CHAPTER ~ 3

MATERIALS AND METHODS

The materials used and the methods followed for conducting the trials have been described in this chapter. The research on the topic entitled "Response of Zinc to Maize Based Cropping System in alfisols of Odisha" was carried out for two consecutive cropping years (2013-14 and 2014-15).

3.1 Delineation of available micronutrients (Zn, Cu, Fe, Mn and B) and S status in soils

For the delineation of available micronutrients (Zn, Cu, Fe, Mn and B) and S status in soils, 154 nos. of soil samples were collected from three blocks viz. Umerkote, Raighar and Jharigaon of Nabarangpur district of Odisha with the help of global positioning system (GPS) and recorded the longitude and latitude of the area. Thematic soil fertility maps were prepared by using GPS and Geographical Information System (GIS).

3.2 Vertical distribution of available Zn in soil profiles of alluvial, black, red and Laterite soil

To study the vertical distribution of available Zn in soil profiles, soil samples were collected from different depth (0-15, 15-30, 30-45 and 45-60cm) of Red, Alluvial, Black and Laterite soils of Orissa. Soil samples were air dried under shade and sieved through 2 mm sieve and put in plastic container for analysis.

3.3 Adsorption characteristics of Zn in alluvial, black, red and Laterite soils

An adsorption study was also made by taking different concentration of Zn varying from 2 to 64 µg/ml as ZnSO₄.7H₂O were added to soil (0.5g) in 50ml polypropylene centrifuged tube with three replication for each then shake for 48 hrs at room temperature. The content was centrifuged and supernatant solution was analyzed for Zn by Atomic Absorption Spectrophotometer. Then amount of Zinc Adsorbed by soil was calculated from the difference of initial and final Zn concentration after
equilibration. The Adsorption isotherm was plotted according to Langmuir Adsorption Equation.

\[ C/x/m+(1/Kb)+(C/b) \]

\( C \) = equilibrium Zn concentration in ppm, \( x/m \) = the amount of Zn adsorption \( \mu/g \) of soil, \( b \) = adsorption maximum and \( K \) = a constant related to bonding energy of the soil. The value of adsorption maxima ‘b’ and bonding Energy 'K' was calculated from the slope and intercept.

### 3.4 Pot Experiment

**Efficacy of Zinc on growth and yield of Maize**

Pot experiment on the efficacy of Zn on maize was conducted in earthen pots of capacity of 5kg soil in Kharif during the second week of month June 2013 and the treatment details are mentioned in Table 1.1

### 3.5 Field Experimental Site

**Response of Zn on Maize based cropping system**

The field experiment on maize was conducted in the farmer's field during Kharif season of 2013 and 2014 (in the last week of June) and Green gram was grown as residual crop during rabi season of 2013 and 2014 (in the third week of December) in the Village- Chingudiguda, Block: Jharigaon, District: Nabarangpur, one of the adopted village of Krishi Vigyan Kendra, Nabarangpur. The experimental site is situated at 19.6864° N latitude and 82.2751° E longitude and it comes under Eastern Ghat high land Zone. The experimental plan is given in the fig.1 There were nine treatment combinations in a randomized block design with row to row and plant to plant spacing of 60cm and 20cm, respectively with plot size (4m x 4m) and Maize crop var. (Bio 9681) was grown followed by Greengram as residual crop.

### 3.6 Climate of Experimental Site

The location is characterized by dry and wet type of climate. The hottest month is April-May and coldest month is December-January.
3.7 Weather Condition during the period of the Experiment.

The weather data pertaining to the cropping seasons of 2013-14 and 2014-15 recorded at meteorological observatory, Regional Research and Technology Transfer Station, Umerkote, Nabarangpur are presented year wise separately in Table 1.

