MATERIALS AND METHODS
3. MATERIALS AND METHODS

To accomplish the objectives of the research problem entitled “Effect of integrated nutrient management on yield, quality and soil properties in pea-okra system in an acid Alfisol”, a field experiment was conducted at the Experimental Farm of the Department of Soil Science, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur during the three cropping cycles from rabi 2008-09 to kharif 2011. The details of the materials used and the experimental methods followed, during the course of this study, have been described in this chapter under the following heads:

3.1 General description of the study area
3.2 Field Studies
3.3 Laboratory Studies
3.4 Economic Analysis
3.5 Statistical Analysis

3.1 General description of the study area

3.1.1 Experimental site
The experimental farm is located at 32° 6’ N latitude and 76° 3’ E longitude at an elevation of about 1280 m above mean sea level in wet-temperate agro-climatic zone of Himachal Pradesh.

3.1.2 Weather and climate

Agro-climatically, Palampur falls under wet-temperate humid zone of Himachal Pradesh, which is characterized by mild summers, severe winters and experiences occasional snowfall during winters. The region receives an average rainfall of 2600 mm per annum, with major portion of it (80 per cent) being received during June to September. Winter rains are received during December to February. October, November, April and May months are usually dry and receive very low or no rainfall. Mean monthly meteorological data recorded at the meteorological observatory of the Department of Agronomy Forage and Grassland Management during the crop season has been presented in Fig 3.1, 3.2 and 3.3.
Fig. 3.1 Mean monthly weather data at Palampur during 2008-09
Fig. 3.2 Mean monthly weather data at Palampur during 2009-10
Fig. 3.3 Mean monthly weather data at Palampur during 2010-11
3.1.3 Soil characteristics

Taxonomically, the soil of the study area falls in the order “Alfisols” and sub-group “Typic Hapludalf” (Verma 1979). In the genetic system of classification, the soils of the area have been classified as “Grey Brown Podozols” and have been developed from fluvioglacial parent material. These soils owe their origin to rocks like slates, phyllites, schists, quartzites and gneisses.

The soil of the experimental site at initiation of experiment was acidic in reaction and silty clay loam in texture. The surface soil of the experimental field was categorized as low in available nitrogen and medium in available phosphorus and potassium. The organic carbon content was also medium in status. Some important soil characteristics of the surface soil (0 - 0.15 m) of the experimental site are given in Table 3.1.

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Physical analysis</strong></td>
<td></td>
</tr>
<tr>
<td>Bulk density (Mg m$^{-3}$)</td>
<td>1.25</td>
</tr>
<tr>
<td>Water holding capacity (%)</td>
<td>52.4</td>
</tr>
<tr>
<td>Particle Size analysis</td>
<td></td>
</tr>
<tr>
<td>o Sand (%)</td>
<td>22.50</td>
</tr>
<tr>
<td>o Silt (%)</td>
<td>44.60</td>
</tr>
<tr>
<td>o Clay (%)</td>
<td>31.70</td>
</tr>
<tr>
<td>Textural class</td>
<td>Silty clay loam</td>
</tr>
<tr>
<td><strong>B. Chemical analysis</strong></td>
<td></td>
</tr>
<tr>
<td>Soil pH</td>
<td>5.35</td>
</tr>
<tr>
<td>Exchangeable acidity [ c mol(p$^+$) kg$^{-1}$]</td>
<td>0.25</td>
</tr>
<tr>
<td>pH dependent acidity [ c mol(p$^+$) kg$^{-1}$]</td>
<td>7.78</td>
</tr>
</tbody>
</table>
Organic carbon (g kg\(^{-1}\)) 9.80
CEC [c mol(p\(^+\)) kg\(^{-1}\)] 10.0

Available Nutrients (kg ha\(^{-1}\))
- Nitrogen 256
- Phosphorus 17.0
- Potassium 195

