Production:-

1. Production Process:-

- Details of production.
- Standard production process (step by step operations)

Slant preparation

(Purified slant and plates.)

Mother culture preparation.

(Preparation flasks with respective to individual organisms.)

Sterilization of mother culture.

(Preparation above broth and distribute equally into 3 parts and sterilize at 121°C at 50 psi for 20 minutes.)

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Inoculation of mother culture.

(Once media is cooled dpown after sterilization inoculation can be done with respective to its own individual organism in laminar air flow.)

Incubation operation.

(5-6 days of incubation are required flasks are kept on shaker (bacterial) or without shaker (fungi) with 28-30°C temperature.)

1

Media sterilization

(100 lit.media has to be sterilized and allowed it to cool down in tanks.)

1

Mother culture inoculation in fermenter or tanks.

(Fully grown mother culture can be added in fermenter or into tanks in equal proportion, allow it to incubate for 5-6 days.)

Quality control of sample.

(Microscopic and total viable count has to be checked for sample before filling.)

Formulation addition

(Formulation has to be added to the tanks 24 hrs. before filling. formulation is evenly mixed in tanks.)

Filling and Curing

(After incubation mat layer on top of media to be crushed separately aseptically and later can be added to tanks and even mixture is created of all organisms in individual tank. Once all addition is done and inner vent caps are placed. Allow

bottles to withstand for curing for 24 hrs.)

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Labelling and packing

(After 24 hrs. of curing bottles can be packed completely with labels on it. Material is then for dispatch.)

PROCEDURE OF MEDIA PREPRATION:

1. Weigh all the ingredients as per the media composition.

2. Take these ingredients in conical flask.

3. Then add hot water.

Then shake it for dissolving all ingredients.

5. Then check its pH and adjust as per requirement.

6. Then autoclave it @ 1 5lbs for 15 mm.

Name of products:-

1. Azophospho.

2. Rhizophospho.

3. Acetobacter.

Decomposing Culture.

5. Micronutrients.

6. EM Solution.

7. Potash mobilizing bacteria.

8. Ferro- Zinco.

9. Humic Acid

10. VAM

11. Trichoderma.

12. Beauveria

Phosphorous Solublizing Bacteria
Metarhizium.

Papu Co

15. Verticilium

16. Sulpho.

17. Silico.

18. Pseudo.

These products are prepared by unit but they gave only 4 products information in detail. These are.....

1.ACETOBACTER:

Acetobacter is also called as acetic acid bacteria. It has the ability to

convert ethanol to acetic acid in the presence of oxygen. It fixes nitrogen non-

symbiotically. It is mostly used in sugarcane crop. It also secretes the useful growth promoting hormones such as Indole Acetic Acid andGibberlin. It is saccharophilic bacteria.

Beneficial properties They are used in the production of vinegar.

 They are used to Internationally acidified beer during long maturation period.

2. It increases the nitrogen up take efficiency of sugarcane.

It is eco-friendly product.

- It fixes the nitrogen in sugarcane crop is 30kg N/ha. It increases crop yield 5-25%.
- 5. It increases the sugar content of the sugarcane.

Composition of media : LGIP Media.

- K2HPO4. 0.6 g.
- K2HPO4. 0.2 g.
- CaCl2 0.02 g.
- FeCl3 0.01 g.
- MgSO47H2O. 0.2 g.
- Na2M0O4 0.002 g.
- BTB 0.5%.
- Cane sugar 100 ml.
- Agar Agar- 1.8 g.
- Distilled water 1000 ml.

Source Of Isolation:-

Sugarcane samples (Roots, leaf, stem & bud)

Procedure of isolation:-

- Take 1 gram of sugarcane sample (root/leaf/stem/bud) are to be washed thoroughly in the running tap water.
- Surface sterilization with 70%ethanol and subsequently washed in changes of sterilized distilled water.
- Surface sterilized samples are to be macerated in sterile blended and serial dilution are prepared up to 10 dilution.
- >1 ml of 10 dilutions is to be inoculated into various enrichment media viz. diluted cane juice semisolid medium, LGIP semisolid medium and acetic LHIP semisolid media.
- 5. Enrichment culture are to be sub cultured for every 2-3 days.
- The isolated cultures grown on acetic LGIP broth are used for further characterization

Colony Characters of Acetobacter:

The white colonies which become yellow orange & finally dark orange and 2-3mm in diameter.

Manufacturing process of Acetobacter liquid, biainoculant:-

Step 1-Pure culture of Acetobacter is maintained at refrigerator (4°C), inoculated 1 ml of the suspension in 250ml volume conical flask containing 100 ml growth media

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<u>Step 2</u> – Inoculated the flask for 48 hrs. – 72 hrs. at 120 rpm on rotary incubator shaker at 28+/-*c checked the growth of purity and biomass used as mother culture.

<u>Step 3</u> – Inoculate the 15-20 ml of culture of Acetobacter to 2000 ml volume flask containing 1800 ml media and incubate on rotary shaker for 120 hrs. (5days) at 150 rpm at 28-30°c. Check the pH, cell growth and contamination periodically and use it for mass production of Acetobacter.

Sterilize 100 lit growth medium at 121°c for 30 min and cool at 28-30°c. inoculate 10 lit culture of acetobacter in fermenter for 5 days. Check the pH, cell growth & contamination periodically.

<u>Step 4</u> – After completion of fermentation, formulate the liquid Acetobacter bioinoculant by adding cell protectants, cell growth boosters, adjutants and fill-packed in plastic bottles or cans of different capacity, informative labels with details of method of applications and others.

Stem 5- Packing, Marking, Storage & Application.

5.1 PACKING:-

Acetobacter Liquid Bio-fertilizers are packed in milky white plastic containers (1/5 ltrs.) of 160gms and 350gms wt. respectively.

