MATERIALS AND METHODS

Chapter- III

The present investigation entitled "**Studies on the efficacy of vermicompost, urea and *Azotobacter* on growth, yield and quality attributes of strawberry (*Fragaria* × *ananassa* Duch.)"** was carried out at the Horticulture Research Farm-I, Babasaheb Bhimrao Ambedkar University,Vidya Vihar, Rae Bareli Road, Lucknow (U.P.) during 2013-2014 and 2014-2015.The details of methodology adopted in the investigations have been presented under the following headings:

3.1 Experimental Location

The experiment was conducted at the Horticultural Research Farm-I, of the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University,Vidya Vihar,Rae Bareli Road, Lucknow.

The experimental plot located approximately 10 km from Lucknow on the Rae Bareli Road towards the south city of the Lucknow. It is also well connected by the Bijnore Road. The farm is situated at an elevation of 129 meters above the mean sea level. Lucknow is geographically situated in the subtropical tract of central U.P. at 26^{o} 46' North latitude and 80^{o} 55' East longitudes.

The climate of the region is subtropical with maximum temperature ranging from 29.3 ^{o}C to 45 ^{O}C in the summer season and minimum temperature ranging from 3.5 ^{O}C to 12 ^{O}C in winter. The relative humidity (RH) is 50 -77 % in different seasons of the year with hot or summer and cold winter. The average rainfall is 700 mm, most of which is received from July to September distributed over a period of about 100 days, with the peak period between July to August. Scattered showers also occur during the winter months. In general, the temperature ranges from 5 ^{O}C to 42 ^{O}C.The coldest month of the year is January, while the maximum temperature is recorded during the months of May and June, respectively.

3.2 Soil Analysis

The soil samples collected from different spots of the strawberry field at the Horticulture Research Farm-I, soil samples collected with the help of soil khurpi from 0-20 cm depth. These samples mixed together, dried in air and powdered and again samples were mixed

thoroughly. The representative samples, each of 5 g soil for each analysis were taken and the same were analyzed in the Laboratory of Central Soil Salinity Research Institute, regional research station Lucknow (U.P.), India.

3.3 Physical constituents of the soil

The soil of the experimental site has been classified as saline. Soil was white to yellow in colour, imperfectly drained with moderately low organic matter content. The soil of experimental area was of open in nature and poor in soil moisture retention at different layers.

3.4 Mechanical and Chemical Analysis

The soil of the experimental field was slightly saline with pH 8.25 to 8.5 electrical conductivity more than 4 and sodium exchangeable percentage less than 15.

3.5 Experimental Materials

The runners of strawberry cultivar 'Sweet Charlie' were collected from M/S Beniwal Strawberry Research Farm, New Delhi, India during the last week of October (Plate-1). Before planting, the runners were kept in shade house for a period 24 hours for proper acclimatization to the experimental site. After which they were planted in well prepared beds under open field condition.

Standard cultural practices were followed during the experimental period for maintaining the runners. The experimental materials i.e. vermicompost were collected from the National Botanical Research Institute, Lucknow; urea was arranged from the local market from Lucknow and *Azotobacter* were collected from Microbiology Laboratory, Indian Agricultural Research Institute, Pusa, New Delhi.

3.6 Meteorological conditions

The experimental site received rainfall of between 0 mm (November 2013) and 47 mm (January 2014) with average minimum and maximum temperature ranging between 8 °C (December 2013 and January 2014) to 31°C (October 2013 and March 2014), where as during 2014-15, the average minimum and maximum temperatures recorded between 6 ^{0}C (December, 2014 and January, 2015) to 31^{0} C (March, 2015) and mean rainfall ranged between 0 to 76 mm during the cropping period (Table 1 and Figure- 3.1) .

Table 3.1: Monthly meteorological data (average) recorded during the course of investigation (2013- 14 and 2014-15)