Exchangeable Ca [c mol(p\(^+\)) kg\(^{-1}\)] 3.20
Exchangeable Mg [c mol(p\(^+\)) kg\(^{-1}\)] 0.51

Available micronutrients (g ha\(^{-1}\))
- Fe 32.4
- Mn 31.0
- Zn 0.84
- Cu 1.63

Different forms ofIron (mg kg\(^{-1}\))
- Exchangeable 23.5
- Extractable 64.2
- Amorphous 2181
- Crystalline 1463

Different forms of Aluminium (mg kg\(^{-1}\))
- Exchangeable 18.9
- Extractable 61.2
- Amorphous 521
- Crystalline 1243

C. Microbiological Analysis

Microbial biomass carbon (µg g\(^{-1}\)) 24
Dehydrogenase activity (µg TPF g\(^{-1}\) hr\(^{-1}\)) 2.3
3.2 Field studies

3.2.1 Experimental details

A field experiment with thirteen treatment combinations of lime, NPK doses and vermicompost was conducted on pea and okra for three years at the experimental farm of Department of Soil Science, CSK HPKV, Palampur with the following treatments:

<table>
<thead>
<tr>
<th>Code</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>Control</td>
</tr>
<tr>
<td>T₂</td>
<td>75 % NPK</td>
</tr>
<tr>
<td>T₃</td>
<td>100 % NPK</td>
</tr>
<tr>
<td>T₄</td>
<td>125 % NPK</td>
</tr>
<tr>
<td>T₅</td>
<td>75 % NPK + Lime</td>
</tr>
<tr>
<td>T₆</td>
<td>100 % NPK + Lime</td>
</tr>
<tr>
<td>T₇</td>
<td>125 % NPK + Lime</td>
</tr>
<tr>
<td>T₈</td>
<td>75 % NPK + vermicompost @ 5 t ha⁻¹</td>
</tr>
<tr>
<td>T₉</td>
<td>100 % NPK + vermicompost @ 5 t ha⁻¹</td>
</tr>
<tr>
<td>T₁₀</td>
<td>125 % NPK + vermicompost @ 5 t ha⁻¹</td>
</tr>
<tr>
<td>T₁₁</td>
<td>75 % NPK + vermicompost @ 10 t ha⁻¹</td>
</tr>
<tr>
<td>T₁₂</td>
<td>100 % NPK + vermicompost @ 10 t ha⁻¹</td>
</tr>
<tr>
<td>T₁₃</td>
<td>125 % NPK + vermicompost @ 10 t ha⁻¹</td>
</tr>
</tbody>
</table>

Lime was applied @ 1/10th of the lime requirement in furrows only to the first crop i.e. pea during all the years.
Treatments : 13
Replications : 3
Total No. of plots : 39
Net plot size : 5 m$^2$
Crops : Pea and Okra
Design : Randomized Block Design

i. **Field preparation**

The individual plot was prepared manually with spade.

ii. **Sowing**

Sowing of pea crops during *rabi* 2008-09, 2009-10 and 2010-11 was done on 15$^{th}$ November, 2008, 28$^{th}$ November, 2009 and 24$^{th}$ November, 2010, respectively. The variety used was Palam Priya in all the seasons. Pea seeds were treated with Bavistin $\theta$ 2.5 g kg$^{-1}$ of seed before sowing to reduce the seed borne diseases. The crops were irrigated only twice and thereafter they met their water requirement through rainfall. Weed control was done chemically with the application of Pendimetheline $\theta$ 1.5 kg AI ha$^{-1}$ as pre-emergence. Okra during *kharif*, 2009, 2010 and 2011 was sown on 4$^{th}$ April, 2009, 13$^{th}$ April, 2010 and 8$^{th}$ April, 2011, respectively and the variety used was P-8. Okra (field trial) during *kharif*, 2010, failed due to excessive rainwater stagnation in the field, so trial was repeated during *kharif* 2011.