5.2 MARKING:-

Each plastic container marked legibly and indelibly with the following information:

a) Name of the product: Acetobacter liquid bio-inoculant.

b) Name of the manufacturer: Yashwantrao Mohite Krishna S.S.K. Ltd, Karad, Satara.

c) Crops for which intended: Sugarcane, wheat, Jowar, Bajara, Maize and all sugar containing crops.

d) Type of the carrier used: Liquid.

e) Count: 1x109.

f) Batch no.:

g) Date of Manufacture:

h) Expiry date : 3 months) Net weight: 1/5 ltrs.)

Storage instruction: Store in cool and dry place and keep away from direct sunlight.



1) Any other information :-.

- Do not mix inoculated seeds/setts or liquid bioinoculant with chemical fertilizer.
- II. Liquid bioinoculant treatment should be followed after fungicidal and insecticidal seed treatments.

III. Use the liquid bioinoculant before the date of expiry.

IV. Do not use any sticker during spraying of LiquidAcetobacter

5.3 Directions for use:-

Sett treatment / ratoon treatment -

1. L in 200 L of water and sell dipping for 30 mins or spraying on ratoon.

Foliar application-

1. L in 200 L of water for 2-4 months old sugarcane or ratoon crop.

5.4 Storage

Acetobacter Liquid bioinoculant are stored in a cool and dry place away from direct heat and temperature is maintained at 20 °C.

Recommended for:- Sugarcane, sweet potato, tea, ragi, coffee, mango, pineapple, etc.

Response: Increase in yield by 15-30 %

2.AZOshakati:

AZOTOBACTER INTRODUCTION:-

Azotobacter species are Gram negative bacteria found in neutral and alkaline soils, in water, and in association with some plants. They are aerobic, free-living soil microbes which play an important role in the nitrogen cycle in nature, binding atmospheric nitrogen, which is inaccessible to plant, and releasing it in the form of ammonium ions into the soil.



Beneficial Properties:-

- Ability to fix atmospheric N₂ 20-40 mg BNF/g of C source in laboratory equivalent to 20-40kg N/ha.
- Production of growth promoting substances like vit . B, IAA, GA. Ability to produce thiamine, riboflavin, pyridoxine, cyanocobalamine, nicotinic acid, pentathonic acid, etc.
- 3. Biological control of plant diseases by suppressing Aspergillus, Fusarium.
- 4. It improves seed germination and plant growth.
- 5. It thrives even in alkaline soils.

PHOSPHATE SOLUBLIZING BACTERIA INTRODUCTION:

It play important role in soil by solubilizing phosphorus and making it available plants. Many fungi, bacteria, actinomycetes and cyanobacteria are potential solubilizer of bound phosphate in soil

So the isolation of efficient PSB required use of proper technique and media depending upon kind of organism to isolated.

Composition of media

Azotobacter media (Jensen's medium g/l)

| FeSO ₄ | Sucrose : 0.1K ₂ HPO ₄ | : 20 : 1.0MgSO ₄ ,7H ₂ O : 0.5NaCl | |
|------------------------|---|---|--|
| : 0.5CaCO ₃ | : 2.0Na ₂ MoO : 1000 mlpH | | |
| Pikovskyaya | 's (g/l) for P Solubilizing E | BacteriaGlucose : | |
| | 10.0Tricalcium Phospha | ite : 5.0 | |

| $(NH_4)_2SO_4$ | : 0.5NaCl | : | 0.2MgSO ₄ 7H ₂ O |
|--|--------------------------|---------|--|
| | : 0.1KCI | : 0.2 | |
| Mn(SO ₄₎ .2H ₂ O | : TraceFeSO ₄ | | : TraceYeast |
| | Extract : 0.5pH: | 6.8-7.0 | |

PROCEDURE

STEP-1- Pure culture of Azatobactorchroococcum, Bacillus megaterium, B. polymyxa and Pseudomonas striatamaintainted at refrigerated (4⁰C), incubate 1 ml of the suspension in 250 ml volume conical flask containing 100 ml growth media. STEP- 2- Incubate the each flask for 96 hours at 120 RPM on rotary incubator shaker at 28±°C. Check the growth for purity and use biomass as Mother Culture.

<u>STEP -3</u> i.Incubate 5-10 ml of culture of *Azatobactor, Bacillus megaterium, Bacilluspolymyxa* and *Pseudomonas striata* to separate 2000 ml volume conical flask containing 1350 ml respective media and incubate on rotary shaker for 96 hours(3 days) at 120 rpm at 28-30^oC.Check the p^H, cell growth and contamination periodically and use it for mass production of bio-fertilizer.

ii. Sterilize the fermenter with respective growth medium at 121°C for 30 min. and cool at 28-30°C. Incubate 10 *l* inoculum of *Azatobactor*, *Bacillus megaterium*, *Bacillus polymyxa* and *Pseudomonas striata* separately in fermenter. Incubate for 3-4 days. Check the p^H, cell growth and contamination periodically.

<u>STEP -4-</u> After completion of fermentation, mix all culture in formulation vessel, formulate the liquidbioinoculant by adding cell protectants, cell growth boosters, adjuvants, cure it for 24-48hrs and aseptically fill-pack by semi-automatic filling machine in plastic bottles or cans of different capacity, informative labels with details of method of application and others fix & put in corrugated boxes.

STEP -5- Packing, marking, storage and use.

5.1 PACKING

Pack liquid biofertilizers in milky white HDPE plastic containers (1/5/20 / capacity of 160 gms,350gms &1.5kg wt.) resp.

5.2 MARKING

Each plastic container mark legibly & indelibly with the following info:

- Name of the product : Azophospho liquid bioinoculant.
- ii. Name of the manufacturer : Y. M. Krishna SSK Ltd, Karad, Satara.

iii. Crops for which intended : S'cane, Wheat, Jowar, Bajara, Maize,

Cotton, Turmeric, Ginger, Potato, Tobacco, Brinjal, Tomato, etc.

- iv. Type of the carrier used : Liquid.
- v. Count :1×10⁹.
- vi. Batch No . :
- vii. Date of Manufacture

viii. Expiry Date : 6 months from packing.

ix. Net wt. : 1/5lit.

 x. Storage instruction : Store in cool & dry place , away from direct heat & sunlight.

