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Production of citric acid from different *Aspergillus* species obtained from soil

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Abstract

The genus *Aspergillus* is considered highly important in the production of various types of enzymes and organic acids. *Aspergillus* species produce organic acids such as citric acid, itaconic acid, and malic acid, which are one of the most important alternate techniques for chemical processes. Citric acid is an essential primary agricultural product that is extensively used in the world. It acts as an intermediate in the tricarboxylic acid cycle (citric acid cycle) during the oxidation of carbohydrates to carbon dioxide. Citric acid has an important role in industries involving the production of food products, beverages, pharmaceutical and agricultural products, detergents, and cosmetics. The production of citric acid, a crucial agricultural commodity with widespread applications in various industries including food, beverages, pharmaceuticals, agriculture, detergents, and cosmetics, heavily relies on microbial fermentation. The great demand for citric acid due to its wide industrial applications and less toxicity. Citric acid can be produced using less expensive substrates that are renewable too. In this work, *Aspergillus* species was isolated from the soil source, collected in different localities of Chhatrapati Sambhajinagar. The total 90 different isolates are isolated among these the 20 different *Aspergillus* species are isolated and pure culture by using potato dextrose agar (PDA) Czapek dox agar medium and study the effect of different media on growth and sporulation of different *Aspergillus* species and the quantitative analysis of citric acid production was done by titration method with help of NaOH and phenolphthalein indicator. The main reason for the study increases is the large number of applications that can be found for citric acid, primarily in the food and pharmaceutical industries

Keywords: Soil; *Aspergillus;* Citric Acid; PDA

1. Introduction

Citric acid is an organic acid that is generally found in a variety of fruits such as limes, lemons, oranges, pineapples, and grapefruits. It is a natural ingredient that aids in detoxification, maintaining energy Levels, and supporting healthy digestion and kidney function. It has a slightly tart and refreshing flavors and is employed for balancing the Sweetness in soft drinks, juices, and other beverages. Citric acid used in food and beverage industry due to its antioxidant properties to preserve the food or as an acidifier enhances the Flavors and aroma of fruit juices, ice cream, and marmalades. In the pharmaceutical Industry, it is used as an antioxidant to preserve vitamins, effervescent, pH corrector, blood preservative, iron citrate tablets as a source of iron for the body, ointments and cosmetic preparations, and so forth [1]. In the chemical industry, for softening and treatment of textiles, it is used as a foaming agent. In metallurgy, certain metals are utilized in the form of citrate. Because of less eutrophic effect, Citric acid is used in the detergent industry as a phosphate substitute [1]. Citric acid is frequently incorporated in facial packs and masks as it naturally brightens and lightens the skin tone, minimizes break-Outs and oiliness, and regenerates the dead skin cells. Currently, the global Citric acid market is projected to reach USD 3.2 billion by 2023 and is expected to witness a Compound Annual Growth Rate (CAGR) of 5.1 % during the forecast period. The global production of Citric acid is estimated to be around 736,000 tones/year, and the entire production is carried out by fermentation. In Brazil, almost

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the entire demand of Citric acid is met through imports. Due to its numerous applications, the volume of Citric acid production by fermentation is continually increasing at a high annual rate of 5% [2, 3] and also witnessing steadily increasing demand/consumption. It is accepted worldwide as a GRAS (Generally Recognized as Safe) as approved by the Joint. The Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Com-Mitte on Food Additives [4]. The Driving factor behind the growth of the global Citric acid market is the expanding application in various industries. Development of Citric acid production increased greatly since the last century due to biotechnology, which provides proper knowledge of fermentation techniques and product recovery; biochemistry, which provides knowledge of different factor That affects synthesis and blockage of Citric acid production; molecular regulatory mechanisms; and strategies that enhance Citric acid production. In the Past 60 years, extensive reviews of literature along with more than thousands of reports have been published in connection to Citric acid production [1, 5, 6, 7]. However, the enhancement of Citric acid production with the recent advancement of the Last few years has not been updated.

