CONCLUSION:

The investigation made under this work can be summarized as follows:

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 type’s soil from representative agricultural fields, waste water saturated zones considered for collection. The present study is based on the isolation of rhizosphere soil for collection of high potential strain of Aspergillus niger. Common trees found in selected equal 54 blocks of entire Nandurbar district. For this propose there is selected 54 villages and 23 trees species found commonly in that region. Fifty four Aspergillus niger strains were isolated from Nandurbar district among the 54 different strains isolated from different soil types only three strain were found to be competent for citric acid production. These strains selected by following method:

First screening: Done in 54 rhizosphere soil sample. It gives higher potential power of strain of A.niger for citric acid production, in this manner 5 strain are selected from 54 rhizosphere soil sample strain of A. niger which are collected from soil sample of village Bhagdari, Khuntamovli, Leghapani, Morakhi and Valmba.

2nd screening: The prepared medium of PDA of 10 ml are filled in petri plate in laminar air flow and at room temperature allowed to cool small quantity of the conidial spot five Aspergillus niger was given aseptically or controlled environment to each of these petri-plates. The petri-plates incubated for 12 hoursat 30\(^0\)C. Yellow zones due to citric acid formation were formed. On the basis of larger citric acid zone compared with control, the best strains of A. niger were picked and transferred for culture to the PAD slants this strains are isolated from soil sample of
Leghapani, Bhagdari and Valmba village from trees of *Bauhinia variegata* L., *Madhuka longifolia* (Koem.) and *Tectona grandis* L. respectively.

The cultures were incubated for 4-6 days at 30\(^0\)C until maximum sporulation. Three *A. niger* isolates were selected from five strains of *A. niger* for further submerged fermentation.

**Abbreviation for these three strains:**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Place</th>
<th>Name of trees</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leghapani</td>
<td><em>Bauhinia variegata</em> L</td>
<td><em>Asperillus</em> niger LBV10</td>
</tr>
<tr>
<td>2</td>
<td>Bhagdari</td>
<td><em>Madhuka longifolia</em> (Koem.)</td>
<td><em>Asperillus</em></td>
</tr>
</tbody>
</table>
In the study of selected carbon sources which are flower of *Madhuka longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba* (200 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH$_2$PO$_4$ (100 mg), NaNO$_3$ (400 mg), MgSO$_4$.7H$_2$O (20 mg), and adjust 4.5 initial pH with HCL and 0.1 NaOH. The flask was autoclaved after flask with cotton plugged at 121$^0$C for 15 minutes. Per medium inoculated by 1.0 ml (6.0 X 106) of *A. niger* (selected strains *Asperillus niger* LBV10, *Asperillus niger* BML6 and *Asperillus niger* VTG18) conidial suspension of *A. niger* after cooling at room temperature and incubated in static incubator for 8 days at 28$^0$ C. Later the fermentation, addition of distilled water (1:4 W/V) for the dilution of medium. The filtrates was used for citric acid detection after medium were filtered. In this study were found that the *A. niger* –B gives the highest yield of citric acid that is 8.3 grams in given condition, medium and incubation time.

Study were done with selected carbon sources which are flower of *Madhuka longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba* (100, 150, 200 and 250 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH$_2$PO$_4$ (100 mg), NaNO$_3$ (400 mg), MgSO$_4$.7H$_2$O (20 mg), and adjust 4.5 initial pH with HCL and 0.1 NaOH. The flask was autoclaved after flask with cotton plugged at 121$^0$C for 15 minutes. Per medium inoculated by 1.0 ml (6.0 X 106) of *A. niger* (selected strains *Asperillus niger* LBV10, *Asperillus niger* BML6 and *Asperillus niger* VTG18) conidial suspension of *A. niger* after cooling at room temperature and incubated in static incubator for 8 days at 28$^0$ C. Later the fermentation, addition of distilled water (1:4 W/V) for the dilution of medium. The filtrates was used for citric acid detection after medium were filtered. In this study there were select four Carbons source which are flower of *Madhuka longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and
flower of *Bombax ceaba*. And there was found that 200 g flower of *Madhuka longifolia* is best for citric acid production in three strains of *A. niger* which are *Asperillus niger* LBV10, *Asperillus niger* BML6 and *Asperillus niger* VTG18, *Asperillus niger* BML6 is best for citric acid production.