Any other information:-.

- i. Do not mix liquid bioinoculant with chemical fertilizers.
- Liquid bioinoculant treatment should be followed after fungicidal ,weedicidal, insecticidal treatments.

iii. Use the liquid bioinoculants befor the date of expiry.

5.3 DIRECTIONS FOR USE :Soil/Field application:

As per recommended dose, add 2.5 *l*/ha (1*l*/acre) of Azophospho liquid bioinaculant in 500 kg of FYM /compost & mix it uniformly. Add water just sufficient to maintain moisture up to 20 to 25 % & keep it for overnight. Apply this mixture in fields eqully before planting.

OR

Add 2.5 *l*/ha of Azophospho liquid inoculants in 500 litr. Water, drench the solution near root zone of crop.

5.4 STORAGE

Azophospho liquid bioinoculant are stored in a cool and dry place away from direct heat and temp. maintained at 20°C.

3.RHIZOSHAKATI:

RHIZOBIUM INTRODUCTION:-

Rhizobiums are special bacteria that can live in soil or in nodules formed to the roots legumes. It formasymbiotic association with the legumes obtaining nutrients form plant and producing nitrogen & the process are called as biological nitrogen fixation. They are broadly classified as fast or slow growing based on their growth on laboratory media.

Beneficial properties

1. Direct contribution of N symbiotically with legumes.

2. Residual nitrogen benefit for the succeeding crop.

3. Yield increase is by 10-35%.

4. Improve soil structure.

5. Produces exopolysaccharides.

6. Produces plant growth hormone.

PHOSPHATE SOLUBLIZING BACTERIA INTRODUCTION

It play important role in soil by solubilizing phosphorus and making it available plants. Many fungi, bacteria, actinomycytes and cyanobacteria are potential solubilizer of bound phosphate in soil.

So the isolation of efficient PSB required use of proper technique and media depending upon kind of organism to isolated.

Composition of Media

YEAST EXTRACT MANNITOL (YEM) media

| Yeast Extract | - | lg |
|---------------------------------------|---|--------|
| Mannitol | ÷ | 10g |
| K 2HPO4 | 2 | 0.5g |
| MgSO ₄ , 7H ₂ 0 | • | 0.2g |
| NaCl | 2 | 0.1g |
| Congo Red | 5 | 2.5m1 |
| Distilled water | - | 1000ml |
| pH | | 7 |

Composition of Pikovskyaya's for P solubilizing bacteria

| Glucose | | - 10. | 0g |
|----------------------|----|-------|----|
| Tricalcium Phosphate | | 5.0g | |
| $(NH_4)_2SO_4$ | 73 | 0.5g | |
| NacI | 25 | 0.2g | |
| | | | |

MgSO₄.7H₂O - 0.1g

| KC1 | • | 0.2g |
|--|----|------------|
| Mn (SO ₄). 2H ₂ O | - | Trace |
| FeSO4 | 20 | Trace |
| Yeast extract | - | 0.5 |
| pН | | 6.8 to 7.0 |

Manufacturing process of Rhizophospho Liquid Bioinoculant

STEP 1- Pure culture of *Rhizobium*, *Bacillus megaterium*, *Bacillus polymyxa* and *Pseudomona striata* maintained at refrigerator (4⁰ C), inoculate 1ml of the suspension in 250ml volume conical flask containing 100 ml following growth media.

STEP 2- Incubate the each flask for 48-72 hours at 120 RPM on rotary incubator shaker at $28\pm2^{\circ}$ Cheek the growth for purity and use biomass as Mother Culture.

STEP 3--i. Inoculate 5-10 ml of culture of *Rhizobium*, *Bacillus megaterium*, *Bacillus polymyxa* and *Pseudomonas striata* to 2000 ml volume conical flask separately containing 1250 ml respective media and incubate on rotary shaker for 72-96 hours (3-4days) at 120 RPM at 28- 30 0C. Check the pH, cell growth and contamination periodically and use it for mass production of biofertilizers.

ii. Sterilize the Fermenter with respective growth medium at 1210C for 30 minutes and cool at 28-30 °C. Inoculate 10 L inoculum of *Rhizobium*, *Bacillus megaterium*, *Bacillus polymyxa* and *Pseudomonas striata* separately in fermenter. Incubate for 3-4 days. Check the pH, cell growth and contamination periodically.

STEP 4-- After completion of fermentation, formulate the liquid *Rhizobium*, *Bacillus megaterium*, *Bacillus polymyxa* and *Pseudomonas striata* bioinoculant, mix it in formulation vessel by adding cell protectants, cell growth boosters, adjuvants and aseptically fill-pack by semiautomatic filling machine in plastic bottles or cans of different capacity, informative labels with details of method of application and others fix and pack in corrugated boxes.

STEP 5. - Packing, Marking, Storage and use

5.1 PACKING

1. DECOMPOSING CULTURE:

COMPOSITION OF MEDIA

Czapec Dox / DC media

| Glucose | - | 10g |
|---------------------------------|---|----------|
| KH ₂ PO ₄ | | 0.875g |
| KNo3 | 8 | 3.5g |
| $MgSO_4$ | 5 | 0.75g |
| Distilled water | | 1000ml |
| pН | ÷ | 5.5to6.0 |

Manufacturing process of Liquid Decomposing Culture

STEP 1- Pure culture of Decomposing culture (Aspergilus awamori, Aspergillus niger, Peniciiiumchrysogenum, Trichoderma viride, 4 cellulomonassps. and 2 Streptomyces sps.) maintained at refrigerator (4 °C), inoculated bit of inoculum from 90 mm petri- dishes containing media (PDA) / 250m1 volume conical flask containing 100 ml growth media.

STEP 2- Incubated the flasks for 6-8 days till sporulation or completion of growth (For fungal growth incubate at stationary phase and for bacterial growth incubate on at 120 RPM on rotary Incubator shaker at 28±°C) checked the growth for purity and biomass used as Mother Culture.