iii. **Application of fertilizers and vermicompost**

Before the sowing of crops, vermicompost was applied in their respective treatments. At the time of sowing of pea, inorganic fertilizers were applied in each plot as per apportioned
to treatment, whereas no fertilizer of any kind was added in control treatment. N, P₂O₅ and K₂O were applied @ 50, 26 and 50 kg/ha through urea, single super phosphate (SSP) and muriate of potash (MOP), respectively in pea. Full doses of N, P and K were applied at the time of sowing of pea. In okra, N, P and K were applied @ 75, 21 and 42 kg/ha through urea, single super phosphate (SSP) and muriate of potash (MOP), respectively. Full dose of N, P₂O₅ and K₂O were applied at the time of sowing.
LAYOUT OF FIELD EXPERIMENT

Pakka road from Gate No. 3 to COVAS Palampur

Kaccha road within the farm

R1

T13 T7 T5 T11 T9 T10 T4 T1 T6 T3 T2 T12 T8

T12 T11 T10 T5 T6 T7 T9 T3 T8 T13 T1 T2 T4

T2 T12 T11 T10 T5 T6 T7 T9 T3 T8 T13 T1 T2 T4

R2

T8 T5 T13 T7 T1 T12

R3

T8 T5 T13 T7 T1 T12

Net Plot Size: 5m²

T1 : Control

T8 : 75% NPK + Vermicompost @ 5t ha⁻¹
<table>
<thead>
<tr>
<th>T_2</th>
<th>75 % NPK</th>
<th>T_9</th>
<th>100% NPK + Vermicompost @ 5t ha(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_3</td>
<td>100% NPK</td>
<td>T_{10}</td>
<td>125 % NPK + Vermicompost @ 5t ha(^1)</td>
</tr>
<tr>
<td>T_4</td>
<td>125 % NPK</td>
<td>T_{11}</td>
<td>75 % NPK + Vermicompost @ 10t ha(^1)</td>
</tr>
<tr>
<td>T_5</td>
<td>75 % NPK + Lime</td>
<td>T_{12}</td>
<td>100% NPK + Vermicompost @ 10t ha(^1)</td>
</tr>
<tr>
<td>T_{13}</td>
<td>125 % NPK + Lime</td>
<td>T_{13}</td>
<td>125 % NPK + Vermicompost @ 10t ha(^1)</td>
</tr>
</tbody>
</table>
iv. Harvesting

The pods of pea/fruits of okra were harvested in four picking and yield of green pea/fruits of okra in each treatment were recorded. To obtain yield per plot, weight of green pods of pea/fruits of okra harvested from different plots from each picking were added and calculated in terms of \( q \text{ ha}^{-1} \). At harvest, whole plants of pea and okra from each plot were harvested and dried in sun for 2 to 3 days till constant weight to obtain the dry matter yield.

3.2.2 Plant sampling and preparation

Plant samples of pea and okra were taken at each picking from each plot and after initial sun drying for a couple of days. Pea samples were dried in an oven at 60\(^\circ\) C. The seeds of pea were taken out from pods and hulls were mixed with stover. The seeds and stover samples were ground separately and stored in paper bags for further analysis. In case of okra, fruit and stover samples were dried in an oven at 60\(^\circ\) C. They were ground and then stored in paper bags for further analysis.

3.3 Laboratory studies

3.3.1 Preparation and analysis of organics

Sample of vermicompost was collected, air dried, sieved and stored for further analysis. The nutrient content of vermicompost is given in Table 3.2 and the detail of chemical analysis is given below:

a) Total nitrogen content

A sample of vermicompost was digested with concentrated \( \text{H}_2\text{SO}_4 \) using digestion mixture and total nitrogen was determined by Modified Kjeldahl’s Method (Jackson 1967).