Citric acid is an essential organic acid naturally presents in various citrus fruits. The citric acid has been first isolated and crystallized by Swedish chemist Carl Wilhelm Scheele. From this incident, the importance of citric acid has been understood and has utilized by various industries like food, cosmetics and pharmaceutical. More than 70% of the citric Acid is used in the food and fermentation stabilizers, flavors enhancers and chelators. Less than 10% is used in cosmetic and pharmaceutical industries. The citric acid is being produced by Microorganisms during fermentation Through their metabolic citric acid cycle [8]. Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid) is the most important commercial product, which is found in almost all plant and animal tissues. It exists widely in the nature and present as a kind of fruit acid in lemon, orange, pine apple, plum, peas, peach and in animal bones, muscles and blood. It has many applications in food, pharmaceutical and cosmetic industries as an acidulant, flavors enhancer, preservative, antioxidant, emulsifier and chelating agent. In recent years, citric acid has been commercially produced by fungal fermentation mainly by *Aspergillus niger*. Citric acid is one of the most common products which have a never-ending demand in the global market. It plays a pivotal role in food and beverage industries and pharmaceutical, chemical cosmetic, and other industries for applications such as acidulation, antioxidant, flavors, enhancement, preservation, and plasticization and as a synergistic agent. Citric acid fermentation is one of the primitive fermentations but still its production is increasing with passage of time. In 2007, its global production has exceeded 1.6 million tons. One of the most important fungi used in industrial microbiology, *Aspergillus niger*, has been employed for many years for the commercial production of citric acid. However, the worldwide demand for citric acid is increasing faster than its production and more economical processes are required. *Aspergillus niger* is most commonly used for citric acid production. This is because of the fact that this organism has capacity to utilize varieties of substrates due to its well-developed enzymatic system. Although *Aspergillus niger* is the traditional producer of citric acid, during the last 30 years the use of yeasts for citric acid fermentation processes has attracted the interest of researchers. Among the yeast species, *Yarrowia lipolytica* is known as a potential producer of citric acid. Citric acids can be produced by well-developed methods in using chemicals. however, microbial fermentations are been used for long time for industrial scale citric acid production. The citric acids can also be used in remediation aspects. Citric acids are used as chelating agents to remove heavy metals in soils [9]. Fungal organic acids usually consisting One or more carboxylic groups which is Responsible for the chelating properties. The citric acids can effectively remove Zinc, chromium and nickel from the soil [10, 11]. One of the most important achievements in the field of Industrial Microbiology is the production of citric acid by fermentation. Today Mycological citric acid is a huge industry in several parts of the world In United States, Europe, Russia, England and Japan. An institute devoted to the development and improvement of this process exists In Russia. Most of the citric acid produced in the United States is fermentation citric acid. While no estimates of the total consumption of citric acid in our country or of the world are available yet it is certain that it must be enormous in amount because of its varied uses in medicinal products, for foods (as flavoring extracts, soft drinks etc.) in candies, inks, silvering, dyeing, calico printing, enlarging etc. The production of citric acid has engaged the attention of a large Number of investigators because of its apparent enormous commercial Potentialities. In spite of all this there is probably less basic established Fundamental knowledge regarding it than in most other industrial Processes [12]. Citric acid formation is probably one of the most widely distributed Metabolic processes known in fungi. But one species of the genus, *Aspergillus niger* van *Tieghem*, is well known as the organism most Suitable for its production. Considering all above literature, it was clear that fungi have potential production citric acid, secondary metabolites, enzymes and different chemicals there for emphasis.

2. Material and methods

2.1. Area of study

This study was conducted at the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar. All experiments were accomplished aseptically in the Microbial culture laboratory.