STEP 3-Inoculate fungal bit of 5-10 mm thick of decomposing cultures to 2000ml volume conical flask containing 2000ml media and incubate it at stationary phase (at 25-28 °C). Check the pH, cell growth and contamination periodically and used it for mass production.

STEP 4--After completion of different microbial growth, collect all mat, aseptically mash it in sterile mixer mix all bioculture formulate the decomposing culture by adding cell protectants, cell growth boosters, adjuants and fill-packed in plastic bottles or cans of different capacity, informative labels with details of method of application and others fixed and put in corrugated boxes.

STEP 5.- Packing, Marking, Storage and use

5.1 PACKING

Liquid Bio-fertilizers are packed in milky white plastic containers (1/5/ 20 ltr capacity of 160gms, 350gms and 1.5kg wt.) respectively

5.2 MARKING

Each plastic container are marked legibly and indelibly with the following information:

a) Name of the product : Liquid Decomposing Culture

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b) Name of the manufacturer : Yashwantrao Mohite Krishna SSK Ltd, Karad, Satara.

.c) Decomposition of : Any agro-industrial waste including sugarcane trash, other crops trash, cowdung, and PMC &spentwash of distilleries of sugar factories.

d) Type of the carrier Used : Liquid

e) Count : 1×10⁹

f) Batch No

g) Date of manufacture :

h) Expiry Date : 6 months.

i)Net weight. : 1/5/20 lit.

j) Storage Instruction : store in cool and dry space & keep away from direct sunlight.

k) Any other Info .:

- i. Do not mix liquid bioinnoculant with chemical fertilizer
- ii. Liquid bio-inoculant treatment should be followed after fungicidal, weedicidal& Insecticidal treatments.
- iii. Use the bio-inoculant before the date of expiry.

5.3 Direction for use:

Pit method & Heap method

Size: pit method: 1m depth×1.5-2m width×10-15m length.

Heap method: 4-5m length (as per required) ×1.5-2m width ×1.5 m height

- Give the first layer of 15 cm with a disposed sugarcane trash ,agril.wastes, garden wastes, garbage etc. and add water up to 60% just to wet the materials
- Then add 8kg urea 16kg SSP in 1 Mt of trash or Agril.residues in each layer
- After 4-8 days add 100 kg dung in 500 lit. of water and 1 lit. Decomposing culture per 4 T of trash ,agril. Residues uniformly on each layer.
- Cover pit/Heap with mixture of soil/press mud cake, dung, trash, grass and water
- While filling the pits the space of 1-2 feet should be kept towards the longer side of pit in order to supply oxygen.
- 2-3 turning should be given at the interval of 45 days.
- In this way good quality compost will be ready within 90-120 days.

5.4 Storage

Liquid decomposing culture are stored in cool and dry place away from direct heat and temp.is 28°C.

 Plant & Machineries: Automized, Semi-automized and manual operating machineries.

1] Autoclave:-

It is an apparatus in which saturated steam under a pressure effects sterilization. The pressure increases the temperature to which water can be heated. Cells are destroyed by the higher temperature and not by the pressure. Most of the organism are killed at temperature 121°C(i.e. 1 lb/in2) in 15 minutes. Sterilization in autoclave is done with saturated steam under pressure.

2] Laminar air flow chamber:-

The cabinet is fabricated out of thick bore of sun mica or stainless steel. Interior surface of working platform is of stainless steel with the sun mica clayed at the top. Side of the panel is of thick transparent plexi glass duly framed. The unit is fitted with both pre filter and high-efficiency particulate air (HEPA) filters. Air is drawn through pre filter and is made to pass through highly effective HEPA filters having efficiency rating as high as 99.99%thus retaining all the particles of size 0.3 pm or larger. A blower and motor assembly of 1.5 HP.

The working area is illuminated by fluorescent light fitted with the unit. A UV light is also fixed underneath the sun mica clayed at the top and it is to be switched on 10 to 20 minutes before working.

Laminar flow provides aseptic environment for performing various activities such as pouring of sterilized media in sterilized plate, isolation, transfer of pathogen.

3] BOD incubators-

Incubators providing controlled conditions (light, temperature, humidity, etc.) required for the growth and development of microorganisms. Multiplication of starter culture can be done in this instrument. Normally temperature in BOD is 27°C to 29°C. in generally temperature inside the BOD is below or above the ambient temperature.

4] Rotary shaker:-

It is used for agitating culture flasks by circular motion under variable speed control. Shaking provides aeration for growth of cultures. Shakers holding

upto20-50 flasks are generally used. The capacity of the shaker may be increased if it is a double- decker type.

5] Hot air oven:

Hot air oven is meant for sterilizing all glassware materials. Dry heat is used in this apparatus to sterilize the materials. Normally 180°C is used for 20 min for sterilizing glasswares.

7] pH meter:-

An instrument for measuring pH of the solution using a 0-14 scale in which seven represents neutral points, less than seven is acidity (excess of H over -OH) and more than seven is alkality (excess of -OH over H) useful in adjusting the pH of the growth medium.

8] Refrigerator:-

Refrigerator is need to maintain the cultures in pure form for further studies. Maintenance of cultures in generally carried out at low temperature (0- 5 °C). The mother culture is periodically sub-cultured and stored in the refrigerator for longterm usage. Temperature maintained inside the refrigerator is always below ambient temperature.

9] Fermentor:-

A fermentor is the equipment, which provides the proper environment for the growth of a desired organism. It is generally a large vessel in which, the organism may be kept at the required temperature, PH, dissolved oxygen concentration and substrate concentration. Different models of fermentors are available depending upon the necessity. A simple version model contains steam generator, sterilization process devices and agitator. A sophisticated fermentor contains pH regulator, oxygen level regulator, anti-foam device, temperature controller, etc₄ 546

9] Colony counter:-

Colony counter are used to estimate a liquid counter's density of microorganisms by counting individual colonies on an agar plate, slide, mini gel or petri dish. It counts the number of colonies of micro-organisms that have grown on an agar plate prepared from a sample. Counting is done by using pen marker.

