**‘’Biofertilizer Production of *Trichoderma asperellum* and *Azollo* spp’’**

**Module Report**

**Submitted to**

**College of Agriculture,Umarkhed**

**In partial fulfilment of the requirements**

**For the**

**Degree of**

**BACHELOR OF SCIENCE**

**IN**

**AGRICULTURE**

**(Hons.)**

**By**

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**COLLEGE OF AGRICULTURE,UMARKHED**

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**Enrollment Number – QQ/2852 2021-22**

**DECLARATION OF STUDENT**

I hereby declare that, the experience learning work and its interpretation the module report file entitled “**Biofertilizer production of *Trichoderma asperellum* and *azolla***” or part of their has neither been derived from any module report/publication of any college or university.

The source of material used all assistance received during the course of experimental learning module have been duly acknowledge.

Place: Umarkhed Miss.Gauri Raju Jamode

Date : Enrollment No : QQ2852

**CERTIFICATE**

This is Certify that the module entitled “**Biofertilizer production of *Trichoderma asperellum* and *Azolla***” submitted in partial fulfilment of the requirement for the degree of “**Bachelor of Science in Agriculture(Plant Pathalogy)**” of College of Agriculture Umarkhed ,Dist.Yavatmal ,is a record of experimental learning module work carried out by Miss. Gauri Raju Jamode,QQ2852 under my guidance and supervision.

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**ACKNOWLEDGEMENT**

Success is not possible lonely without the involvement of many minds and hands to beautify.

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These words falls short to appropriately and sufficiently thank her.

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I am very much thankful to authors whose article helps me in organizing my module work properly using proper tools for interpretations of the result.

I was fortunate enough to receive the all kind of co-operation from almost everyone in one way or other during my stay at this institute above all I bow my head before almighty “LORD KRISHNA and GANESH”, whose blessings always gave me the strength to achieve this goal.

Place-Umarkhed Gauri Raju Jamode

Date- QQ-2852

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1) % - percentage

2) e.g - example

3) i.e – That is

4) GRT – Giant retinal tern

5) etc - et.cetera

Title of module – **“ Biofertilizer production of *Trichoderma asperellum* and *Azolla*”**

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**MODULE ABSTRACT**

**MODULE ABSTRACT**

Module work on “ Biofertilizer production on Trichoderma asperellum and Azolla “was carried out during 2021-2022.

In this all the work and this result can be mentioned . Soil sample collected from the rhizosperic soil from field and isolate on the Potato dextrose agar media .Trichoderma is fungicide used to protect seed from fungal diseases . Trichoderma is takes 7 days for incubation . The colour of trichoderma culture is dark greenish .

Take the culture on pure agar medium to grow while the whole procedure precautions must taken and all the instrument and media sterilized to protect from contamination ,spray ethanol on your hand before handeling the instrument while transfering the culture from petriplates to main fresh agar media .

Usually about 1 part by weight of broth is required to 2 parts of dry carrier . Final moisture content varies from 30-50 depending on quality of carrier .

After adding the broth culture to carrier powder in 1:2 proportion by weight then it is kept for curing at roo temprature for 5 to 10 days in 10 cm deep trays of convenient size .

**CHAPTER – I**

**INTRODUCTION**

**CHAPTER –I**

**INTRODUCTION**

1.1 Biofertilizer – ‘Biofertilizer is a fertilizer which contain the living or latent cells of micro-organism which fixes nitrogen

n and improve fertility.’

1.2 Types of biofertilizer

1) Bacterial

a) Nitrogen fixer

1.symbiotic e.g – Rhizobium

2. Non – symbiotic e.g - Azotobacter

3. Assosiative e.g – Azosprillium

b) phosphorus fixer

1. Non symbiotic e.g – Bassillus pseudomonas

2) Algal

e.g - BGA

3) Fungal

Phosphate solubilizer –

1.symbiotic mycorrhizae e.g - Glumose

2.Non symbiotic mycorrhizae e.g – Aspergillus penicllum

4) Actinomycets e.g – Frankia

1.3 Importance in Agriculture

1.Biofertilizer helps in the establishment and growth of crop ,plants and trees .

2.They inhance biomass production and yield by 20%.