2013-14						2014-15					
Month	Average Temperature		Average Relative Humidity (%)	Average Rainfall (mm)	Average wind speed (km/h)	Month	Average Temperature		Average Relative Humidity (%)	Average Rainfall (mm)	Average wind speed (km/h)
	Minimum (^{0}C)	Maximum (^{0}C)					Minimum (^{0}C)	Maximum (^{0}C)			
April	19.3	37.8	42.1	0	8.7	April	17.9	39.3	34.5	0	9.5
May	24.5	41.3	47.4	0	7.9	May	23.1	41.6	40.7	0.2	10.8
June	24.6	35.8	77.8	10.6	10.34	June	25.5	43.1	51.6	0.9	8.2
July	24.8	34.2	84.2	6.6	8	July	24.5	36.1	80.2	9.5	7.4
August	23.9	33.7	85.3	6.6	7.1	August	24.5	36.7	76.7	5.9	6.5
September	23.5	35.1	75.7	1.4	7.6	September	22.4	36.1	77.6	3.5	6.1
October	19.5	31.2	76.9	1.5	6.3	October	18.1	32.5	72.6	3.4	3.1
November	10.1	28.5	66.8	0	4.3	November	10.2	29.1	60.6	0	2.5
December	7.3	24.7	71.1	0.2	5.3	December	6.3	21.4	79.8	0.5	2.4
January	7.7	19.9	85.4	1.7	6.8	January	8.4	19.4	83.5	1.1	3.5
February	8.7	24.1	73.1	1.1	7.6	February	11.9	27.2	69.9	1.1	4.1
March	13.3	31.6	55.4	0.2	7.8	March	15.2	31.1	61	2.4	5.9

3.7 Treatment combinations

Recommended dose of vermicompost, urea and *Azotobacter* were applied at the rate of 100 q/ha, 100 kg/ha and 30 ml per litre water, respectively. Vermicompost and urea were applied by broadcasting in the plots and mixes with the soil manually before planting of runners. Vermicompost and urea were applied at the rate of 1.62 kg (100%), 1.21 kg (75%), 0.81 kg (50%), 0.40 kg/plot (25%) and 17g (100%), 12.75g (75%), 8.5g (50%) and 4.5g (25%), respectively. The details of treatment combinations employed in present investigation were as follows:

Table 3.2 Details of Treatment Combinations

S.No.	Symbol	Combinations
1	T_1	Control
2	T_2	Vermicompost (100%)
3	T_3	*Azotobacter* (100%)
4	T_4	Urea (100%)
5	T_5	Vermicompost (75%) + Urea (25%)
6	T_6	Vermicompost (50%) +Urea (50%)
7	T_7	Vermicompost (25%) + Urea (75%)
8	T_8	*Azotobacter (*75%) + Vermicompost (25%)
9	T_9	*Azotobacter* (50%) + Vermicompost (50%)
10	T_{10}	*Azotobacter* (25%) + Vermicompost (75%)
11	T_{11}	*Azotobacter* (75%) + Urea (25%)
12	T_{12}	*Azotobacter* (50%) + Urea (50%)
13	T_{13}	*Azotobacter* (25%) + Urea (75%)
14	T_{14}	Vermicompost (25%) + *Azotobacter* (25%) +Urea (50%)
15	T_{15}	Vermicompost (50%) + *Azotobacter* (25%) +Urea (25%)
16	T_{16}	Vermicompost (25%) +*Azotobacter* (50%) +Urea (25%)

3.8 Agronomical practices

3.81 Preparation of experimental field

The experimental field was ploughed to the depth of 30 cm with the help of tractor. The field was kept open to sun for atleast 10 days for killing the weeds and eggs of insects by repeated plough followed by planking to obtain fine tilth. Required area was marked and prepared

according to the lay out plan. A total 48 plots were made with a size 2.1 x 1.2 m^2 of each plot. 0.5 m wide drainage channel were made between the two replications. Each plot contains 6 rows (rows were raised by 15 cm from main field) and runners were planted at distance of 30 x 15 cm (6 plants in each row), accommodated 36 plants in each plot. The strawberry runners were planted on 29th October in the evening 2013-14 and 2014-15, respectively. The details of experimental layout are presented in Table 3.3 and Figure 3.2.

Table 3.3 The details of experimental field layout.

Design	Randomized Block Design
Treatments	16
Replication	3
Number of plots	48
Net Plot size	1.80 x 0.90 m
Gross Plot Size	2.10 x 1.20 m
Spacing	Plant to plant: 15 cm
	Row to Row: 30 cm
Number of runners per plot	36
Total number of runners	1728
Variety	Sweet Charlie

3.82 Irrigation

The first irrigation was applied immediately after planting and the subsequent irrigation were given at an interval of 7-10 days during winter and 4-5 days during summer.

3.83 Intercultural operation

The beds were kept clean by regular weeding and hoeing. Weeding and hoeing were done manually with the help of hand hoe and khurpi. Generally four to five weeding and hoeing were done during the crop period.

3.84 Observations recorded

3.841 Plant characters

3.842 A. Vegetative growth parameters:

3.843 Plant height (cm)

The average plant height of 5 plants was measured from the ground level of the plant with the help of digital vernier callipers and data were expressed as plant height in cm.

3.844 Plant spreads (cm)

The spread of the plants was recorded from North-South and East-West direction was measured with the help of digital vernier callipers at 15 days interval and their mean values were expressed as the spread of the plant in cm.

