S.R.R GOVERNMETN ARTS & SCIENCE COLLEGE

KARIMNAGAR

DEPARTMENT OF MICROBIOLOGY

BEE FREE HONEY PRODUCTION USING ASPERGILLUS NIGER

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S.R.R. GOVT ARTS & SCIENCE COLLEGE (A), KARIMNAGAR

JIGNASA STUDENT RESEARCH STUDY PROJECT

SUBJECT: MICROBIOLOGY

BEE FREE HONEY PRODUCTION USING ASPERGILLUS NIGER

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Title

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STATEMENT OF PROBLEM OR HYPOTHESIS:

The demand for honey is ever increasing. To meet the demand, adulteration of honey is at its peak. An attempt is made to prepare bee free honey with microbial enzymes. It is aimed to increase the fructose and reduce the glucose from sucrose hydrolysate. *Aspergillus Niger* was isolated producing invertase and glucose oxidase. Microbial fermentation conditions are optimized for maximum production of invertase and glucose oxidase. Immobilized *Aspergillus niger* and enzymes are used for conversion of sucrose syrup to honey, at 40°C. The prepared honey is characterized by 42.6% fructose and 23.1% glucose and further concentrated to 80 brix with boiling.

Objectives:

- Isolation of *Aspergillus niger*.
- Bee free honey produced with help of immobilized Organisms.
- To reduce glucose from sucrose and fructose.

INTRODUCTION:

Honey is the natural sweet product of *Apis melifera* bees obtained from plant nectars. Honey bees collect pollen grains and nectar from various flowering plants, and convert them into wax and honey. Due to its sweet taste, Nutritive value and medicinal qualities of honey, it has high demand. Raw honey has been used as folk remedy throughout history and has variety of medical uses like it acts as Antibacterial, Antifungal, Antioxidant. Invertase and glucose oxidase are secreted by the hypopharyngeal glands of honeybees for the hydrolysis of sucrose and the preservation from microbial effect.

Deforestation is in the momentum to meet the requirements of increased population and subsequently bee populations are depleted. Decreased bees honey production and increased usage has yielded huge demand. To meet the demand, honey is being adulterated with sugary syrups. In the present study microbial enzymes invertase, glucose oxidase was used for honey preparation. Invertase catalyze the hydrolysis of α-1,4-glycosidic bonds of sucrose and release equimolar mixtures of monosaccharides D- glucose and D- fructose called invert sugar. Glucose oxidase an enzyme which oxidizes glucose to gluconic acid. This enzyme is generally present in all microorganisms. Different strains of microorganisms are used for the production of invertase. Mostly used microorganisms for production of invertase are Aspergillus niger, Saccharomyces cerevisiae, Candida utilis. As both the enzymes, Invertase and glucose oxidase are present in Aspergillus niger, we used A. niger in the production of invertase and glucose oxidase for bee free honey preparation. Invertase converts sucrose to glucose and fructose whereas, glucose oxidase converts glucose to gluconic acid.

Gluconic acid has been designed as GRAS and it is a part of Gluconic acid imparts refreshing sour taste.

Materials and Methodology used:

Materials required: Potato dextrose agar, sucrose, sugarcane bagasse, test tubes, flasks.

Methodology:

Isolation of fungi (Aspergillus niger): potato dextrose agar was prepared by mixing 500g potato infusion, 20g sucrose and 20g agar in one liter of distilled water. Petri plates and plugged flasks were autoclaved at 15lbs, 121°C for 15 minutes. After cooling, the media was poured into the plates. A small section of sugarcane bagasse (having a black fungal portion) was placed on the potato dextrose agar and incubated at 30°C for 48 hours.

BEE FREE HONEY PREPARATION:

Bee free honey is prepared by using two methods they are:

1) **Immobilized Aspergillus niger:** The immobilized *Aspergillus niger* is prepared by taking 2.5% of sodium alginate in water, to that 2% *Aspergillus niger* spore suspension is added and mixed well, add 0.5% of glycerol to it. Drop it in ice cold 2M cold $CaCl_2$. Bee free microbial honey is prepared by taking 1litre of 20% sucrose syrup, to that add 150g of *Aspergillus niger* beads, and incubate it at 40°C for 1 hour.

CHARACTERIZATION OF BEE FREE HONEY:

1. DENSITY TEST:

A) Blot test: Honey is allowed to flow on blot paper. If honey flows through the blot paper without wetting, then it is regarded as pure. If blotting paper gets wet it is said to be impure or adulterated honey.

B) Water drop test: A spoon of honey is dipped into a glass of water, if the honey is dissolved in water, then it is said to be adulterated. If the honey settles at the bottom of glass, then it is said to be pure honey.

