WORK PLAN AND METHODOLOGY:

For the proposed research work the investigator has framed work into different parts.

1) **ISOLATION OF ORGANISM (Aspergillusniger):**

   There will be wide screening schedule for the isolation of potent strains in this work soil properties are considered as major functional aspect. With this regard entire Nandurbar district is divided in equal block in squares and that geographical zone is divided in to 54 squares. The further consideration is with collection of rhizosphere soil of forest trees. With this regard dominant tree species belongs to Nandurbar forests are considered for rhizosphere soil collection. The above approach is new approach and never discussed in any research communication, people have collected rhizosphere soil samples, but the way of collection is not scientific which represents somehow the proper representation of soil. With this respect one study the soil parameters in randomized block design. There will be variation in different soils throughout the district. In addition to natural soil types soil from representative agricultural fields, waste water saturated zones of sugar factory, textile mill is also considered for collection.

2) **SCREENING ISOLATED CULTURES FOR CITRIC ACID PRODUCTION:**

   To study the *Aspergillusniger* strain producing organic acid from carbon substrates can be detected by incorporation of pH indicators dye bromocresol green in Czepeck’sDox agar /Potato Dextrose agar medium and growing fungal strain on it. A color change of medium from blue to yellow in the vicinity of colony indicates organic acid production.

3) **DESIGNING OF MEDIA FOR FERMENTATION OF CITRIC ACID PRODUCTION:**

   Growth and production of microorganism are strongly affected by the medium composition such as concentrations of carbon, nitrogen, phosphorous, potassium, trace elements and stimulators. Thus, citric acid productivity by *A. niger* can be improved by optimizing the medium composition.

4) A) **EFFECT OF MEDIA COMPONENTS:**

   To study the effect of media components such as percentage of carbon sources, nitrogen, phosphate magnesium source and effect pH, methanol and initial pH with H₃PO₄ in growth medium on citric acid fermentation all the components, except one to be studied, are kept constant with respect to control medium and one component concentration is changed in
particular range. These broths were then tested for citric acid production by selected strains of *A. niger*.

**B) EFFECT OF PHYSICAL PARAMETERS IN GROWTH MEDIUM:**

To study the effect of physical parameters such as Effect of shaking + electric current, without electric current, Fermentation temperature, Moisture content on fermentation and UV treatment on strain.

**5) ESTIMATION OF CITRIC ACID PRODUCTION:**

The fermentation broth was filtered through filter paper in order to remove mycelia. The filtrate was analyzed for total citric acid content. Total acid was determined by titration with 0.1429 mol/l NaOH using phenolphthalein as indicator.

**SIGNIFICANCE OF STUDY:**

Citric acid has a wide range of applications. The fields of citric acid application include Beverages, food and candy industry, Pharmaceutical industry, cosmetic industry, soap industry and textile industry etc. The food industry consumes about 70% of total citric acid produced, while other industries consumer remaining 30% (Khadijah-Al-Khadir et al, 2011). It is accepted worldwide as GRAS (generally recognized as safe), approved by the Joint FAO/WHO Expert Committee on Food Additives (Carlos R. Soccol et al., 2006). Due to this pleasant acid taste and high water solubility, the most extensive application is in beverages, Jams and jellies as well as in sweets, but there are numerous additional uses in this field. Almost every carbonated soft drink contains citric acid because it gives cool taste and maintains carbonation. In frozen foods, citric acid neutralizes residual lye and protects ascorbic acid from oxidation. It also inactivates oxidative enzymes by lowering the pH to prevent changes in color and flavor.