2.2. Collection of soil sample

The Soil sample was collected from different localities of Chhatrapati Sambhajinagar region. The soil was collected in depth of 15 cm in a sterilized paper bag. Each paper bag was labelled properly by indicating the site of collection, Date, time and then sample were taken to the laboratory for further analysis.

2.3. Isolation of *Aspergillus* Fungi

For studying the *Aspergillus* fungi present in soil the serial dilution plate method developed by [13] used for the isolation of *Aspergillus* fungi from different region in Chhatrapati Sambhajinagar. For the isolation of *Aspergillus* fungi serial dilution factor 10^{-3} used. At the time of serial dilution labeled the dilution blank as 10^{-1}, 10^{-2} and 10^{-3} marked with a marker pen. 1gm of air-dry soil sample from a composite sample was added in 10ml sterilized distilled water and a dilution test tube was labeled 10^{-1}. Thus, dilute the original sample 10 times (1:10) and shake thoroughly for 10 minutes to obtain a uniform distribution and release of microorganisms from adhering soil particles. From the first dilution 1ml of suspension was added in 9ml sterilized distilled water to dilute test tube number 10^{-2} by sterile pipette. The dilution number second was shaken for 5 minutes. The same procedure was repeated till the original sample has been diluted 10^{-3} times, every time a fresh sterile pipette was used. Finally, from the dilution number 10^{-3} of suspension was transferred with the help of a sterile pipette to sterilized petri plates containing Potato Dextrose Agar (PDA), Czapek Dox Agar (CZA), Rose Bengal Agar (RBA) Medium. The 1ml of wilted crop soil sample suspension was added in three sterile poured petri plates. The inoculated petri plates were incubated in inverted position at room temperature at 30˚C for 3 to 4 days.

2.4. Selection of Media for Isolation

During investigation usually Potato Dextrose Agar (PDA), Czapek dox agar (CZA) and Rose Bengal Agar (RBA) medium was used for the isolation and maintenance of pure cultures. The constituents of the medium are as follows

2.4.1. Potato Dextrose Agar (PDA) [14]

Peeled Potatoes - 200g, Dextrose - 20g, Agar – Agar - 20g, streptomycin - 0.2 g, Distilled Water - 1000 ml, pH – 5.6

2.4.2. Czapek dox agar (CZA) [15]

Sucrose - 30g, Sodium Nitrate ($NaNO_3$) - 2.0g, Potassium Dihydrogen Phosphate (KH_2PO_4) - 1.0g, Magnesium Sulphate Heptahydrate ($MgSO_4$.7 H2O) - 0.5g, Potassium Chloride (KC)l -0.5g, Ferrous Sulphate ($FeSO_4$) - 0.01g, Agar-Agar - 20g, Distilled water - 1000 ml, pH - 5.6.

2.4.3. Rose Bengal Medium [16]

Dextrose – 10.0g, Peptone – 5.0g, Monopotassium Phosphate (KH_2PO_4) – 1.0 g, Magnesium sulphate ($MgSO_4$) - 0.5g, Agar – Agar 15.0g, Streptomycin – 0.2g, Rose Bengal – 0.05g, Distilled Water - 1000 ml, pH – 7.2.

2.5. Microscopic Identification *Aspergillus* species

After the incubation period the growth of *Aspergillus* on the petri plates. The Macroscopic observation was done by external feature, texture, colony color, growth rate and microscopic characteristics are arrangement of conidia. Macroscopic and Microscopic features of *Aspergillus* fungi are helpful in the accurate identification of *Aspergillus* fungi. The culture characteristics were observed and the growth was examined microscopically to confirm its purity using lactophenol cotton blue stain technique [17]. The identification of *Aspergillus* Fungi was done by using the various research paper, monographs and other literature such as Manual of soil fungi [18], Handbook of soil Fungi [19], The Illustration of Fungi [20].