C) Heat test: Honey is heated in this process. When honey is heated, if it quickly broils then it is considered as pure honey. If bubbles appear then it is said to be adulterated honey.

D) Vinegar and water test: In this test a spoon full of honey is mixed with two spoons of vinegar and little amount of water, if foam appears then it is considered as adulterated honey. If foam does not appear then it is pure honey.

2. SELIWANOFF'S TEST:

Take 1ml of honey in a test tube and add 2ml of seliwanoff's reagent (Dissolve 5ml resorcinol in 33 ml concentrated HCl and make it to 100 ml with water)and heat it.

3. ACIDITY TEST:

The total acidity was determined by the titrimetric method as follows: The addition of 0.05 M NaOH was stopped at pH 8.50 (free acidity), immediately a volume of 10 mL 0.05 M NaOH was added, without delay, back-titrated with 0.05 HCl to pH 8.30 (lactonic acidity). Results were expressed in meq NaOH kg.

RESULTS:

1. Isolation of Aspergillus niger:

Aspergillus niger was isolated from sugarcane bagasse and cultivated on PDA. The growth of organism is seen the following are the colony and microscopic characteristics.

1) Colony characteristics: Aspergillus niger grown on Potato dextrose agar (PDA) showing colony diameter 55- 68mm, cottony appearance, white colour during early stages, later acquired black colour due to spore growth.
2) Microscopic characteristics: The microscopic observation of Aspergillus niger – Aspergillus with conidia and conidiophores are observed.

2. **Bee free honey preparation:**

Using two methods, honey was prepared. The appearance, texture, colour, taste,and nutritional value of the honey also resembled the natural one.

Characterization:

Density tests:

1. **Blot test:** In the blot test, bee free honey went on the paper without wetting it as wild honey, which indicates its purity.
2. **Water drop test:** The bee free honey had a thick texture which settled at the bottom of a glass as wild honey, displayed its purity
3. **Heat test:** When the bee free honey was heated, it quickly caramelized as wild honey, hence the honey is pure.
4. **Vinegar and water test:** When vinegar and water was added to honey, foam didn't appear as wild honey, which represents the pure nature of bee free honey.
5. **Seliwanoff's test:**

   This test is used to verify the presence of ketose in honey. On addition of seliwanoff's reagent and heating, the mixture turned into red colour which indicates the presence of ketose and indicates the presence of fructose.
6. **Acidity:** The values of total acidity present in different honey samples was 29meq/kg (pure honey/control), 28 meq/kg (bee free honey). The sample

was in the safe range, which represents the non-appearance of undesirable fermentation.

CONCLUSION:

Hence, we conclude that honey can also be prepared by immobilizes Aspergillus niger showing similar characteristics of natural honey. It is less expensive, less time consuming, No adulteration, less labor cost.

