyzed at the initial and at the post harvesting stage of to know the fertility status of the soil. The processed samples were analyzed for pH, electrical conductivity, nitrogen, phosphorus and potassium and the methods of analysis were given in Table I.

3.11. Vermicompost analysis

The applied vermicompost was analyzed to know the status of organic carbon, calcium and magnesium levels. The details of the methods used were given in Table I.

3.12. Statistical analysis

Statistical analysis like arithmetic mean and analysis of variance (ANOVA) were employed to predict the results of the experiment.

TABLE I

ANALYSIS OF BIOCHEMICAL PARAMETERS, ANTIOXIDANT STATUS IN PLANT AND ENZYME ANALYSIS IN SOIL

Parameters	Parts used	Method of analysis	Reference	Appendix number
Biochemical analysis				
Chlorophyll	Fresh leaves	Spectrophotometry	Witham *et al.*, 1971	I
Carotenoid	Fresh leaves	Spectrophotometry	Zakaria *et al*, 1979	II
Protein	Fresh leaves	Spectrophotometry	Lowry *et al.*, 1951	III
Total phenol	Fresh leaves	Spectrophotometry	Malick and Singh, 1980	IV
Reducing sugar	Fresh leaves	Spectrophotometry	Somogyi, 1952	V
Phosphorous	Plant dry sample	Spectrophotometry	Fiske and Subbarow, (Oser, 1971)	VI
Iron	Plant dry sample	Spectrophotometry	Wong's (Oser, 1971)	VII
Total free amino acid	Fresh leaves	Spectrophotometry	Moore and Stein, 1948	VIII

Parameters	Parts used	Method of analysis	Reference	Appendix number
Enzymic antioxidants				
Catalase	Fresh leaves	Spectrophotometry	Luck, 1974	IX
Peroxidase	Fresh leaves	Spectrophotometry	Reddy *et al.*, 1995	X
Superoxide dismutase	Fresh leaves	Spectrophotometry	Misra and Fridovich, 1972	XI
Glutathione-S-transferase	Fresh leaves	Spectrophotometry	Habig *et al.*, 1974	XII
Glutathione peroxidase	Fresh leaves	Spectrophotometry	Rotruck *et al.*, 1973	XIII
Non Enzymic antioxidants				
Ascorbic acid	Fresh leaves	Spectrophotometry	Roe and Keuther, 1953	XIV
Tocopherol	Fresh leaves	Spectrophotometry	Rosenberg, 1992	XV
Reduced glutathione	Fresh leaves	Spectrophotometry	Moron *et al.*, 1979	XVI

Parameters	Parts used	Method of analysis	Reference	Appendix number
Analysis of soil				
Amylase	Soil	Colorimetry	Peter, 1955	XVII
Cellulase	Soil	Colorimetry	Sumner,1955	XVIII
Dehydrogenase	Soil	Colorimetry	Chendrayan *et al.*, 1980	XIX
Phosphatase	Soil	Colorimetry	Tabatabai and Bremner,1969	XX
Urease	Soil	Colorimetry	Sumner, 1955	XXI
Nitrogen	Soil	Titrimetry	Humphries, 1956	XXII
Phosphorus	Soil	Colorimetry	Raguramulu *et al.*, 2003	XXIII
Potassium	Soil	Flame photometry	Jackson, 1975	XXIV
Analysis of vermicompost				
Organic carbon	Vermicompost	Titrimetry	Jackson,1973	XXV
Calcium Magnesium	Vermicompost	Titrimetry	Cheng and Bray, 1951	XXVI

PLATE I

GROWTH OF *Hibiscus surattensis* (L.) ON THE 30TH DAY AFTER SOWING

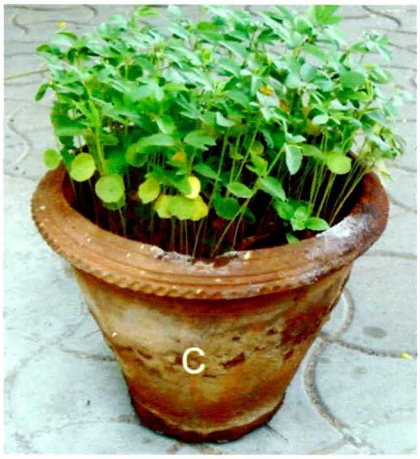

PLATE II

GROWTH OF *Hibiscus surattensis* (L.) ON THE 45TH DAY AFTER SOWING

4.0. RESULTS AND DISCUSSION

Leafy vegetables are generally good sources of nutrients. They are important protective foods and highly beneficial for the maintenance of health and prevention of diseases as they contain valuable food ingredients which can be utilized to build up and repair the body. The varieties of leafy vegetables utilized are diverse, ranging from leaves of annuals and shrubs to leaves of trees (Kubmarawa *et al.*, 2009).

Green leafy vegetables (GLV) have long been recognized as the cheapest and most abundant potential sources of vitamins and minerals. The ethno botanical reports offers information on medicinal properties of GLVs like antidiabetic, antihistaminic, anticarcinogenic, hypolipidemic and antibacterial activity. GLVs are also rich sources of carotenoids (Raju *et al.*, 2007).

Leafy vegetables are also known for their therapeutic value. They also contain antioxidant vitamins and pigments. Green leafy vegetables hold important place in well balanced diet. The idea of a well balanced diet changed in recent years and lesser amount of red meat and more vegetables and fruits are advised (Latif and Elaal, 2007). Due to their dietary importance, many scientific studies have been carried out on the nutritive values of green leaves (Gayathri *et al.*, 2006).

Hence a study was carried out to find the "**Effect of Liquid Biofertilizers, Chemical fertilzrers and Vermicompost on the Growth and Yield of** *Hibiscus surattensis* (L.)". The study was carried out as pot culture with three replications for each treatment. The experiment was laid out in completely randomized block design. Each pot was filled with 8 kg of soil and treated with liquid biofertilizers such as *Azospiriilum, Phosphobacteria, Azophos* and *Azophosmet* and different percentage of chemical fertilizer. Vermicompost was mixed at the rate of 5 tonnes ha^{-1}.

At the end of the 30^{th} and 45^{th} day of growth, plants were uprooted carefully and were subjected to biometric, biochemical and antioxidant analysis. The soil was analyzed for the soil enzyme activity and physicochemical properties at the initial and at the post harvesting stage.