Table 1. Meteorological data of Umerkote, Nabarangpur from June 2013 to February 2014 and June 2014 to February 2015

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature</th>
<th>RH (%)</th>
<th>Rainfall (mm)</th>
<th>No of Rainy Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum</td>
<td>Minimum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013-14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June</td>
<td>36.3</td>
<td>22.5</td>
<td>76.8</td>
<td>163.5</td>
</tr>
<tr>
<td>July</td>
<td>29.5</td>
<td>22.6</td>
<td>88.5</td>
<td>299.5</td>
</tr>
<tr>
<td>August</td>
<td>29.9</td>
<td>23.5</td>
<td>84.3</td>
<td>293.6</td>
</tr>
<tr>
<td>September</td>
<td>33.9</td>
<td>22.7</td>
<td>86.4</td>
<td>155.8</td>
</tr>
<tr>
<td>October</td>
<td>31.9</td>
<td>19.5</td>
<td>87.8</td>
<td>58.5</td>
</tr>
<tr>
<td>November</td>
<td>29.3</td>
<td>16.8</td>
<td>86.2</td>
<td>38.8</td>
</tr>
<tr>
<td>December</td>
<td>28.4</td>
<td>13.4</td>
<td>73.6</td>
<td>0.0</td>
</tr>
<tr>
<td>January</td>
<td>29.6</td>
<td>15.6</td>
<td>71.9</td>
<td>0.0</td>
</tr>
<tr>
<td>February</td>
<td>35.5</td>
<td>18.7</td>
<td>75.4</td>
<td>0.0</td>
</tr>
<tr>
<td>2014-15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June</td>
<td>36.7</td>
<td>23.1</td>
<td>75.2</td>
<td>171.5</td>
</tr>
<tr>
<td>July</td>
<td>30.2</td>
<td>22.7</td>
<td>87.5</td>
<td>399.5</td>
</tr>
<tr>
<td>August</td>
<td>29.8</td>
<td>24.1</td>
<td>84.4</td>
<td>297.6</td>
</tr>
<tr>
<td>September</td>
<td>33.9</td>
<td>22.9</td>
<td>85.4</td>
<td>157.8</td>
</tr>
<tr>
<td>October</td>
<td>31.8</td>
<td>19.7</td>
<td>85.8</td>
<td>64.5</td>
</tr>
<tr>
<td>November</td>
<td>30.1</td>
<td>16.9</td>
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<tr>
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<td>February</td>
<td>35.8</td>
<td>18.9</td>
<td>75.1</td>
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</tr>
</tbody>
</table>
3.8 Experimental Details

The experiment was conducted in a randomized block design having four replications in both the years. Nine different treatments were randomly allotted to the plots following random number table (Gomez and Gomez, 1976). There were nine
treatment combinations consisting of zinc, lime, organic matter and nano zinc. Treatment combinations are presented as follows in table 1.1

**Table 1.1 Treatment details (field experiment)**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Treatment details</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>NPK alone</td>
</tr>
<tr>
<td>T2</td>
<td>NPK + 2.5kgZn/ha</td>
</tr>
<tr>
<td>T3</td>
<td>NPK + 2.5kgZn/ha + lime10% of LR</td>
</tr>
<tr>
<td>T4</td>
<td>NPK + 5.0 kgZn/ha</td>
</tr>
<tr>
<td>T5</td>
<td>NPK + 2.5kgZn/ha + 3 spray of ZnSO₄ @ 0.5%</td>
</tr>
<tr>
<td>T6</td>
<td>NPK + Organic Matter @ 5t/ha</td>
</tr>
<tr>
<td>T7</td>
<td>NPK + Organic Matter + 2.5kgZn/ha</td>
</tr>
<tr>
<td>T8</td>
<td>NPK + Organic Matter + 2.5kgZn/ha + 3 spray of ZnSO₄ @ 0.5%</td>
</tr>
<tr>
<td>T9</td>
<td>NPK + Organic Matter + Nano Zn @ 0.3% spray before flowering</td>
</tr>
</tbody>
</table>

**REPLICATION**

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**REPLICATION**

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**REPLICATION**

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**FIELD IRRIGATION CHANNEL**