2.6. Growth pattern and Morphological Characteristics of *Aspergillus* species

In order to study growth pattern and morphological characteristics of different *Aspergillus species* which are isolated from different soil sample, were grown on solid Potato Dextrose Agar (PDA) Media. These isolates where incubated room temperature at 30˚C for Nine days on Potato Dextrose Agar (PDA) Media and the result were in the form of colony color, colony pattern and colony diameter.

2.7. Composition of Potato Dextrose Agar (PDA) Media [14]

Potato – 200g, Dextrose – 20g, Agar-Agar – 20g, Distilled Water (D/W) – 1000ml, p^H – 5.5 to 5.6

2.8. Growth pattern of *Aspergillus* species on Liquid Media

In order to compared the growth pattern and sporulation of different *Aspergillus* species isolates which were isolated from different soil sample. The flask was incubated room temperature at 30˚C for Nine days on Glucose Nitrate (GN) Liquid Media and the result were in the form of Mycelial Color, Liquid Media Colour, Mycelial Dry Weight and Sporulation.

2.9. Composition of Glucose Nitrate (GN) Liquid Media

Glucose – 10g, Potassium Nitrate (KNO_3) – 2.5g, Potassium Dihydrogen Phosphate (KH_2PO_4) -1.0g, Magnesium Sulphate Heptahydrate (MgSO4.7H2O) -0.5g And Distilled Water (D/W) – 1000ml.

2.10. Culturing *Aspergillus* Species

Prepare the citric acid medium broth & dispense about 50ml in 250ml conical flask, then Autoclave at 121ºC for 15 Minute and allow it cool at 40 to 45ºC and inoculate the spores of *Aspergillus* species on conical flask & incubate it on a shaker water bath at 25^0C with gentle shaking for 3-5 days. After Incubation, filter the mycelium using Whatman filter & measure the amount of citric acid in the filtrate by titrimetric methods.

2.11. Composition of Citric Acid Production Medium [21]

Sucrose - 15.0 g, Ammonium nitrate (NH_4NO_3) - 2.5g, Potassium Dihydrogen Orthophosphate (KH_2PO_4) - 1.0 g, Magnesium sulphate heptahydrate ($MgSO_4$) - 0.25g, Distilled water -1000ml, pH - 3.5.

2.12. Estimation of Citric acid using phenolphthalein as indicator

The 100ml of the culture filtrate is pipetted into a conical flask and 2-3 drops of phenolphthalein indicator is added to it. This is titrated against 0.1N NaOH taken in the burette till a pale pink colour is formed. The titration is repeated till concordant values are obtained. The volume of alkali used for neutralization is used to find the normality and the percentage of acid in the sample.

2.13. Calculation of percentage of Citric acid

Formula

Normality of Citric acid = [N(NaOH)×V(NaOH)] / V (Citric acid)

% of Citric acid = [Normality Equivalent weight of citric acid 100] / Volume of filtrate

(N-Normality, V – Volume, Equivalent weight of Citric acid – 64)

3. Results and discussion

The present investigation of Production of Citric Acid from Different *Aspergillus* species Obtained from Soil. During this investigation the soil mycoflora analysis from different localities like Daulatabad, Khultabad, Sillod, Kannad, and Paithan, it was clear from the table no.01. The fungi show diversity the total 90 fungal species isolate among these the 30 different fungal species were isolated from the all localities. The highest occurrence of Fungal species from the Daulatabad locality these are 22 isolates among these 19 different Fungi were isolated. Total 18 fungi were isolated in Paithan locality. Similarly, the 17 different fungal species were isolated in Kannad locality and 13 fungal species are isolated in Khultabad locality. The lowest occurrence of fungal species in Sillod locality these are 12 different fungal species.