Studies were done with selected carbon sources which are flower of *Madhuka longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba* (200 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH$_2$PO$_4$ (100 mg), NaNO$_3$ (300,350,400 and 450 mg), MgSO$_4$.7H$_2$O (20 mg), and adjust 4.5 initial pH with HCL and 0.1 NaOH. The flask was autoclaved after flask with cotton plugged at 121$^0$C for 15 minutes. Per medium inoculated by 1.0 ml (6.0 X 106) of *A. niger* (selected strains *Asperillus niger* LBV10, *Asperillus niger* BML6 and *Asperillus niger* VTG18) conidial suspension of *A. niger* after cooling at room temperature and incubated in static incubator for 8 days at 28$^0$C. Later the fermentation, addition of distilled water (1:4 W/V) for the dilution of medium. The filtrates was used for citric acid detection after medium were filtered. In this study were found that the *A. niger* –B gives the highest yield of citric acid that is 7.92 grams in given condition, medium and incubation time.

Study was done with selected carbon sources which are flower of *Madhukalongifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba* (200 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH$_2$PO$_4$ (50,75,100 and 125 mg), NaNO$_3$ (MgSO$_4$.7H$_2$O (20 mg), and adjust 4.5 initial pH with HCL and 0.1 NaOH. The flask was autoclaved after flask with cotton plugged at 121$^0$C for 15 minutes. Per medium inoculated by 1.0 ml (6.0 X 106) of *A. niger* (selected strains *Asperillus niger* LBV10, *Asperillus niger* BML6 and *Asperillus niger* VTG18) conidial suspension of *A. niger* after cooling at room temperature and incubated in static incubator for 8 days at 28$^0$C. Later the fermentation, addition of distilled water (1:4 W/V) for the dilution of medium. The filtrates was used for citric acid detection after medium were filtered. In this study were found that the *A. niger* –B gives the highest yield of citric acid that is 8.11 grams in given condition, medium and incubation time.
Study was done with selected carbon sources which are flower of Madhukalongifolia, flower of Bauhinia variegate, pithy pulp of fruit of Adansonia digitata and flower of Bombax ceaba (200 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH$_2$PO$_4$ (100 mg), NaNO$_3$ (MgSO$_4$.7H$_2$O (15, 20, 25, and 30 mg), and adjust 4.5 initial pH with HCL and 0.1 NaOH. The flask was autoclaved after flask with cotton plugged at 121$^0$C for 15 minutes. Per medium inoculated by 1.0 ml (6.0 X 106) of A. niger (selected strains Asperillus niger LBV10, Asperillus niger BML6 and Asperillus niger VTG18) conidial suspension of A. niger after cooling at room temperature and incubated in static incubator for 8 days at 28$^0$ C. Later the fermentation, addition of distilled water (1:4 W/V) for the dilution of medium. The filtrates was used for citric acid detection after medium were filtered. In this study were found that the A. niger –B gives the highest yield of citric acid that is 7.92 grams in given condition, medium and incubation time.

Study was done with selected carbon sources which are flower of Madhukalongifolia, flower of Bauhinia variegate, pithy pulp of fruit of Adansonia digitata and flower of Bombax ceaba: on citric acid production (200 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH$_2$PO$_4$ (100 mg), NaNO$_3$ (MgSO$_4$.7H$_2$O (20mg), and adjust different initial pH (3.5; 4.0; 4.5 and 5.0) with Hydrochloric acid and 0.1 NaOH. The flask was autoclaved after flask with cotton plugged at 121$^0$C for 15 minutes. Per medium inoculated by 1.0 ml (6.0 X 106) of A. niger (selected strains Asperillus niger LBV10, Asperillus niger BML6 and Asperillus niger VTG18) conidial suspension of A. niger after cooling at room temperature and incubated in static incubator for 8 days at 28$^0$ C. Later the fermentation, addition of distilled water (1:4 W/V) for the dilution of medium. The filtrates was used for citric acid detection after medium were filtered. In this study were found that the A. niger –B gives the highest yield of citric acid that is 7.92 grams in given condition, medium and incubation time.

