CHAPTER- III
MATERIALS AND METHODS
MATERIAL AND METHODS.

Field experiments were conducted to comparatively evaluate impact of the biofertilizer and chemical fertilizer on Soya bean (*Glycine max*) and French bean (*Phaseolus vulgaris*) in an areca nut garden at Shimoga. The experimental site was selected on the basis of suitability of land for conducting experiments on Soya bean and French bean during both kharif and summer seasons (2009 to 2011). The details of materials used and experimental techniques adopted during the course of investigation are detailed in this chapter.

Study Area

Shivamoga district is a part of the malnad region in Karnataka and is also known as “Gateway to malnad” or “Malenaadu Hebbagilu” in Kannada. The district ranks 9th in terms of total area among the districts of Karnataka. It is spread over an area of 8465 km². Shivamoga district lies between the latitudes 13° 27' and 14° 39' north and between the longitudes 74° 38' and 76° 4' east at mean latitude of 640 meters above the sea level. The two major rivers flow through this district are Tunga and Bhadra which meet at koodli near Shivamoga city to gain the name Tungabhadra, which later joins the river Krishna. Shivamoga is a place known for its scenic beauty, lush green forest, eye catching waterfalls, and cool climate. Malnad region is known for one of the biodiversity hotspots with a rich diversity of fauna and flora.
Fig. 3.1. Description of the study area
Soil characteristic of the experimental site

Composite soil samples were collected at a depth of 15cm before transplantation and were analyzed for important physical and chemical characteristics. Standards methods were followed for soil analysis and obtained results are presented below.

Table 3.1 Soil characteristic of the experimental site.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Particulars</th>
<th>Values obtained in %</th>
<th>Methods employed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Physical Characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Sand</td>
<td>10.8</td>
<td>International pipette method</td>
</tr>
<tr>
<td>c.</td>
<td>Clay</td>
<td>26.8</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Chemical characteristic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Soil pH</td>
<td>7.5</td>
<td>pH meter (Piper, 1966)</td>
</tr>
<tr>
<td>b.</td>
<td>Electrical conductivity (µmhos/cm)</td>
<td>0.23</td>
<td>Conductiometry (Jackson, 1967)</td>
</tr>
<tr>
<td>c.</td>
<td>Available Nitrogen (kg/ha)</td>
<td>341</td>
<td>Kjeldhal method (Subbaiah &amp; Asija, 1956)</td>
</tr>
<tr>
<td>d.</td>
<td>Available Phosphorus (kg/ha)</td>
<td>25</td>
<td>Olson’s method (Jackson, 1967)</td>
</tr>
<tr>
<td>e.</td>
<td>Available Potassium (kg/ha)</td>
<td>250</td>
<td>Flame photometer (Jackson, 1967)</td>
</tr>
</tbody>
</table>

Soil nutrients analysis

The samples were air dried and properly sieved. Soil pH was determined by pH meter (Piper, 1966). Soil chemical properties were determined before and after harvest of each crop. Chemical properties like available nitrogen was determined by Kjeldhal method (Subbaiah and Asija, 1956), available phosphorus was determined by Olsen’s methods (Jackson, 1967) and available potassium was estimated by flame photometer (Jackson, 1967).
Climatic conditions

The data on monthly rainfall, maximum and minimum temperature during 2009-2011 was obtained from nearest Meteorological Observatory, Shimoga.

Rainfall

During the study period 2009-2010, the maximum rainfall was recorded in the month of August 2010 i.e. 81.6mm and minimum was recorded in the month of February and March 2010, which was 0.0 mm and 0.4mm respectively. The average rainfall was 29.8mm. In the subsequent year, 2010-11 the highest rainfall was recorded in the month of September and November 2010 i.e., 68.2mm and 68.6mm and minimum was found in the month of December 2010 and January, February and March 2011 i.e. 0,0,1.8mm and 0 mm respectively. The average rainfall was 28.2mm.

Temperature

Temperature is an important factor that influences the growth and production of any crops. Maximum temperature was recorded in the month of April 2010 measuring 41.5°C and minimum was recorded in the month of September 2009 and August 2010 i.e., 31 and 31°C. The average temperature was 35.6° C. In the year 2010-11 the maximum temperature was recorded in the month of April and May 2011 i.e. 42 and 42°C and minimum was found in the month of July and August 2011 i.e. 31 and31°C. The average temperature was 36.2°C.
Table 3.2. Meteorological data for the period of two years from 2009-2011