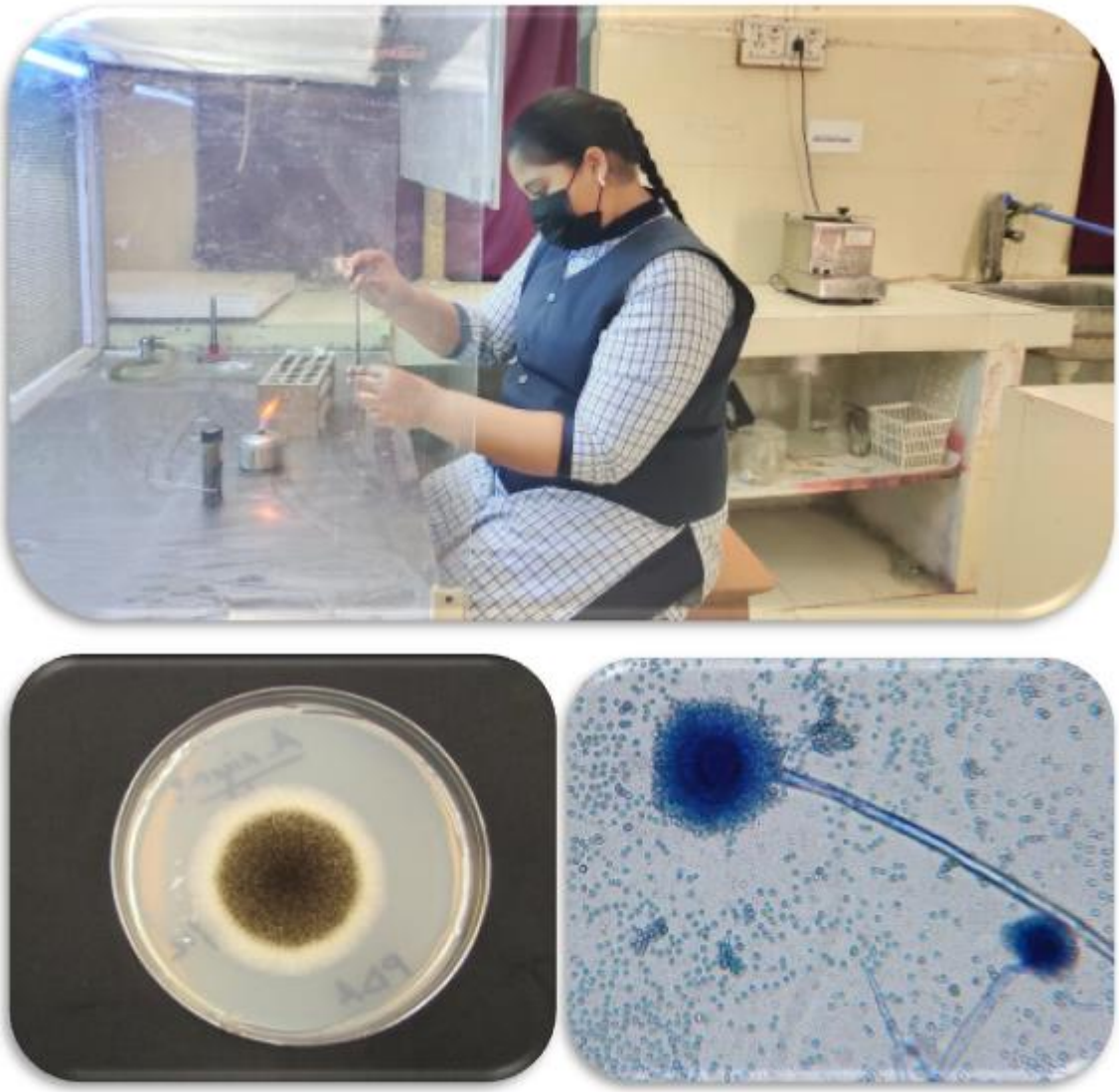

Figure 1: Isolation of *Aspergillus niger*

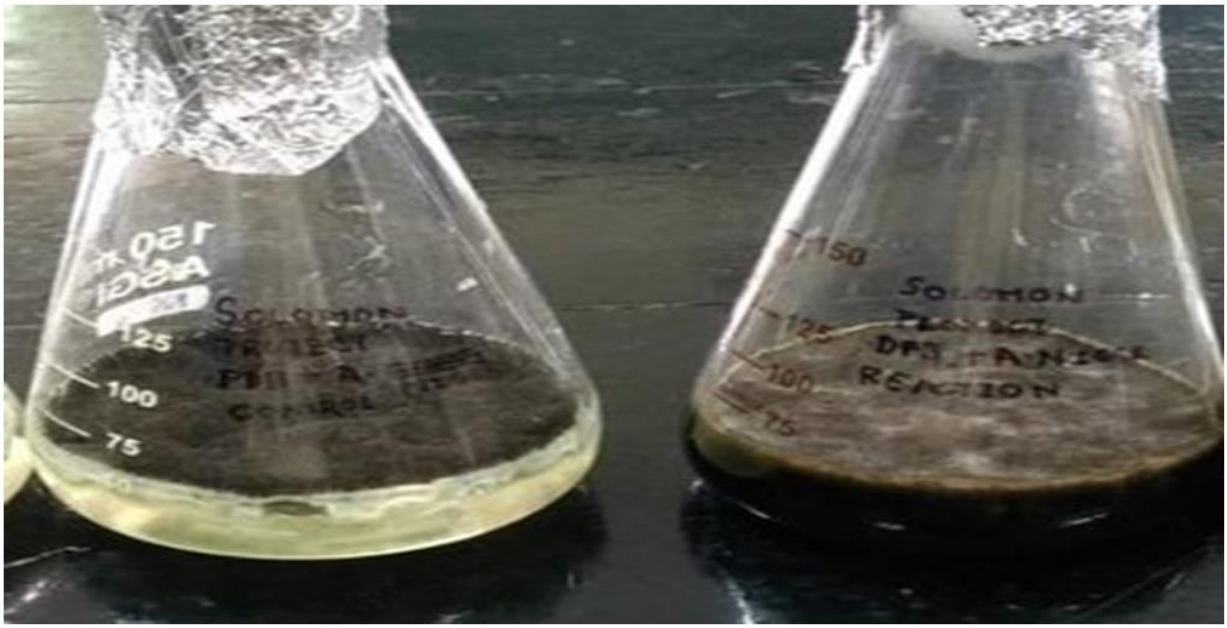

Figure 2: Submerged fermentation of *Aspergillus niger*

Figure 3: Preparation and Immobilization of *Aspergillus niger*

Figure 4: Testing of Honey prepared from *Aspergillus niger*

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