Table 3.2 Nutrient content of Vermicompost

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Parameters</th>
<th>Vermicompost</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisture (%)</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>Nitrogen (%)</td>
<td>1.46</td>
</tr>
<tr>
<td>3</td>
<td>Phosphorus (%)</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td>Potassium (%)</td>
<td>0.74</td>
</tr>
<tr>
<td>5</td>
<td>Calcium (%)</td>
<td>0.53</td>
</tr>
<tr>
<td>6</td>
<td>Magnesium (%)</td>
<td>0.041</td>
</tr>
</tbody>
</table>
b) **Total phosphorous content**
Vermicompost sample was digested with diacid mixture of HNO₃ and HClO₄ in the ratio of 4:1 and the extract was made to a definite volume. Total phosphorous was determined by Vanadomolybdate Phosphoric Acid Yellow Colour method at 730 nm (Jackson 1967).

c) **Total potassium content**
It was determined using flame-photometer from the extract obtained by digestion with diacid mixture (Chapman and Brown 1950).

d) **Total Calcium content**
It was determined using Flame-emission spectrophotometer from the extract obtained by digestion with diacid mixture (Black 1965).

e) **Total Magnesium Content**
It was determined on atomic absorption spectrophotometer (AAS – 4129) from the extract obtained by digestion with diacid mixture (Jackson 1967).

f) **Micronutrients (Fe, Cu, Zn, Mn)**
Micronutrients in the vermicompost were determined on atomic absorption spectrophotometer (AAS-4129) from the extract obtained by digestion with diacid mixture (Jackson 1967).

### 3.3.2 Collection and preparation of soil samples

Plot wise composite soil samples were collected from 0-0.15 m depth after pea (*rabi*, 2008-09, 2009-10 & 2010-11) and okra (*kharif*, 2009, *kharif*, 2010 & *kharif*, 2011) harvest. The soil samples were air dried, processed and passed through 2 mm sieve and properly stored in polyethylene bags for further analysis.

### 3.3.3 Analysis of soil sample

Soil samples before the start of experimentation were analyzed for pH, CEC, texture, organic carbon, available N, P, K, exchangeable Ca and Mg, available Fe, Mn, Cu & Zn.

**Table 3.3 Various methods used for soil analysis**

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Parameters</th>
<th>Method employed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical property</td>
<td>i)  Particle Size analysis</td>
<td>International pipette method (Piper 1966)</td>
</tr>
<tr>
<td></td>
<td>ii) Bulk Density</td>
<td>Core sampler method (Piper 1950)</td>
</tr>
<tr>
<td></td>
<td>iii) Water holding capacity</td>
<td>Keen’s Method (Piper 1950)</td>
</tr>
<tr>
<td>Chemical property</td>
<td>i)  Soil pH (1:2.5)</td>
<td>1:2.5 soil water suspension using glass electrode pH meter (Jackson 1967)</td>
</tr>
<tr>
<td></td>
<td>ii) Organic carbon</td>
<td>Rapid Titration Method (Walkley and Black 1934)</td>
</tr>
<tr>
<td>iii) Cation exchange capacity</td>
<td>Neutral N NH₄OAc Method (Jackson, 1967)</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------------------------------------</td>
<td></td>
</tr>
<tr>
<td>iv) Available nitrogen</td>
<td>Alkaline Permanganate method (Subbiah and Asija 1956)</td>
<td></td>
</tr>
<tr>
<td>v) Available phosphorus</td>
<td>0.5M NaHCO₃, pH 8.5 (Olsen et al. 1954)</td>
<td></td>
</tr>
<tr>
<td>vi) Available potassium</td>
<td>Ammonium acetate (pH 7.0) method (Black 1965)</td>
<td></td>
</tr>
<tr>
<td>vii) DTPA extractable micronutrients</td>
<td>Extracted with DTPA and analyzed by atomic absorption spectrophotometer (Lindsay and Norvell 1978)</td>
<td></td>
</tr>
</tbody>
</table>

**Microbiological property**

| i) Microbial Biomass C        | Fumigation-extraction method (Vance et al. 1987) |
| ii) Dehydrogenase activity   | Triphenyl formazan method (Casida et al. 1964) |

Mn and Cu, exchangeable, extractable, amorphous, crystalline Al and Fe, exchangeable and pH dependent acidity, bulk density, water holding capacity, microbial biomass carbon and dehydrogenase activity.