3.845 Number of leaves per plant

The number of leaves was recorded at 15 days interval from the plants kept for observation purpose and expressed as average number of leaves per plant.

3.846 Leaf length (cm)

The length of leaf was measured with the help of digital vernier callipers from the base of leaf to the apex of leaf and expressed in cm.

3.847 Leaf width (cm)

The length of leaf was measured with the help of digital vernier callipers from the broadest side of the leaf and expressed in cm.

3.848 Leaf area (cm^2)

Leaf area was measured with the help of portable leaf area meter (LI 3006) and expressed in cm^2.

3.849 Number of runners per plant

The number of runners was calculated manually from the representative plants and expressed as average number of runners per plant.

3.8.4.2.1 B. Physical Parameters:

3.8.4.2.2 Number of flowers per plant

The number of flowers was recorded from the representative plants and expressed as average number of flowers per plant.

3.8.4.2.3 Number of fruits per plant

The total number of fruits was counted from each plant kept for observation purpose and their average was expressed as number of fruits per plant.

3.8.4.2.4 Fruit length (cm)

Fruit length was measured from the base of pedicel to the apex of fruit with the help of digital vernier callipers.

3.8.4.2.5 Fruit width (cm)

Fruit width was measured at the broadest area of the fruits with help of digital vernier callipers.

3.8.4.2.6 Fruit weight (g)

The average weight of fruits from each treatment was weighed individually with the help of electronic balance.

3.8.4.2.7 Fruit volume (ml)

The volume of fruit was measured by using wide mouth jar filled up to brim with water. Fruit was immersed in the jar and the run off collected was measured which gave the fruit volume and average value was calculated as suggested by Rangana (1997).

3.8.4.2.8 Specific gravity.

Specific gravity was calculated with the help of fruit volume and fruit weight by using the following formula as suggested by Rangana (1997).

$$\text{Specific gravity} = \frac{\text{Weight of fruit } (g)}{\text{Volume of water displaced by fruit (ml)}}$$

3.8.4.2.9 Fruit yield per plant (g)

For the determination of yield per plant, the number of fruits was counted for each replication and average number of fruits per plant was calculated. Average weight of fruits was worked out by weighing ten fruits from each treatment. Final data was calculated by multiplying the total number of fruits per plant by the average weight of fruits.

Yield per plant (g) =Total number of fruits per plant x average weight of fruits in gram.

3.8.4.3 Fruit yield per plot (kg)

For the determination of yield per plot, the number of fruits was counted for each replication and average number of fruits per plot was measured with the help of electronic balance.

3.8.4.4 C. Bio-Chemical Parameters:

3.8.4.4.1 TSS (Total soluble solids)

The total soluble solids of the berry juice determined with the help of Erma Hand Refractometer, the values were corrected applying correction factor at 20 ^{0}C and expressed in ^{O} Brix of fresh juice.

3.8.4.4.2 Titratable Acidity (%)

The titratable acidity was determined by the method as suggested by Rangana (1997). Twenty- five grams of berry pulp were taken in water blended homogenized in distilled water and volume was made up to 250 ml. The contents were filtered through Whatman No.1 filter

paper.10 ml of filtered juice were titrated against N/10 NaOH solution, using phenolphthalein as an indicator. The appearance of pink colour indicated the end point.

$$\text{Acidity (\%)} = \frac{\text{Titer value x Normality of NaOH X 64 X volume makeup}}{\text{Alliquot taken x weight af sample x 1000}} \text{ x } 100$$

The total titratable acidity was calculated in terms of citric acid on the basis of 1 ml of N/10 NaOH equivalent to 0.0064 g of anhydrous citric acid. The result was expressed in terms of per cent acidity.

3.8.4.4.3 Vitamin A (I.U. / 100 g)

The Vitamin A was determined by the method as suggested by Rangana (1997).

Regents: Acetone, Anhydrous sodium sulphate, petroleum ether.

Procedure:

Take 5 g of fresh sample and crush in 10-15 ml acetone, adding a few crystal of anhydrous sodium sulphate, with the help of pastel and mortar. Decant the supernatant into a beaker. Repeat the process twice and transfer the combined supernatant to a seperatory funnel, add 10-15 ml petroleum ether and mix thoroughly, two layers will separate out on standing. Discard the lower layer and collect upper layer in a 100 ml volumetric flask, make up the volume up to 100 ml with petroleum ether and record optical density at 452 nm using petroleum ether as blank.

$$\beta\text{- Carotene } (\mu/100 \text{ g}) = \frac{O.D.\ X\ 13.9\ X\ 104\ X\ 100}{\text{Weight of sample x 560 x 1000}}$$