10] Bottling Unit:-

Once comment is given 1/5 lit. of liquid bioinoculant is released through bottling unit and packed aseptically. Semi-automized bottling unit is used to fill pack correct amount of content under aseptic conditions.

11] Weighing balance:-

It is instrument which is used to determine the weight and mass of an object. They are used to measure solids, liquids, etc. exact mass is then determined using an analytical balance.



Microbiology Departments Production



Biofertilizers



Biofertilizers

राजारामबापु कॉलेज ऑफ टेक्नॉलाजी ॲड रिसर्च सेंटर इस्लामपूर

बायोफर्टिलायजर फिडबॅक फॉर्म

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| पत्ताः : | स. व | T. जागढा | हो ता.च | ल्हुश्न | |
| ्। मो. नं.: | | 835-44 | 0-29 | | |

वापरलेल्या फर्टिलायझरचे नाव:—

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लॅब इनवार्ज सही

Adros प्रोडक्शन घेणाऱ्या शेतकऱ्याची सही



राजारामबापु कॉलेज ऑफ टेक्नॉलाजी ॲड रिसर्च सेंटर इस्लामपूर

बायोफर्टिलायजर फिडबॅक फॉर्म

| शेतकऱ्याचे नाव:- की. आभीजीत | भाते |
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| 4 | फवारणी केलेल्याची दिनांक | 1718/2021 |
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| C | पी.एच.(PH) | 7:00 |

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प्रोंडव ची सही शतकऱ्या

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Rajarambapu College of Sugar Technology, Islampur Hand Sanitizer Unit

GUIDE TO LOCAL PRODUCTION (As per WHO)

It is intended to guide a local producer in the actual preparation of the formulation. Materials required (small volume production)

| REAGENTS FOR FORMULATION 1 | REAGENTS FOR FORMULATION 2 | |
|--|--|--|
| Ethanol 96% | Isopropyl alcohol 99.8% | |
| Hydrogen peroxide 3% | Hydrogen peroxide 3% | |
| Glycerol 98% | Glycerol 98% | |
| Sterile distilled or boiled cold water | Sterile distilled or boiled cold water | |

- ✓ 10-litre glass or plastic bottles with screw-threaded stoppers or 50-litre plastic tanks (preferably in polypropylene or high density
- ✓ polyethylene, translucent so as to see the liquid level) or Stainless steel tanks with a capacity of 80–100 litres
- (for mixing without overflowing) Wooden, plastic or metal paddles for mixing
- ✓ Measuring cylinders and measuring jugs
- ✓ Plastic or metal funnel
- ✓ 100 ml plastic bottles with leak-proof tops
- ✓ 500 ml glass or plastic bottles with screw tops
- An alcoholometer : the temperature scale is at the bottom and the ethanol concentration (percentage v/v) at the top



Note

- Glycerol: used as humectant, but other emollients may be used for skin care, provided that they are cheap, widely available and miscible in water and alcohol and do not add to toxicity or promote allergy.
- Hydrogen peroxide: used to inactivate contaminating bacterial spores in the solution and is not an active substance for hand antisepsis.
- Any further additive to both formulations should be clearly labelled and be non-toxic in case of accidental ingestion.
- A colorant may be added to allow differentiation from other fluids, but should not add to toxicity, promote allergy, or interfere with antimicrobial properties. The addition of perfumes or dyes is not recommended due to risk of allergic reactions.

METHOD: 10 LITRE PREPARATIONS

These can be prepared in 10-litre glass or plastic bottles with Screw - threaded stoppers.

| FORMULATION 1 | FORMULATION 2 |
|------------------------------|----------------------------------|
| Ethanol 96%: 8333 ml | Isopropyl alcohol 99.8%: 7515 ml |
| Hydrogen peroxide 3%: 417 ml | Hydrogen peroxide 3%: 417 ml |
| Glycerol 98%: 145 ml | Glycerol 98% : 145 ml |

Recommended amounts of products

Step by step preparation:

- The alcohol for the formula to be used is poured into the large bottle or tank up to the graduated mark.
- Hydrogen peroxide is added using the measuring cylinder.
- 3) Glycerol is added using a measuring cylinder. As glycerol is very viscous and sticks to the wall of the measuring cylinder, it should be rinsed with some sterile distilled or cold boiled water and then emptied into the bottle/tank.



- The bottle/tank is then topped up to the 10-litre mark with sterile distilled or cold boiled water.
- The lid or the screw cap is placed on the tank/bottle as soon as possible after preparation, in order to prevent evaporation.
- 6) The solution is mixed by shaking gently where appropriate or by using a paddle.
- 7) Immediately divide up the solution into its final containers (e.g. 500 or 100 ml plastic bottles), and place the bottles in quarantine for 72 hours before use. This allows time for any spores present in the alcohol or the new/re-used bottles to be destroyed.

Final products

| FORMULATION 1 | FORMULATION 2 |
|-----------------------|------------------------------|
| Final concentrations: | Final concentrations: |
| Ethanol 80 % (v/v), | Isopropyl alcohol 75% (v/v), |
| Glycerol 1.45% (v/v) | Glycerol 1.45% (v/v), |
| Hydrogen peroxide | Hydrogen peroxide |
| 0.125% (v/v) | 0.125% (v/v) |

Quality Control:

- Pre-production analysis should be made every time an analysis certificate is not available to guarantee the titration of alcohol (i.e. local production). Verify the alcohol concentration with the alcoholmeter and make the necessary adjustments in volume in the preparation formulation to obtain the final recommended concentration.
- Post-production analysis is mandatory if either ethanol or an isopropanol solution is used. Use the alcoholmeter to control the alcohol concentration of the final use solution. The accepted limits should be fixed to ± 5% of the target concentration (75%-85% for ethanol).
- 3) The alcoholmeter shown in this information pamphlet is for use with ethanol: if used to control an isopropanol solution, a 75% solution will show 77% (± 1%) on the scale at 25°C.