<table>
<thead>
<tr>
<th>Months</th>
<th>Rainfall 09-10</th>
<th>Temp 09-10</th>
<th>Months</th>
<th>Rainfall 10-11</th>
<th>Temp 10-11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sep 2009-10</td>
<td>38.0</td>
<td>31.0</td>
<td>Sep 2010-2011</td>
<td>68.2</td>
<td>35.0</td>
</tr>
<tr>
<td>Oct 2009</td>
<td>31.8</td>
<td>35.0</td>
<td>Oct 2010</td>
<td>43.4</td>
<td>35.0</td>
</tr>
<tr>
<td>Nov 2009</td>
<td>28.6</td>
<td>34.0</td>
<td>Nov 2010</td>
<td>68.6</td>
<td>35.0</td>
</tr>
<tr>
<td>Dec 2009</td>
<td>26.6</td>
<td>35.0</td>
<td>Dec 2010</td>
<td>-</td>
<td>34.0</td>
</tr>
<tr>
<td>Jan 2010</td>
<td>36.6</td>
<td>35.0</td>
<td>Jan 2011</td>
<td>-</td>
<td>35.0</td>
</tr>
<tr>
<td>Feb 2010</td>
<td>-</td>
<td>37.0</td>
<td>Feb 2011</td>
<td>1.8</td>
<td>37.0</td>
</tr>
<tr>
<td>Mar 2010</td>
<td>0.4</td>
<td>39.0</td>
<td>Mar 2011</td>
<td>-</td>
<td>39.0</td>
</tr>
<tr>
<td>Apr 2010</td>
<td>16.8</td>
<td>41.5</td>
<td>Apr 2011</td>
<td>56.8</td>
<td>42.0</td>
</tr>
<tr>
<td>May 2010</td>
<td>24.2</td>
<td>39.5</td>
<td>May 2011</td>
<td>16.8</td>
<td>42.0</td>
</tr>
<tr>
<td>June 2010</td>
<td>32.2</td>
<td>38.0</td>
<td>June 2011</td>
<td>24.8</td>
<td>39.0</td>
</tr>
<tr>
<td>July 2010</td>
<td>41.8</td>
<td>32.0</td>
<td>July 2011</td>
<td>28.6</td>
<td>31.0</td>
</tr>
<tr>
<td>Aug 2010</td>
<td>81.6</td>
<td>31.0</td>
<td>Aug 2011</td>
<td>30.2</td>
<td>31.0</td>
</tr>
<tr>
<td>Average of 12 Months</td>
<td>29.8</td>
<td>35.6</td>
<td>Average of 12 Months</td>
<td>28.2</td>
<td>36.2</td>
</tr>
</tbody>
</table>

Note: Rainfall in mm, Temp: Temperature in degree centigrade

Fig. 3.2 shows the meteorological data.
Experimental Details

Experiment I: Soya bean (Glycine max)

Treatment Details

This study included ten treatments with three replications.

T1 = Control

T2 = NPK (Nitrogen Phosphorus Potassium) (25:50:25)

T3 = FYM (Farm Yard Manure) (5 ton/ha)

T4 = NPK+FYM

T5 = NPK+PSB (Phosphate Solubilizing Bacteria)

T6 = Vermicompost (2.5 ton/ha)

T7 = Vermicompost +NPK+PSB

T8 = Rhizobium (60gm/ha)

T9 = Rhizobium+ NPK

T10 = FYM+ Rhizobium +Vermicompost +PSB

Experiment -II – French bean (Phaseolus vulgaris)

Experimental details
Treatment Details

This study included ten treatments with three replications.

\[
\begin{align*}
T1 & = \text{Control} \\
T2 & = \text{NPK (Nitrogen Phosphorus Potassium) (25:50:25)} \\
T3 & = \text{FYM (Farm Yard Manure) (5 ton/ha)} \\
T4 & = \text{NPK+FYM} \\
T5 & = \text{NPK+ PSB (Phosphate Solubilizing Bacteria)} \\
T6 & = \text{Vermicompost (2.5 ton/ha)} \\
T7 & = \text{Vermicompost +NPK+ PSB} \\
T8 & = \text{Poultry manure (2.5 ton/ha)} \\
T9 & = \text{Poultry manure + NPK} \\
T10 & = \text{FYM+ Poultry manure + Vermicompost +PSB}
\end{align*}
\]

Experimental details

- Design = Randomized complete block design
- Replication = Three (3)
- Treatments = ten (10)
- Total number = Thirty (30)
- Of plots
Plot size = 12m x 9m
Gross plot size = 1.8m X 1.4m
Spacing = 30 cm x 10cm
Variety (Soya bean) = Cv.JS 335
Variety (French bean) = Arka komal (S-9)

Plot lay out for experiment
Seed source

The breeder seeds of soya bean Cv. JS-335 were obtained from university of Agricultural science, Dharwad.

![Soya bean seeds](image)

**Fig. 3.3 shows Soya bean seeds**

Description of variety

Soya bean Cv. JS-335 has been released for general cultivation. It is a short duration (85-90 days) variety with yielding ability of 25-35 q per hectare. The leaves are dark green and purple flowers, yellow seed coat, and semi determinate, tolerate to pod shattering up to 8-10 days after maturity.