After 60 days of sowing and at harvest of pea and okra, soil samples were analyzed for pH, available N, P, K, exchangeable Ca and Mg, available Fe, Zn, Mn and Cu during all the years.

At the start and at end of the experiment exchangeable, extractable, amorphous, crystalline Al and Fe, exchangeable acidity, pH dependent acidity, bulk density, water holding capacity were also be determined.
Table 3.4  Methods used for determination of different forms of acidities

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Determination</th>
<th>Method Followed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Exchangeable acidity: Soil was leached with 1N unbuffered KCl solution and leachate was titrated with 0.01 N NaOH using phenolphthalein indicator</td>
<td>Yuan’s Method (1959) and improved by Amedee and Peech (1976)</td>
</tr>
<tr>
<td>2.</td>
<td>pH dependent/ non-exchangeable acidity: 1N KCl pre-leached soil was extracted with BaCl$_2$–TEA (pH 8.2) and the extract was titrated with 0.2N HCl using mixed indicator</td>
<td>Coleman et al. (1959)</td>
</tr>
<tr>
<td>3.</td>
<td>Total acidity: Soil extracted by BaCl$_2$–TEA (pH 8.2) and extract was titrated with 0.2N HCl using mixed indicator</td>
<td>Mehlich’s Method (1948) and improved by Peech et al. (1962)</td>
</tr>
</tbody>
</table>

Determination of different forms of Al and Fe

The detailed procedure as proposed by Ballard and Fiskell (1974) for the determination of different forms of Al and Fe is described as under:

Pre- treatments of extracts

A pre-treatment of 10 ml mixture of HNO$_3$: H$_2$SO$_4$ (10:3) was given to a known amount of citrate-dithionite-bicarbonate extract in silica crucible. The crucibles were kept on a sand bath until the residue attained brownish colour. Later, the residue in the silica crucibles was kept in the muffle furnace at 550°C to get rid of citrate dithionite, which interferes during the determination of Al. The oxalate extract was treated with 5 ml of HNO$_3$ and 2 ml of HClO$_4$ acid and were digested on hot sand bath till the residue attained white colour, so as to remove the interference of oxalate during the determinations of Al and Fe.

Table 3.5  Methodology for determination of different forms of Al and Fe

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extractant</th>
<th>pH</th>
<th>Soil</th>
<th>Shaking time</th>
<th>Forms of Al and Fe</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Solution</th>
<th>Ratio</th>
<th>Time</th>
<th>Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1N KCl</td>
<td>7.0</td>
<td>1:10</td>
<td>Exchangeable Al</td>
</tr>
<tr>
<td>2</td>
<td>1N NH₄OAc</td>
<td>4.8</td>
<td>1:5</td>
<td>Exchangeable Al and Fe</td>
</tr>
<tr>
<td>3</td>
<td>0.3 M Ammonium Oxalate</td>
<td>3.2</td>
<td>1:50</td>
<td>Amorphous and extractable Al and Fe</td>
</tr>
<tr>
<td>4</td>
<td>Citrate dithionite bi-carbonate</td>
<td>8.2</td>
<td>1:50</td>
<td>Kept for 15 minute on water bath at 80°C</td>
</tr>
<tr>
<td>5</td>
<td>Conc. HCl (11.3N)</td>
<td>-</td>
<td>1:125</td>
<td>Total Al and Fe</td>
</tr>
</tbody>
</table>

**Determination**

After the pre-treatments to citrate dithionite-bicarbonate and oxalate extracts, a known amount of each was taken in 0.5 N HCl and volume was made to 50ml. Aluminium was determined by the method of aluminon as proposed by Herwitz (1975), whereas iron was determined in atomic absorption spectrophotometer.