Table 1 Isolation of fungi from different localities

Sr. No	Name of Fungi	Daulatabad	Khultabad	Sillod	Paithan	Kannad
1	*Aspergillus japonicus*	++	+	-	++	+
2	*Trichoderma viride*	-	+	-	-	+
3	*Aspergillus foetidus*	+	-	-	+	-
4	*Alternaria alternata*	-	+	-	+	+
5	*Aspergillus carbonarium*	+	-	++	-	-
6	*Aspergillus brasiliensis*	+	-	-	+	+
7	*Fusarium oxysporum*	+	++	-	+	-
8	*Aspergillus aureus*	-	-	+	+	+
9	*Aspergillus carbonarius*	+	-	-	+	+
10	*Penicillium chrysogenum*	-	+	+	-	-
11	*Aspergillus tubingenesis*	+	-	-	-	+
12	*Aspergillus niger-1*	-	-	+	+	-
13	*Cladosporium cladosporioides*	+	-	+	-	-
14	*Aspergillus glaucus*	++	+	-	-	++
15	*Colletotrichum capsica*	-	-	-	+	+
16	*Aspergillus oryzae*	+	++	-	-	-
17	*Aspergillus ellipticus*	+	-	+	+	+
18	*Aspergillus species*	+	-	+	-	-
19	*Rhizopus stolanifer*	+	-	-	+	+
20	*Aspergillus fischeri*	+	-	+	+	-
21	*Aspergillus paraciticus*	-	+	++	-	++
22	*Penicillium notatum*	+	+	-	-	+
23	*Aspergillus wentii*	-	+	-	+	-
24	*Aspergillus aculeatus*	++	-	-	+	+
25	*Aspergillus niger 2*	-	-	+	+	-
26	*Penicillium glabrum*	-	+	-	-	+
27	*Aspergillus phoenicus*	+	-	-	+	-
28	*Alternaria brassicae*	+	-	+	+	+
29	*Aspergillus fumigatus*	+	++	+	+	-
30	*Aspergillus ustus*	-	+	-	-	+

Table 2 Effect of Growth and Sporulation of Different *Aspergillus* Species on Glucose Nitrate Broth (GNB)

Sr.no	Name of Fungi	Sporulation	Mycelial colour	Culture filtrate Colour	Mycelial dry weight
1	*Aspergillus japoniccus*.	+	Blackish	Yellowish	0.04g
2	*Aspergillus foedidus*	++	Whitish black	Yellowish	0.09g
3	*Aspergillus carbonarium*	++++	Blackish	Yellowish	0.13g
4	*Aspergillus brasiliensis*	++	Dark blackish	Yellowish	0.07g
5	*Aspergillus aureus*	++	Blackish	Yellowish	0.09g
6	*Aspergillus carbonarius*	++++	Blackish	Yellowish	0.12g
7	*Aspergillus tubingenesis*	+++	Dark Blackish	Yellowish	0.11g
8	*Aspergillus niger*-1	++	Blackish	Yellowish	0.05g
9	*Aspergillus glaucus*	++	Brown	Dark brown	0.06g
10	*Aspergillus oryzae*	++	Whitish black	Off white	0.09g
11	*Aspergillus ellipticus*	++++	Blackish	Off white	0.12g
12	*Aspergillus species*	+++	Dark Blackish	Pale yellow	0.11g
13	*Aspergillus fischeri*	++	Blackish	Pale yellow	0.05g
14	*Aspergillus paraciticus*	+	Greenish	Brownish	0.09g
15	*Aspergillus wentii*	++	Brownish	Dark yellowish	0.02g
16	*Aspergillus aculeatus*	+	Blackish	Off white	0.08g
17	*Aspergillus niger-2*	++	Blackish white	Pale yellowish	0.04g
18	*Aspergillus phoenicus*	+	Whitish brown	Off white	0.08g
19	*Aspergillus fumigatus*	+++	Whitish green	Off white	0.13g
20	*Aspergillus ustus*	++	Creamish white	Dark brown	0.11g

During the study of soil fungi, the total 30 fungi were isolated from different localities of Chhatrapati Sambhajinagar. Among the 30 isolates 20 different *Aspergillus* fungal species were selected for the further study (PHOTO PLATE NO. 01 and 02). The 20 different *Aspergillus* spp. Were Study different character like as sporulation, Mycelia colour, Culture filtrate colour and Dry weight on Glucose Nitrate (GN) broth. The selected 20 different *Aspergillus* species are the *Aspergillus carbonarium, A. ellipticus, A. ustus, A. carbonarius, A. tubingenesis, A. species and A. fumigatus* show highest growth, sporulation and show the different variation in mycelia colour, culture filtrate colour and dry weight. It was clear from the table no. 02. (PHOTO PLATE NO.03).