Study were done with selected carbon source which is flower of flower of Madhukalongifolia, flower of Bauhinia variegate, pithy pulp of fruit of Adansonia digitata and flower of Bombax ceaba (200 g). Were cut in small spices and crushed in mixture machine with 50 ml
distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH₂PO₄ (100 mg), NaNO₃ (MgSO₄.7H₂O (20mg), different percentage of methanol (1.0, 1.5. 2.0, and 2.5) before autoclave and adjust initial pH (4.5) with HCL and 0.1 NaOH. The flask was autoclaved after flask with cotton plugged at 121°C for 15 minutes. Per medium inoculated by 1.0 ml (6.0 X 10⁶) of A. niger (selected strains Asperillus niger LBV10, Asperillus niger BML6 and Asperillus niger VTG18) conidial suspension of A. niger after cooling at room temperature and incubated in static incubator for 8 days at 28°C. Later the fermentation, addition of distilled water (1:4 W/V) for the dilution of medium. The filtrates was used for citric acid detection after medium were filtered. In this study were found that the A. niger –B gives the highest yield of citric acid that is 7.77 grams in given condition, medium and incubation time.

Study were done with selected carbon sources which are flower of Madhuka longifolia, flower of Bauhinia variegata, pithy pulp of fruit of Adansonia digitata and flower of Bombax ceaba (200 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH₂PO₄ (100 mg), NaNO₃ (MgSO₄.7H₂O (20mg), different percentage of methanol (1.0, 1.5. 2.0, and 2.5) before inoculation and adjust initial pH (4.5) with HCL and 0.1 NaOH. The flask was autoclaved after flask with cotton plugged at 121°C for 15 minutes. Per medium inoculated by 1.0 ml (6.0 X 10⁶) of A. niger (selected strains Asperillus niger LBV10, Asperillus niger BML6 and Asperillus niger VTG18) conidial suspension of A. niger after cooling at room temperature and incubated in static incubator for 8 days at 28°C. Later the fermentation, addition of distilled water (1:4 W/V) for the dilution of medium. The filtrates was used for citric acid detection after medium were filtered. In this study were found that the A. niger –B gives the highest yield of citric acid that is 6.29 grams in given condition, medium and incubation time.

Study were done with selected carbon sources which are flower of Madhuka longifolia, flower of Bauhinia variegata, pithy pulp of fruit of Adansonia digitata and flower of Bombax ceaba (200 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH₂PO₄ (100 mg), NaNO₃ (MgSO₄.7H₂O
(20mg), and adjust different initial pH (3.5, 4.0, 4.5, and 5.0) with H$_3$PO$_4$ and 0.1 NaOH. The flask was autoclaved after flask with cotton plugged at 121$^0$C for 15 minutes. Per medium inoculated by 1.0 ml (6.0 X 106) of A. niger (selected strains Asperillus niger LBV10, Asperillus niger BML6 and Asperillus niger VTG18) conidial suspension of A. niger after cooling at room temperature and incubated in static incubator for 8 days at 28$^0$ C. Later the fermentation, addition of distilled water (1:4 W/V) for the dilution of medium. The filtrates was used for citric acid detection after medium were filtered. In this study were found that the A. niger –B gives the highest yield of citric acid that is 5.74 grams in given condition, medium and incubation time.