**Computation of different forms of Al and Fe**

Different forms of Al and Fe were calculated as described below:

Amorphous Al = Oxalate Al - KCl Al

Crystalline Al = Citrate-dithionite-bicarbonate-Al - Oxalate Al

Amorphous Fe = Oxalate Fe - 1N NH₄OAc Fe

Crystalline Fe = Citrate-dithionite-bicarbonate Fe - Oxalate Fe

**3.3.4 Preparation and analysis of plant samples**

Samples of stover and seed/fruit were collected, first air dried, then dried in oven for 3-4 days at 60°C till constant weight. The dried samples were then powdered and stored for further analysis. The detail of chemical analysis is given below:
a) **Total nitrogen content**

Powdered plant samples and seed samples were digested with concentrated $\text{H}_2\text{SO}_4$ using digestion mixture and total nitrogen was determined by micro Kjeldahl’s method (Jackson 1967).

b) **Total phosphorous content**

Plant and seed samples were digested with diacid mixture of $\text{HNO}_3$ and $\text{HClO}_4$ in the ratio of 9:4 and the extract was made to a definite volume. Total phosphorous was determined by vanadomolybdate phosphoric acid yellow colour method at 730 nm (Jackson 1967).

c) **Total potassium content**

It was determined by using Flame-photometer from the extract obtained by digestion with diacid mixture (Chapman and Brown 1950).

d) **Total Calcium content**

It was determined using Flame-emission spectrophotometer from the extract obtained by digestion with diacid mixture (Black 1965).

e) **Total Magnesium Content**

It was determined on atomic absorption spectrophotometer (AAS – 4129) from the extract obtained by digestion with diacid mixture (Jackson 1967).

f) **Micronutrients (Fe, Cu, Zn, Mn)**

It was analysed on atomic absorption spectrophotometer from the extract obtained by digestion with diacid mixture (Jackson 1967).

g) **Nutrient uptake**

The concentration of nitrogen, phosphorous, potassium and micronutrients were determined in stover and seed samples and uptake was calculated as follows:

Uptake (kg/ha) = [% concentration of nutrient X yield of crop in q ha (on oven dry weight basis)]. Total uptake was calculated as follows:

Total uptake = uptake in stover + uptake in seed/fruit

h) **Protein content in grains**
Protein content in grains was estimated by multiplying the nitrogen content by factor 6.25.

i) **Total Soluble Solids (TSS)**

The TSS contents were determined with Zeiss Pocket refractometer (0 to 32.0 brix) by putting a drop of extracted juice of fresh pea grains on the prism and taking the reading. Temperature corrections were applied when the readings were made at temperature other than 20°C (A.O.A.C. 1975).

j) **Ascorbic Acid Content**

Ascorbic acid content was determined by the 2, 6-Dichlorophenol-Indophenol Visual Titration method given by Johnson (1948). The detail of the method is given below:

Took 10 g of sample, blended it with 3 per cent metaphosphoric acid (HPO$_3$) and made up to 100 ml with HPO$_3$. Took 10 ml of the HPO$_3$ extract of the sample and titrated with the standard dye to a pink end point which persisted for at least 15 seconds.

**Ascorbic acid standard:** Weighed accurately 100 mg of L-ascorbic acid and made up to 100 ml with 3 per cent HPO$_3$. Diluted 10 ml to 100 ml with 3 per cent HPO$_3$ (1 ml= 0.1 mg of ascorbic acid).

Dye Solution: Dissolved 50 mg of the sodium salt of 2, 6-dichlorophenol-indophenol in approximately 150 ml of hot glass distilled water containing 42 mg of sodium bicarbonate. Stored in a refrigerator and standardized every day.