General information

Labelling should be in accordance with national guidelines and should include the following:

- 1) Name of institution
- 2) WHO-recommended hand rub formulation
- For external use only
- 4) Avoid contact with eyes



- 5) Keep out of the reach of children
- 6) Date of production and batch number
- Use: Apply a palmful of alcohol-based handrub and cover all surfaces of the hands. Rub hands until dry
- 8) Composition: ethanol or isopropanol, glycerol and hydrogen peroxide
- 9) Flammable: keep away from flame and heat

Production and storage facilities:

- Production and storage facilities should ideally be air conditioned or cool rooms. No naked flames or smoking should be permitted in these areas.
- WHO-recommended handrub formulations should not be produced in quantities exceeding 50-litres locally or in central pharmacies lacking specialised air conditioning and ventilation.
- 3) Since undiluted ethanol is highly flammable and may ignite at temperatures as low as 10°C, production facilities should directly dilute it to the abovementioned concentration. The flashpoints of ethanol 80% (v/v) and of isopropyl alcohol 75% (v/v) are 17.5°C and 19°C, respectively.
- National safety guidelines and local legal requirements must be adhered to the storage of ingredients and the final product.



| DSTA/LAB/TR/F21 | 100 | ST REPORT | Page 1 of 2 |
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| Name & Address of the customer : Rajarambapu College of Sugar Technology , New Bahe Naka,Ba Road,Islampur, Tal- Walwa, Dist- Sagli: 415409 Phone: 788010299 Email:rcstcollege2010@gmail.com | | | 15409 |
| Name of Sample : Sanitizer | | | |
| Sample condition: | Light Blue Tra | nsparent Liquid | |
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| Particulars | Details |
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| Justomer Letter No. / Reference No. | KSPM/RCST/4160/2020-21 Dt.22/07/2020 |
| Date of Receipt | 04/08/2020 |
| nalysis Start Date | 04/08/2020 |
| nalysis End Date | 14/08/2020 |

| r. 0. | Parameters | Results | Control | Methods |
|----------|--|-------------------------|-------------------------|-------------------------|
| 0. | Microbial population by turbidity measurement | 84.60% | 100% | By Spectrophotometer |
| | Swab Test | Unit (CFU/ml/sq.ft.) | Unit (CFU/ml/sq.ft.) | Total Viable Count |
| | A. Wooden Surface: | | | |
| 2 | For Bacteria | 1x 10 ³ | 2 x 10 ³ | |
| | For fungi | 5 x 10 ² | 8 x 10 ² | _ |
| 1 | B. Floor Surface: | | | |
| | For Bacteria | 2×10^{2} | 2 x 10 ³ | |
| | For fungi | 1×10^2 | $2 \ge 10^2$ | |
| | C. Metal Surface | | | |

DSTA House', 17/1&2, Opp. Shivajinagar S.T. Bus Stand, Shivajinagar, Pune – 411 005 as of suger | E Mail: dstalab@gmail.com | Website: www.dsta.in | Telephone: 020-255360 | E Mail: dstalab@gmail.com

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| | For Bacteria | $5 \ge 10^2$ | 1 x 103 | |
|-------|---------------------|---------------------|---------------------|---------------------------|
| | For fungi | 3×10^2 | 6 x 10 ² | |
| | D.Rubber surface: | | | |
| - (4) | For Bacteria | 4 x 10 ² | 2 x 10 ⁵ | |
| | For fungi | 5 x 10 ² | 6 x 10 ¹ | |
| 3. | Zone of Inhibition: | | | Spread plate Technique |
| | For Bacteria | 0 cm | 0 cm | |
| . 7 | For fungi | 0 cm | 0 cm | |

Remarks:

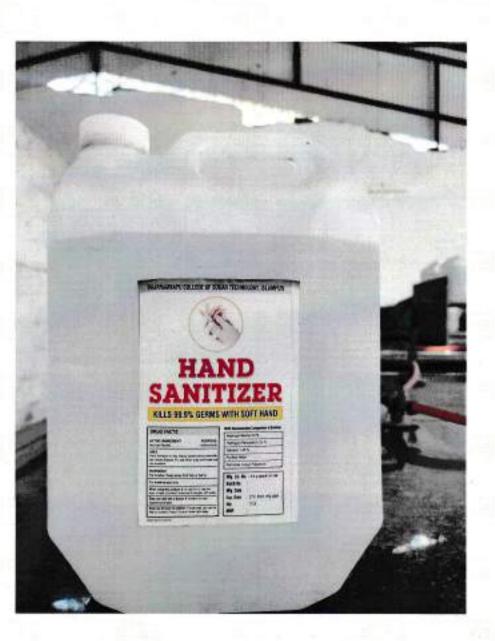
- The above results reported pertain to the samples submitted by the customer.
- The samples are not drawn by Deccan Sugar Technologists' Association, PUNE.
- The test report should not be reproduced except in full without written approval of laboratory.