Studies were done with selected carbon sources which are flower of Madhukalongifolia, flower of Bauhinia variegate, pithy pulp of fruit of Adansonia digitata and flower of Bombax ceaba (200 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks. After add KH$_2$PO$_4$ (100 mg), NaNO$_3$ (MgSO$_4$.7H$_2$O (20mg), and adjust different initial pH (4.5) with HCL and 0.1 NaOH and medium treated with electric current (500mA for 10 min). The flask was autoclaved after flask with cotton plugged at 121$^0$C for 15 minutes. Per medium inoculated by 1.0 ml (6.0 X 106) of A. niger (selected strains Asperillus niger LBV10, Asperillus niger BML6 and Asperillus niger VTG18) conidial suspension of A. niger after cooling at room temperature and incubated in static incubator for 8 days at 28$^0$ C. Later the fermentation, addition of distilled water (1:4 W/V) for the dilution of medium. The filtrates was used for citric acid detection after medium were filtered. In this study were found that the A. niger –B gives the highest yield of citric acid that is 6.79 grams in given condition, medium and incubation time.

Study were done with selected carbon sources which are flower of Madhuka longifolia, flower of Bauhinia variegate, pithy pulp of fruit of Adansonia digitata and flower of Bombax ceaba (200 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks. After add KH$_2$PO$_4$ (100 mg), NaNO$_3$ (MgSO$_4$.7H$_2$O (20mg), and adjust different initial pH (4.5) with HCL and 0.1 NaOH and medium treated with different minute electric current (500mA for 10 min). The flask was autoclaved after flask with cotton plugged at 121$^0$C for 15 minutes. Per medium inoculated by 1.0 ml (6.0 X 106) of A.
niger (selected strains Asperillus niger LBV10, Asperillus niger BML6 and Asperillus niger VTG18) conidial suspension of A. niger after cooling at room temperature and incubated in static incubator for 8 days at 28°C. Later the fermentation, addition of distilled water (1:4 W/V) for the dilution of medium. The filtrates was used for citric acid detection after medium were filtered. In this study were found that the A. niger –B gives the highest yield of citric acid that is 6.79 grams in given condition, medium and incubation time.

Study were done with selected carbon sources which are flower of Madhuka longifolia, flower of Bauhinia variegata, pithy pulp of fruit of Adansonia digitata and flower of Bombax ceaba (200 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH₂PO₄ (100 mg), NaNO₃ (MgSO₄·7H₂O (20mg), and adjust different initial pH (4.5) with HCL and 0.1 NaOH and medium treated with different minute electric current (500mA for 10 min). The The flasks were cotton plugged and sterilized in autoclaved for 15 minutes at 121°C minutes. After medium were cooled at room temperature. Each medium inoculated with 10 minute UV traded 1.0 ml (6.0 X 10⁶) of A. niger (selected strains Asperillus niger LBV10, Asperillus niger BML6 and Asperillus niger VTG18) conidial suspension of A. niger and incubated in static incubator for 8 days at 28°C. Later the fermentation, addition of distilled water (1:4 W/V) for the dilution of medium. The filtrates was used for citric acid detection after medium were filtered. In this study were found that the A. niger –B gives the highest yield of citric acid that is 5.94 grams in given condition, medium and incubation time.

Study were done with selected carbon sources which are flower of Madhuka longifolia, flower of Bauhinia variegata, pithy pulp of fruit of Adansonia digitata and flower of Bombax ceaba (200 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH₂PO₄ (100 mg), NaNO₃ (MgSO₄·7H₂O (20mg), Sucrose (5 g) and adjust 4.5 initial pH with HCL and 0.1 NaOH. The flask was autoclaved after flask with cotton plugged at 121°C for 15 minutes. Per medium inoculated by 1.0 ml (6.0 X 10⁶) of A. niger (selected strains Asperillus niger LBV10, Asperillus niger BML6 and Asperillus niger VTG18) conidial suspension of A. niger after
cooling at room temperature and incubated in static incubator for 8 days at 28°C. Later the fermentation, addition of distilled water (1:4 W/V) for the dilution of medium. The filtrates was used for citric acid detection after medium were filtered. In this study were found that the A. niger –B gives the highest yield of citric acid that is 8.13 grams in given condition, medium and incubation time.