Standardization of Dye: Took 5 ml of standard ascorbic acid solution and added 5 ml of HPO$_3$. Titrated with the dye solution to a pink colour which persisted for 15 seconds. Determined the dye factor, i.e. mg of ascorbic acid per ml of the dye, using the formula:

$$\text{Dye factor} = \frac{0.5}{\text{Titre}}$$

Calculation

Calculated the ascorbic acid content of the sample from the following formula:
Ascorbic acid

\[
\text{Titre x Dye factor x Volume made up x 100 (mg/100g)} = \frac{\text{Aliquot of extract taken x weight of sample taken}}{\text{Sample %}}
\]

**k) Carbohydrate content**

100 mg of the sample was weighed into a boiling tube. It was hydrolyzed by keeping it in a boiling water bath for 3 hr with 5 ml of 2.5 N HCl and cooled to room temperature. It was neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and then centrifuged. The supernatant was collected and 0.5 and 1 ml aliquots were taken for analysis. The standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8, 1.0 ml of working standards. ‘0’ served as blank. The volume was made upto 1 ml in all the tubes including the sample tubes by adding distilled water. Then 4 ml of anthrone reagent was added. It was heated for 8 min in boiling water bath. After cooling rapidly and the green colour was read at 630 nm. A standard graph was drawn by plotting concentration of the standard on the X-axis verses absorbance on Y-axis. From the graph the amount of carbohydrates present in the sample tube was calculated.

**Calculation**

\[
\text{Amount of CHO present in sample (%)} = \frac{\text{Sugar value from graph (mg)}}{\text{Total vol. of extract x 100}} \times \frac{\text{Aliquot sample used (0.5 or 1 ml)}}{\text{Wt. of sample (mg)}}
\]

This method has been given by Sadasivam and Manickam (1996).

**l) Reducing sugars**

100 mg of the sample was weighed and the sugars were extracted with 80% alcohol twice (5ml each time). Then the supernatant was collected and evaporated on water bath. After that 10 ml of water was added and the sugars were dissolved. Aliquots of 0.5 or 3.0 ml of alcohol-free extract were pipetted out into test tubes and the volume was made to 3ml with water in all the tubes. 3ml of DNS reagent was added and mixed. Then it was heated for 5 minutes in boiling water bath. After the colour has developed, 1ml of 40% Rochelle salt solution (when the contents are still warm) was added and mixed. The tubes were cooled under running tap and the absorbance was measured at 510 nm using reagents. Blank was adjusted to zero absorbance. The amount of reducing sugar in the sample was calculated.
using a standard graph prepared from working standard glucose solution (0 to 500µg) in the same manner.

**Calculation**

Reducing sugar value from graph (µg) \[\text{Total vol. of alcohol-free extract (10 ml)} \times 1\]

\[\text{Sugars in sample (\%)} = \frac{\text{Aliquot of alcohol} \times \text{Wt of sample (100mg)}}{\text{free extract used (ml)} \times 1000} \]

The above method was given by Sadasivam and Manickam (1996).

m) **Non-reducing sugars**

The content of non-reducing sugars was calculated by subtracting the reducing sugars from total sugar content.

n) **Total sugar content**

1 g of ground sample was taken and placed in a 100 ml conical flask. Sugars were extracted twice with 50 ml of 80% ethanol, followed by complete extraction four times with 70% ethanol by refluxing on a boiling water bath for 30 min. each time. The contents in the flask were stirred occasionally. The combined alcoholic extract of each sample obtained was concentrated to an aqueous syrup on boiling water bath. The last traces of concentrated sugar solution were transferred to a 100 ml volumetric flask and volume was raised to about 98 ml with distilled water. 1 ml of saturated solution of lead acetate was added to remove proteins and the volume was made to 100ml. The contents were filtered through whatman No.1 filter paper and the excess of lead ions from the filtrate was removed by the addition of sodium oxalate crystals followed by filtration. The clear extract so obtained was used for the estimation of free sugars.