![Soya plant](image)

**Fig. 3.4. Shows Soya bean**
Chapter 3

Material and methods

Seed source (French bean)

Seeds were obtained from agricultural research station, Navile, Shimoga.

Fig: 3.5. Shows French bean seeds

Description of variety

The high yielding genotype Arka komal (S-9) was used in the experiment. It is characterized by straight growth, dwarf with long, straight and slender green pods. It is possible to get first picking green pods at 45 days. It is expected to yield 20 q per hectare and is having higher yield potential over other string less cultivars.

Fig: 3.6. Shows French bean plant
Cultural practices

Land preparation

The land is brought to the fine tilth with one deep ploughing and three harrowing. The residues of the previous crop and weeds were removed from experimental area. The land was leveled with plank.

Manure and fertilizer application

The manures and fertilizers were applied as per the treatments combinations. The farm yard manure, vermicompost, Rhizobium, PSB and recommended dose of fertilizers were incorporated in soil 15 days before sowing. Recommended fertilizer dose of 25kg N, 50kg P₂O₅ and 25 kg K₂O per hectare was applied.
Seeds and sowing

Breeder seed of soya bean were obtained from the University of Agricultural science, Dharwad. The healthy seeds were sown by dibbling with the spacing of 10 cm between the seeds and 30 cm between rows. The seedlings were thinned out at 15 days after sowing in order to maintain one plant per hill.
After care

Irrigation was given at critical stages of crop growth and earthing up were done at 30 days after sowing. The plots were kept weed free by hand weeding at 20 and 30 days after sowing.
Harvesting

The crop was harvested treatment wise at physiological maturity based on visual observation (yellowing of pods and leaves). The harvested plants were sun dried separately and seeds were clean.

Collection of experimental data

Five plants were selected randomly from each plot for recording various morphological observations at different stages.
Plant height (cm)

The plant height on five randomly selected and tagged plants were measured from the base or lower cotyledonary node to the tip of the shoot apex at 20 days, 40 days and at harvest. The average height of the five plants was worked out and expressed in centimeters (cm).

Number of branches

The number of branches arising on the main stem in the five selected and tagged plants was counted at 20, 40 days and at harvest. The mean number of branches per plant was worked out and expressed in number of branches per plant.

Number of leaves

The number of fully opened leaves present in each of the five randomly selected and tagged plants was recorded at 20, 40 days and at harvest. The number of leaves per plants was worked out and expressed in number per plant.

Yield and yield component

Number of pods per plant

The number of pods harvested from randomly selected and tagged plants in each treatment was counted and average was worked out and expressed as number of pods per plant.
Pod yield per plant

The pods yield from five randomly selected crops was recorded and average pod yield was calculated.

Pod yield (Kg ha\(^{-1}\)). Net pod yield was recorded and worked out for hectare.

Dry matter production

Randomly selected five plants samples were separated into leaf, stem, and reproductive parts. These were air dried and then transferred to hot air oven at 80\(^\circ\) C for 72 hours (or until constant weight was obtained) and their dry weight was recorded. Total dry matter was calculated by adding the dry weights of different plant parts and expressed as gram per plant. The dry weight of different plant parts and total dry weight was recorded at 20, 40 days and at harvest after sowing and expressed on per plant basis.

Bio chemical parameters

Chlorophyll content

The method of Amon(1949) was followed for estimating chlorophyll a, chlorophyll b, and total chlorophyll contents. Fully opened leaves selected from top of the canopy were brought from the field in ice box. They were cut in to small pieces and 0.25g of fresh weight of leaf was weighed from each sample and homogenized with pure aceton. The extract was filtered through Whatman No. 1 filter paper and washed 2-3 times using 80 percent of acetone. Finally, the volume of the extract was made up to 25ml. The absorbance of the extract was read at 645,652 and 663 nm in spectrophotometer and for blank 80 percent acetone was used. Total chlorophyll,
chlorophyll a, and chlorophyll b, were calculated by using the formula as given below and expressed in mg/ g fresh weight.

Chlorophyll a = (12.7X A663) - (2.69 x A645) x V/1000 X a X W
Chlorophyll b = (22.9 x A 645) - (4.68 X a663) x V/1000 X Ax w
Total chlorophyll = Chlorophyll a + Chlorophyll b

where,

A = Absorbance at specific wave lengths (645 and 663nm)
V = Final volume of chlorophyll extract
W = Fresh weight of the sample (g)
A = Path length of light (1cm).

Statistical Analysis

Experimental data was analyzed by applying the standard methods known as Analysis of variance as applicable in the randomize block design in kharif and summer season as described by Panse and Suchatme (1967). The significance of the treatments was tested by (t test) and standard error probability was worked out for comparison between treatments. Correlation co-efficient (r) were calculated and tested for their significance at 5% and 1 percent as per the procedure outlined by Panse and Suchatme (1967).