<table>
<thead>
<tr>
<th>BUND 0.05M</th>
<th>4m*4m</th>
<th>1M</th>
<th>4m*4m</th>
<th>1M</th>
<th>4m*4m</th>
<th>1M</th>
<th>4m*4m</th>
<th>1M</th>
<th>4m*4m</th>
<th>1M</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUND 0.05M</td>
<td>T1</td>
<td>T9</td>
<td>BUND 0.05M</td>
<td>T7</td>
<td>BUND 0.05M</td>
<td>T5</td>
<td>BUND 0.05M</td>
<td>T3</td>
<td>BUND 0.05M</td>
<td>T1</td>
</tr>
<tr>
<td>BUND 0.05M</td>
<td>T2</td>
<td>T6</td>
<td>BUND 0.05M</td>
<td>T5</td>
<td>BUND 0.05M</td>
<td>T8</td>
<td>BUND 0.05M</td>
<td>T2</td>
<td>BUND 0.05M</td>
<td>T7</td>
</tr>
<tr>
<td>BUND 0.05M</td>
<td>T3</td>
<td>T7</td>
<td>BUND 0.05M</td>
<td>T8</td>
<td>BUND 0.05M</td>
<td>T6</td>
<td>BUND 0.05M</td>
<td>T1</td>
<td>BUND 0.05M</td>
<td>T4</td>
</tr>
<tr>
<td>BUND 0.05M</td>
<td>T4</td>
<td>T8</td>
<td>BUND 0.05M</td>
<td>T3</td>
<td>BUND 0.05M</td>
<td>T2</td>
<td>BUND 0.05M</td>
<td>T9</td>
<td>BUND 0.05M</td>
<td>T9</td>
</tr>
<tr>
<td>BUND 0.05M</td>
<td>T5</td>
<td>T2</td>
<td>BUND 0.05M</td>
<td>T4</td>
<td>BUND 0.05M</td>
<td>T7</td>
<td>BUND 0.05M</td>
<td>T1</td>
<td>BUND 0.05M</td>
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<td>T5</td>
<td>BUND 0.05M</td>
<td>T1</td>
<td>BUND 0.05M</td>
<td>T6</td>
<td>BUND 0.05M</td>
<td>T9</td>
<td>BUND 0.05M</td>
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<tr>
<td>BUND 0.05M</td>
<td>T7</td>
<td>T4</td>
<td>BUND 0.05M</td>
<td>T9</td>
<td>BUND 0.05M</td>
<td>T3</td>
<td>BUND 0.05M</td>
<td>T8</td>
<td>BUND 0.05M</td>
<td>T8</td>
</tr>
</tbody>
</table>

**Fig.3: Plan of layout for experiments during 2013-14 and 2014-15**
Fig.4: Plan of layout for experiments during 2013-14 and 2014-15

1. Design of layout  Randomized Block Design
2. Number of treatments  Nine (9)
3. Number of replication  Four (4)
4. Plot size  4 m x 4m
5. Total number of plots  36
6. Spacing
   a) Row to row  60cm
   b) Plant to plant  20 cm
7. Crop (Variety)  Maize (Bio 9681)
8. RDF (Recommended dose of NPK fertilizer)  NPK (150-80-80)
9. Seed rate(kg/ha)  Green gram
10. Residual crop in rabi  (var,Tram-1)

3.9 Methods of Application

Nano-zinc was procured from CIFA and Farm yard manure @ 5 t/ha were incorporated in plots as per different treatments details.

Fertilizers

The recommended dose of N, P$_2$O$_5$ and K$_2$O @ NPK (150-80-80) kg/ha were applied through urea, single superphosphate and muriate of potash, respectively. The nitrogen was applied @20%, full dose of phosphorus and potassium were applied as basal preferably drilling of fertilizers in bands along the seed using seed-cum-fertilizer drills. The balanced quantity of nitrogen was applied in four splits for higher productivity. The Zinc as a source of ZnSO$_4$, lime, organic matter and nano zinc were applied as per treatment details and foliar spray of zinc through ZnSO$_4$ and nano-zinc respectively.

Seeds were sown in the field at a spacing of (60cm x 20 cm) after treating the seed with Bavistin @2g/kg of seed. As weeds are serious problem in maize, so pre-emergence application of Atrazine @ of 1.0-1.5 kg a.i/ha in 600 litres of water were
applied to control the weeds. At critical stages the irrigation were ensured though it was cultivated during monsoon season.

Foliar spray of 0.1% Endosulfan 10 days after germination of seeds was applied to control the stalk borer attack in maize. In subsequent stages, Fipronil granules @ 20 kg/ha were applied to control the Termite. Irrigation was given as when required.

3.10 Biometric Observations

3.10.1 Sampling technique

The plants (10 numbers) were selected in each plot to record the biometric observations time to time on different growth parameters like cob yield including other yield attributing characters.