Table 3 Effect of Growth and Sporulation of Different *Aspergillus* Species on Citric Acid Medium (CAM)

Sr. No	Name of fungi	Sporulation	Colony Colour	C.F colour	Mycelial dry Weight
1	*Aspergillus japoniccus*.	+++	Brownish black	Creamish white	0.19g
2	*Aspergillus foedidus*	++	Greenish black	Whitish yellow	0.14g
3	*Aspergillus carbonarium*	+++	Blackish	Pale yellow	1.53g
4	*Aspergillus brasiliensis*	+++	Greenish black	Yellowish	0.62g
5	*Aspergillus aureus*	++	Greenish	Whitish yellow	0.16g
6	*Aspergillus carbonarius*	++	Whitish black	Pale yellow	0.40g
7	*Aspergillus tubingenesis*	+++	Dark black	Creamish white	0.48g
8	*Aspergillus niger*-1	+++	Greenish	Pale yellow	1.35g
9	*Aspergillus glaucus*	+++	Greenish	Pale yellow	0.49g
10	*Aspergillus oryzae*	+++	Brownish Black	Pale yellow	0.20g
11	*Aspergillus ellipticus*	++	Greenish Black	Pale yellow	0.19g
12	*Aspergillus species*	+++	Dark Black	Whitish yellow	0.28g
13	*Aspergillus fischeri*	+++	Whitish Black	Creamish yellow	0.27g
14	*Aspergillus paraciticus*	++	Greenish	Yellowish	0.16g
15	*Aspergillus wentii*	+++	Yellowish	Dark yellow	0.16g
16	*Aspergillus aculeatus*	++	Whitish	Creamish white	0.40g
17	*Aspergillus niger-2*	+++	Dark Black	Pale yellow	0.52g
18	*Aspergillus phoenicus*	++	Yellowish	Yellowish	0.24g
19	*Aspergillus fumigatus*	+++	Brownish Black	Creamish white	0.31g
20	*Aspergillus ustus*	++	Whitish Black	Yellow	0.28g

During the screening of citric acid production. Selected 20 *Aspergillus* species shows difference in growth, sporulation, mycelia colour and mycelia dry weight on Citric acid production medium (PHOTO PLATE NO. 04). Maximum *Aspergillus species* shows high sporulation but dry weight *Aspergillus carbonarium* shows highest mycelial dry weight (1.53gm) after that *Aspergillus niger*-1 shows (1.35gm) dry weight the lowest mycelial weight was seen *Aspergillus foetidus* (0.14gm).

Table 4 Quantitative Analysis of Citric Acid Production by Different *Aspergillus* Species

Sr. No	Name of Fungi	Initial Burette reading	Final Burette Reading(ml)	Vol of NaOH used(ml)	Percentage of citric acid (%)
1	*Aspergillus japoniccus*.	0	4.5	4.5	28.08
2	*Aspergillus foedidus*	0	11.4	11.4	72.96
3	*Aspergillus carbonarium*	0	6.7	6.7	42.88
4	*Aspergillus brasiliensis*	0	7.4	7.4	47.36
5	*Aspergillus aureus*	0	2.8	2.8	17.92
6	*Aspergillus carbonarius*	0	4.5	4.5	28.08
7	*Aspergillus tubingenesis*	0	3.5	3.5	22.04