D. S. T. A. Signature: Gr Julz Name: Mr.S. LABORATORY Designation: Officiating Executive Secretary

Page 2 of 2

End of Test Report*





Sanitizer Production

PREPARATION OF THE SUGAR CANE JAM FROM SUGAR CANE JUICE

Patil Minal

ABSTRACT

India is producing surplus sugarcane and sugar. Therefore, diversifying the cane juice is inevitable. Product diversification is need of the hour. Cane jam is a unique product from sugarcane juice used in daily diet and is not available in the market till date. Cane jam is purely from sugarcane juice and easily blended with flavoring agents. The product is having huge potential as a commercial product like the fruit jams. The process of preparation of sugarcane jam from sugarcane juice is discussed in this paper. Keywords : sugarcane, sugarcane juice , jam , brix , acidity sucrose contain

INTRODUCTION

The high sugar and low acid blend of makes it delicious and palatable. The of ripen sugarcane accounts for 33% to 55%, while the juice is 45% to 67%, The juice is the part of the sugarcane and is composed of water 84% to 90% and sucrose 10% to 15%. The pH value of juice ranged 4.0 to 4.5 and the very low acidity 0.05% to 0.18% in citric acid of the juice, which strongly influences the processing operations. Sugars range from 17°Brix to 18°Brix and are mainly of the reducing type. (Kumar K. et al., 2018)



Fig.1.1sugarcane jam

Assistant prof. - Rajananthapu college of sugar technology Islampor Email al-minalp757@gmail.com

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- Saccharum officinarum is a large, strong-growing species of <u>grass</u> in the genus <u>Saccharum</u>. Its stout stalks are rich in <u>sucrose</u>, a simple <u>sugar</u> which accumulates in the <u>stalk internodes</u>. It originated in <u>New Guinea</u>, and is now cultivated in tropical and subtropical countries worldwide for the <u>production</u> of <u>sugar</u>, <u>ethanol</u> and other products.
 - Saccharum officinarum is one of the most productive and most intensively cultivated kinds of <u>sugarcane</u>. It can interbreed with other sugarcane species, such as <u>Saccharum</u> <u>sinense</u> and <u>Saccharum barberi</u>. The major commercial <u>cultivars</u> are complex <u>hybrids</u>. About 70% of the sugar produced worldwide comes from S. officinarum and hybrids using this species.

| Classification of S. officinarum | | |
|----------------------------------|----------------|--|
| Kingdom: | Plamae | |
| Order: | Poales | |
| Family: | Poaceae | |
| Subfamily: | Panicoideae | |
| Genus: | Saccharum | |
| Species: | S. officinarum | |

1.2 uses

- Portions of the stem of this and several other species of sugarcane have been used from ancient times for chewing to extract the sweet juice. It was cultivated in New Guinea about 8,000 years ago for this purpose. Extraction of the juice and boiling to concentrate it was probably first done in India more than 2,000 years ago.
- Saccharum officinarum and its hybrids are grown for the production of sugar, ethanol, and other industrial uses in tropical and subtropical regions around the world. The stems and the <u>byproducts</u> of the sugar industry are used for feeding to <u>livestock</u>. Pigs fed on sugarcane juice and a <u>soy</u>-based <u>protein supplement</u> produced stronger piglets that grew faster than those on a more conventional diet. As its specific name (officinarum, "of dispensaries") implies, it is also used in traditional medicine both internally and externally.

| Sr. No. | Parameter | Content in fresh fruit |
|---------|---------------------|------------------------|
| 1 | Water | 70-88% |
| 2 | Sucrose | 10-16% |
| 3 | Reducing sugar | 0.5-2%% |
| 4 | Oraganic substances | 0.5-19 |
| 6 | Inorganic | 0.2-0.6% |
| 7 | Nitrogen body | 0.5-19 |
| 8 | Ash | Nil |
| 9 | Fiber | Nil |

Table 1.2 : Composition of Sugarcane Juice





1.3 Fruit Yield

The average yield of cane stalk is 60–70 tonnes per hectare (24–28 long ton/acre; 27–31 short ton/acre) per year, but this figure can vary between 30 and 180 tonnes per hectare depending on knowledge and crop management approach used in sugarcane cultivation.

1.4 Value Added Products of sugarcane juice

The value added products of sugarcane should be promoted as cottage industry. These products viz. **jaggery**, sugarcane juice concentrate, powder jaggery, rab, vinegar etc. are not only nutritious but also have great export potential in International market.

1.5 Justification

Sugarcane juice comes with an abundance of nutrients that includes: potassium, calcium, magnesium, iron, magnesium, zinc, thiamin, riboflavin and several amino acids. A glass of sugarcane juice (240ml) comes with 180 calories. 30 grams of sugar, and is also high in dietary fibre. There is needed to make awareness about the importance of cactus fruit because of the high nutritional values as well as medicinal uses.

The juice is rich in vitamin C, antioxidants, potassium, fairing calcium, magnesium and phosphorus. It is also used to fight viral infections, build strong bones, and boost the immune system, optimize metabolic activities, improve the skin health, protect heart health, prevent cancer, improves wound healing, cure insomnia, reduce inflammation throughout the body.

Keeping all above views, research project has undertaken decided to develop the value added products from the cactus fruit with following objectives:

1.6 Objectives

To prepare value added products from Sugarcane plant ...

1.7 Nutritional properties of sugarcane plant

Sugarcane juice comes with an abundance of nutrients that includes: potassium, calcium, magnesium, iron, magnesium, zinc, thiamin, riboflavin

1.8 Medicinal properties of sugarcane plant

According to medical science, sugarcane juice can effectively provide relief in fatigue and increase body temperature during summers.

Sugar cane juice is nourishing, it is a natural diuretic. Sugarcane is cold in nature. So Sugar cane juice is one of the best natural coolant ...

sugarcane juice has plentiful medicinal properties, and it is considered to strengthen the stomach, kidneys, heart, eyes, brain, ... etc.

MATERIALS AND METHODS

The present project work on "sugarcane jam" was carried out in the Department of Alcohol Technology, Rajarmbapu college of sugar technology Islampur. Sangli during the year 2019-20,





The main objective of this work is to standardize the process technology for making value added products of sugarcane juice i.e. sugarcane juice , sugarcane juice jam, jelly etc .

The methods used for conducting the research work, experiment used, processing steps, material used for conducting the quality analysis of the developed products.

2.0 Ingredients

2.1.1Sugarcane Juice

Freshly harvested sugarcane collected from the field to the farmer .

2.1.2 Sugar

Good quality, clean, crystalline, white cane sugar purchased from local market and used as sweetening agent.

2.1.3 Pectin Powder

Pectin is used as a thickening agent in jams. The classical application is giving the jelly like consistency to jams.