3.8.4.4.5 Ascorbic acid (mg/ 100 g)

Ascorbic acid content was estimated by grinding 5 g fruit pulp with three percent metamorphic acid as buffer. The extract was filtered and 100 ml volume was made. Five ml aliquot was titrated against 2-6-dichlorophenol indophenols dye solution till the light pink colour appeared. The result was expressed as mg ascorbic acid per 100 g of fruit pulp Rangana (1997).

$$\text{Ascorbic acid} = \frac{Titer\ value\ x\ dye\ facter\ X\ volume\ makeup}{\text{Alliquot of extract taken x weight af sample taken for estimation}} \text{ x } 100$$

3.8.4.6 Pectin (%)

The pectin percent in fresh fruit pulp was estimated by using method as reported by Rangana (1997).

Principle: Addition of calcium chloride results in the precipitation of pectin as calcium pectate

From an acid solution. Calcium pectate was washed with water to make free from chloride and is dried and weighed.

1. **Acetic acid**: Normal solution: 30 ml glacial acetic acid in 500 ml water.

2. Calcium chloride: Normal solution: by adding 55 g anhydrous $CaCl_2$ in water, dissolved and diluted to 100 ml.

3. Silver nitrate-1%: By dissolving 5 g $AgNO_3$ in water and diluted to 500ml.

Procedure:

By taking 25 g of the sample in one litre beaker and added 400 ml water and it was boiled for one hour. Replaced the evaporated water by addition of distilled water. Cooled it and transferred to 500 ml volumetric flask. It was filtered through Whatman No.4 filter. Took 100 ml of the filtrate in two beakers. Further, added 300ml distilled water to each. Then 10 ml of 1/N NaOH solution was added and kept overnight. 50 ml 1N acetic acid also added and waited for 5 minutes. After this added $CaCl_2$ solution and kept it for one hour and same was boiled for one minute. Now by taking two whatman No.4 filters wash was done with distilled water and dried on an oven at 100 ^{O}C for two- hour and then weighed solution. This was filtered through Whatman No.4 filter, washing was done with distilled water to make free from chloride ions and a few drops of silver nitrate solution were added and the white precipitate (on filter paper in a Petridish) was kept in an oven, dried and weighed again.

$$\text{Pectin (\%)} = \frac{Weig\,ht\ Ca\ pectate\ \ X\ \ 100}{\text{Weight of sample}}$$

3.8.4.7 Anthocyanin (I.U. / 100 g)

The Anthocynin was determined by the method as suggested by Rangana (1997).

Resents: 0.1NHCL

Procedure:

Dilute 10 ml of juice to 50 ml, with 0.1NHCL and allow equilibrating in the dark for one hour. Record the absorbance (O.D.) at 510 nm.

Total O.D. / 100ml = $\frac{O.D.\ X\ Volume\ \ made\ \ up\ X\ \ 100}{\text{ml of juice taken}}$

Total anthocyanin ($\frac{mg}{100\ ml}$) = $\frac{Total\ \ O.D./100\ ml}{87.3}$

3.8.4.8 Total sugars (%)

Out of 100 ml sample, 5 ml aliquot was taken mixed with 3- drop of HCL and kept overnight. Next day, 2-3 drop phenolphthalein indicator was added and neutralized with 30 per cent sodium hydroxide (NaOH) solution. It was titrated against 1.0 per cent glucose in boiling solution using methylene blue as indicator. The appearance of brick red colour was marked as the end point and the results were expressed as per cent for total sugars.

8.1 Reducing sugar (%)

To determine the reducing sugar, 10 g pulp was crushed with distilled water. Filtered with muslin cloth and volume was maintained up to 100 ml. Five ml aliquot was taken with 5 ml Fehling solution 'A' and 'B' in 100 ml conical flask and was titrated against 1 per cent glucose solution , while boiling by using methylene blue as indicator. The end point was marked by the appearance of brick red colour.

8.2 Non –reducing sugar (%)

Non-reducing sugar was estimated by deducting the quantity of reducing sugar from total invert sugar and multiplied by factor 0.95.The results were expressed as per cent for non-reducing sugar.

Non-reducing sugar (%) = Total sugars (%) – reducing sugar (%) x 0.95

3.10 Statistical analysis

The data collected on various parameters of the crops during two consecutive years were subjected to statistical analysis by applying the procedure given by Gomez and Gomez (1984). The standard error of mean (SE m ±) was calculated for each item of study and critical difference (CD) at 5% level of significance was also worked out for comparing the treatment means, wherever 'F' test was found significant. The data have been illustrated with the help of suitable figures wherever felt necessary.