8	*Aspergillus niger-1*	0	10.5	10.5	67.02
9	*Aspergillus glaucus*	0	3.2	3.2	20.48
10	*Aspergillus oryzae*	0	3.7	3.7	23.68
11	*Aspergillus ellipticus*	0	3.0	3.0	19.02
12	*Aspergillus species*	0	12.5	12.5	80.00
13	*Aspergillus fischeri*	0	3.8	3.8	24.32
14	*Aspergillus paraciticus*	0	3.4	3.4	21.76
15	*Aspergillus wentii*	0	4.5	4.5	28.08
16	*Aspergillus aculeatus*	0	4.8	4.8	30.72
17	*Aspergillus niger-2*	0	11.2	11.2	71.68
18	*Aspergillus phoenicus*	0	7.6	7.6	48.64
19	*Aspergillus fumigatus*	0	10.2	10.2	65.28
20	*Aspergillus ustus*	0	5.5	5.5	35.02

The quantitative analysis of citric acid production was done by titration method with help of NaOH and phenolphthalein indicator (PHOTO PLATE NO. 05). The screening of citric acid production by different *Aspergillus* species these are *Aspergillus Japonicus, Aspergillus foetidus, Aspergillus carbonarium, Aspergillus brasiliensis, Aspergillus aureus, Aspergillus carbonarius, Aspergillus tubingenesis Aspergillus niger-1, Aspergillus glaucus, Aspergillus oryzae, Aspergillus ellipticus, Aspergillus* species, *Aspergillus fischeri, Aspergillus paraciticus, Aspergillus wentii, Aspergillus aculeatus, Aspergillus niger-2, Aspergillus phoenicus, Aspergillus fumigatus and Aspergillus ustus* were taken for screening of citric acid production it was clear for table no. 04. The quantitative analysis of citric acid the *Aspergillus* species have shown highest production (80%) quantitatively. Next to that *Aspergillus foetidus* shows the maximum citric acid production 72.96%. The *Aspergillus niger* have a produces 71.68% citric acid quantitatively. Among the 20 species of *Aspergillus* the lowest citric acid production was by the *Aspergillus ellipticus* (19.02%). The *Aspergillus glaucus* Produced 20.47% and *Aspergillus paraciticus* Produced citric acid 21.76%. It was clear from table no. 04.

Figure 5 Titration of citric acid production

4. Conclusion

The present investigation more fungal diversity is seen in the soil. *Aspergillus* genera was dominant in soil. During this study Among 5 different localities of Chhatrapati Sambhajinagar like Khultabad, Daulatabad, Sillod, Paithan, and Kannad. The highest occurrence of Fungal species from the Daulatabad locality these are 22 isolates. The total 20 different *Aspergillus* species were screened for citric Acid Production and Media are important role in growth and sporulation of different *Aspergillus* species. Citric acid production can be easily done by using microorganism that has

the ability to produce citric acid efficiency such as *Aspergillus.* The result of this study indicates that the use of *Aspergillus* fungi to production of citric acid might represent an efficient method of cost reduction in the production and concomitantly producing organic acid of valuable importance. Citric acid production now reaches 1.4 million tons per year and continues to increase every year. The main reason for the study increases is the large number of applications that can be found for citric acid, primarily in the food and pharmaceutical industries. Significant optimization of all citric acid processes can be seen with genetic improvement of producing strains, which is a powerful tool of the citric acid market today.

Compliance with ethical standards

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Disclosure of conflict of interest

This statement is to certify that all authors have seen and approved the manuscript being submitted. We warrant that the article is the Authors' original work. We warrant that the article has not received prior publication and is not under consideration for publication elsewhere. On behalf of all Co-Authors, the corresponding Author shall bear full responsibility for the submission. All authors agree that author list is correct in its content and order and that no that the Editor-in -Chief's decisions over acceptance or rejection or in the event of any breach of the Principles of Ethical Publishing in the World Journal of Advanced Research and Reviews being discovered of retraction are final.

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