0.2 ml of the extract was taken in each test tube and the volume was made to 1 ml. Glucose standards (10 to 60 mg) and blank were also taken. After adding 1 ml of 5 % phenol to each test tube, the test tubes were placed in ice cold water and 5 ml of 95.5% of sulphuric acid was added swiftly. The content were mixed and equilibrated to room temperature. The Absorbance of pink colour developed was read at 490 mm. The concentration of total sugar was calculated from the test extracts as glucose from the standard curve for glucose.

o) **Crude fiber content (per cent)**
2 g of finally dried powdered and moisture free sample was taken in a paper thimble. It was extracted with petroleum ether (60-90°C BP) in a Soxhlet apparatus for fifteen to eighteen hours to make the sample free from fats. After caring out the ether extraction the sample was digested with 200 ml of 1.25 per cent sulphuric acid (25 ml of 10 per cent acid + 175 ml water) for 30 minutes in a conical flask under reflux. Then it was filtered through muslin cloth (45 threads to an inch) using suction to hasten filtration and washed with water to render it free of acid. The residue was transferred to a beaker and 200 ml of 1.25 per cent Sodium hydroxide (25 ml of 10 per cent NaOH + 175 ml water) was added and boiled for half an hour. It was then filtered and washed to make free it from the alkali using in turn (i) hot water (ii) 1 per cent HNO₃ (iii) hot water for washing. The residue was transferred to a weighed dish and dried to a constant weight at 100°C. Then the residue was ignited to get ash and recorded its weight again. The loss in weight due to ignition is equal to crude fiber. So,

\[
\text{Crude fiber} = \text{Weight of residue} - \text{Weight of ash}
\]

This was expressed as a percentage of original samples taken for ether extraction.

**p) Chlorophyll content**

200 g fresh fruits were homogenized with 15 ml of 80% acetone and centrifuged at 4000 rpm for 20 min. Supernatant was collected and volume was made to 25 ml with 80% acetone. Absorbance (A) of the sample was measured at 645 nm and 663 nm.

\[
\begin{align*}
\text{Chlorophyll a (mg/g fr. Wt.)} & = 12.7 \times A_{663} - 2.69 \times A_{645} \times 1000 \times \text{weight of sample} \\
\text{Chlorophyll a (mg/g fr. Wt.)} & = 22.9 \times A_{645} - 4.68 \times A_{663} \times 1000 \times \text{weight of sample} \\
\text{Total chlorophyll (mg/g fr. Wt)} & = 20.2 \times A_{645} + 8.02 \times A_{663} \times 1000 \times \text{weight of sample}
\end{align*}
\]

The above method was given by Hiscox and Israelstam (1979).

**3.4 Economic analysis**

\[
\text{Value of produce} = \text{Yield (kg)} \times \text{Price (Rs kg}^{-1}\text{)}
\]

**a) Net returns (NR)**

\[
\text{NR} = \text{Value of produce} - \text{Cost of input}
\]

**b) Benefit : Cost ratio (B:C ratio)**

\[
\text{B: C ratio} = \frac{\text{Value of produce}}{\text{Value of produce}}
\]
Cost of input

**Price of vermicompost, fertilizer, green pea and okra fruit**

1) Vermicompost Rs kg\(^{-1}\) : 5
2) Lime Rs kg\(^{-1}\) : 10
3) Fertilizer price Rs kg\(^{-1}\)
   - N : 11.6
   - P\(_2\)O\(_5\) : 22.8
   - K\(_2\)O : 8.6
4) Green pea price Rs kg\(^{-1}\) : 15
5) Okra fruit price Rs kg\(^{-1}\) : 10

**3.5 Statistical analysis**

The data generated from field and laboratory analysis was subjected to statistical analysis using the technique of analysis of variance for randomized block design for the interpretation of results as described by Gomez and Gomez (1984).