2.1.4 Citric Acid

Citric acid used as a preservative. It is a natural preservative and is also used to add an acidic (sour) taste to food.

2.1.5 Water

Fresh potable water used for cleaning the prickly pear fruit.

2.1.6 Instrument/Equipment/Glassware used

2.1.7 Weighing Balance

Electronic weighing balance (Manufactured by SF-400) fig. 3.1 (a) used for weighing the raw materials and ingredients during the preparation of the products. The capacity of weighing balance is 10 kg having least count 0.01 g.

2.1.8 Refractometer

Refractometer (Manufactured by- Bellingham and Stanley) (b) was used for measuring brix of solution

2.1.9 Vernier Caliper

Vernier caliper (Manufactured by ATS) is used to measure outer dimension of prickly pear fruit.

2.1.10 Knife

Stainless steel knife of 18 cm long was used for cutting/slicing the prickly pear fruit.

2.2.1Spatula

It was used for detaching the pulp from the peel of prickly pear fruit.

2.2.2 Food Processor

An electronic food processor (Manufactured by- BAKEMAN plus) fig. 3.1 (d) used for blending the pulp in homogeneous manner.

2.2.3 Strainer





Stainless steel strainer is used for separating seeds from the pulp.It is fig. 3.1 (c)

2.2.4 Beaker

Beaker was used for measuring amount of mixture.

2.2.5 Stirrer

Stirrer was used for stirring the mixture.

2.2.6 Utensils

It was a small hand held tool used for prickly pear fruit for jam preparation.

2.2.7 Measuring Cylinder

Measuring cylinder was used to measure true volume of prickly pear fruit.

Gas Cylinder

2.2.8 It was used for boiling the water for preparing sugar syrup.

2.2.8 Glass Bottles

Glass bottles as fig.3.1 (f) were used for storing semi-liquid products

2.2.9 Preparation of sugarcane juice Jam

Jam is a product made by boiling juice with sufficient sugar to a reasonably thick consistency, firm enough to hold the juice tissues in position. Jam contains 0.5-0.6 percent as a citric acid and invert sugar should not be more than 40 percent.

For the preparation of sugarcane jam, fresh and brown color, well matured,cane selected. These pieces were blended with the help of food processor for making it homogeneous. The homogeneous juice was filtered through stainless steel strainer. It was boiled for five minutes. Sugar was added into the boiled homogenous juice by continuously stirring. Pectin was added in to it. End Point (65-68 % TSS) of boiled mixture was adjusted. Immediately after end point filled it in sterilized glass bottles and stored at cool and dry place. The process flow chart for preparation of jam is given as below:

Matured sugarcane I Washing I Crushing Juice collecting I Straining Addition of Sugar Boiling (with Continuous Stirring) Addition of Citric Acid I Addition of Pectin

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Judging of End Point (65-68 % TSS) Filling in hot condition in sterilized bottles Cooling Capping Storage (At ambient temperature)

Fig. 3.3.1 Flow Chart for Preparation of sugarcane Jam

For development of jam conducted the three trials by selecting the various concentrations of sugar and pectin. Quality of developed jam having various concentrations of sugar and pectin was tested by sensory evaluation.

RESULT AND DISCUSSION

The aim of study is to utilize sugarcane juice is promoted for treating diabetes, high cholesterol, obesity and hangovers. It's also used for its antiviral and anti-inflammatory properties. Therefore the present study was undertaken to use the sugarcane as food in the form of jam. The result so far obtained from sugarcane which presented and discussed.

Quality Optimization

The quality of jam is determined by the proportions of sugar and pectin added to the sugarcane juice. Ideally jams contain 50-65% of sugars which gives the product a bright color and natural stability. To optimize the sugar and pectin content in the final product, the trial jams were developed using different variations as tabulated below (Table 1). Table 1:-

Table 4.2 : Optimization of Sugar and Pectin Content Percentage for Jam

| Trials | Colour | Flavour | Texture | Taste | Overall acceptability |
|--------|--------|---------|---------|-------|-----------------------|
| T.I | 8 | 6 | 6 | 7.1 | 6.8 |
| T2 | 9 | 7,5 | 7.2 | 7,8 | 8.3 |
| T3 | 8 | 6.8 | 6.5 | 7.4 | 7.5 |

(T1: Sugar-40%, Pectin-2%) (T2: Sugar-60%, Pectin-5%) (T3 Sugar-85%, Pectin-2%)

Trial 1

The jam with ingredient Sugar concentration 40% and pectin concentration 2% were insipid slightly harder to eat and its appearance was not too good. Jams with less sugar content were also susceptible to spoilage due to microbial fermentation and may need chemical preservatives.

Trial 2

In 2nd trial we used 60% sugar concentration and 5% Pectin concentration for 1 kg of prickly pear fruit pulp. The color and texture of the jam was pleasant and consistency is good as compare to others and taste was too good. . So it was preferred by everyone.

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Trial 3

The jam with Sugar concentration 75% and pectin concentration 2%, the consistency of this jam was quite good but their taste was not so dominant. Whereas high sugar content cause crystallization in jam.

On the basis of this sensory evaluation, we decided that the overall acceptability was jam having 60% concentration of sugar and 5% of pectin powder because got high ranking. Following table shows the sensory characteristics of the jam having different proportions of sugar and Pectin concentration.

CONCLUSION

The present research work gives an idea to utilize fresh sugarcane, which is promoted for treating diabetes, high cholesterol, obesity and hangovers. It is also touted for its antiviral and anti-inflammatory properties. The nutritional, medicinal and human health properties are factors that could contribute to an increase in cactus pear consumption. Sugarcane is an important food source in satisfying the nutritional needs. The difference caused by jam and other fruit jam is too small for a consumer of sensory evaluation panel. It is very useful fruit to increase value added products for to consume nutritional and Medicinal properties.

Keeping all above views, we decided to develop the value added products from the sugarcane by taking the following objectives:

- To prepare value added products from Sugarcane.
- To evaluate quality and study the cost economics of developed value added products.

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