3.10.2 Growth parameters

Observation on the following characters were recorded on selected plants of each plot for both the trials carried out in 2013-14 and 2014-15.

3.10.3 Cob diameter (cm)

The cob diameter (cm) was measured and expressed in centimeter for all the sample plants of each individual treatment.

3.10.4 Total cob yield (q/ha)

Measured as weight of total quantity of cob per plants for each treatment in the replication and then calculated and expressed in q/ha.

3.10.5 Dry matter content of plant (q/ha)

The plant of ten sampled plants from each plots were chopped separately and composite of 100g was taken from each plot. Samples were air dried and then oven dried at 60°C till a constant weight was obtained. The weight of the dried materials was recorded and expressed in percentage.

The dry matter production per hectare was calculated by using the following formula.

Dry matter production of plant per hectare (q)  

\[ = Plant \ yield \ per \ hectare \ (q) \times Dry \ matter \ percentage \]
3.10.6 Method of collection of plant and Cob Sample:

a) **Plant sample**

The plant samples were collected at the harvesting stage and washed thoroughly with deionised water. The samples were air dried and finally dried in the oven at 60°C temperature till a constant weight was recorded. The oven dried plant samples were ground manually and kept for analysis.

b) **Cob sample**

The cob samples were collected at the harvesting stage and then washed thoroughly with deionized water. The cob samples were analyzed freshly. The results expressed on oven dry basis.

3.11 Methods of Analysis:

**A. Soil:**

3.11. A1 Soil pH

The pH of the soil was determined in 1:2.5 of soil and water suspensions using a glass electrode digital pH meter (Jackson, 1973).

3.11. A2 Organic carbon

The organic carbon content of soil was determined by wet digestion method as outlined by Walkley and Black (1934).

3.11. A3 Available nitrogen (N)

Available nitrogen content of soil was estimated by alkaline KMnO4 method as outlined by Subbiah and Asija (1956) using Kelplus nitrogen auto analyzer. (Kelplus:Model classic DX)
3.11. A4 Available phosphorous (P)

Available phosphorous in the soil was determined by Brays I method (Bray and Kurtz, 1945) as outlined by Page et al., (1982).

3.11. A5 Available potassium (K)

Available potassium was determined by shaking 5g soil in 25ml neutral normal ammonium acetate for five minutes and the available potassium in extractant was determined by flame photometre (Muhr et al., 1965).

3.11. A6 Available boron (B)

The available boron in soil was extracted by hot water reflux method and determined spectrophotomerically using azomethrin-H (Page et al., 1982).

3.11. A7 Available Sulphur(S)

Soluble Sulphate was determined in 0.15% CaCl₂ extract of Soil (Tabatabai, 1982). Sulphate in the extract was determined turbidmetrically (Hoeft et al, 1973)

3.11. A8 Available micronutrient (Zinc, Iron, manganese and Copper)

The available zinc (Zn), iron (Fe), manganese (Mn) and Copper (Cu) in soil were determined by using DTPA (Diethylene Triamine Penta Acetic Acid) extraction method as described by Lindsay and Norvel (1978). Twenty gram soil with 40ml of DTPA was shaken for 2 hours in an environmental shaker. Then filtered through Whatman Filter Paper 42. The aliquot was estimated by Atomic Absorption Spectrophotometer.

3.11. B. Plant:

Total N, P, K and Zn of plant samples (leaves, cob and root) were estimated by using standard procedures. Nitrogen was estimated by distillation in Kelplus N analyzer as suggested by Tandon (1998). The total P was analyzed by vanadomolybdophosphoric acid yellow colour method as described by Jackson (1973). Total potassium was
estimated by using Flame Photometer after diacid digestion (Jackson, 1973). The total zinc content in plant parts were estimated by Atomic Absorption Spectrophotometer.

3.12. Uptake of nutrients

The uptake of Zn was calculated by multiplying the dry matter yields of various treatments with their respective concentration of nutrients. The nutrient uptake was expressed in g/ha.

3.13 Statistical Analysis

The field experiment data generated were arranged in appropriate tables according to the treatments and were subjected to statistical analysis as per analysis of variance technique applicable for randomized block design (Gomez and Gomez, 1976). The treatments variations were tested for significance of F test. The standard error of mean (SEM) and critical difference (CD) at 5% probability were calculated.