3. They are useful in sustainable agriculture .

4. They are input containing micro-organism which capable of mobilizing nutritive elememt from non-available form through biological process.

5. They are less expensive and ecofriendly .

6.Biocontrol is exceside in wide range and is safe for human and animal health .

7. for bioagent farmer dose not require any special treatment procedure ,except microbial formulation .

1.4 General growth charecterstics of Biofertiizer agent

1] Rhizobium

1.Rhizobia invade legume roots through root hairs.

2. Form effective pink colour nodule in roots.

3. Live symbiotically inside the nodule and fix N2

4. Convert atmospheric nitrogen to plant accessible form

5. Most rhizobia are host specific in nature

2] Acetobacter

1. They are bacilli

2. They are gram –ve

3. They are obligate aerobes

4. They can fix nitrogen

3] Azosprillium

1. Azospirillium are gram –ve , do no form spore

2. Azospirillium have at least one flagellum and some time multiple flagella

3. Azospirillium are aerobic

4. Growth of Azospirillium is possible between 50C & 420C

4] PSB – Phosphate Solubilizing Bacteria

1. Phosphorus is very important nutrient for plants

2. These insoluble soil phosphate are solubilize by a group of micro-organism are called PSB

3. It can also be used to enrich compost

4. After the thermophillic phase of composting is over

**INTRODUCTION OF AZOLLA**

Azolla is free floating water ferm fungi which fixes atmospheric nitrogen .It is also used for the animal feeding .

Azolla multiplies vegetatively and its growth is prolific .

Species of Azolla

There are five species of azolla

1] Azolla cariana

2] Azolla nolofinal

3] Azolla microphylla

4] Azolla Mexicana

5] Azoll Pinnata

Most common species is Azolla pinnata.

**CHAPTER – II**

**MATERIAL AND METHOD**

**CHAPTER – II**

**MATERIAL AND METHOD**

2.1 MATERIAL USED

2.1.1 Glassware

The glassware used were petriplates , conical flask of different capacity , burette , funnel , Round bottom flask , pipetts , test tube

2.1.2 Equipment

BOD Incubator , Refrigerator , laminar airflow chamber , Autoclave , centrifuge , Hot air oven , microwave oven

2.1.3 Miscellaneous material

Spirit lamp, glass slide ,cover slip, cork borer , Aluminium foil Incubating needle , weighing balance, measuring cylinder, Bunsen burner ,

Cotton etc.

2.2 METHOD

2.2.1 Sterilization of glassware , media , filter paper , water other mterrial

Petriplates ,conical flask of different capacity were used .The glassware were sterilized in hotair oven at 180 degree celcious for 1 hour .

The media and distilled water were sterilized in autoclave at 15 psi for 15 min

.

Preparation of cultural media

2.2.2 - Potato dextrose broth

1) Distilled Water – 100 ml

2) Dextrose – 20 gm

3) Peeled potato – 200 gm

2.2.3 – Potato Dextrose agar media

1) Peeled potato – 200 gm

2) Dextrose – 20 gm

3) Agar – 20 gm

4) Distilled water – 100 ml

2.3 PRECAUTION TO ELIMINATE CONTAMINATION

- All isolation and inoculation work of microbial culture was carried out aseptically under laminar air flow. The laminar air flow was sterilized by blowing ultra violet lamp for ½ hour, prior to comencement of the work use spirit lamp. Use Handglubs where it is necessary.

2.4 MAINTAINENCE OF FUNGAL CULTURE

- Fungal culture were isolated by serial dillution isolation method and culture were maintained on potato dextrose agar at room temperature by adopting subsequent sub culturing at periodical regular interval 7 days old culture was used for further studies.

2.5 Methodology for isolation of *trichoderma asperellum*

1 collection of soil sample from rhizosperic soil .

2 The collected soil sample were air dried in study and finely grind before serial dilution

3 prepare potato dextrose agar media were used for isolation .

4 Add 9 ml tap water in test tube and test tube pluged with non-absorbent cotton and sterilized in autoclave at 15 psi for 15 min .

5 Addition of 1 gm of soil sample in 100 ml of sterilized water and vigoursly shaken for 30 min .

6 prepare serial dilution from 10 -1  to 10-7 .

7 Transferred 1 ml water from 10-7 dilution on potato dextrose agar media

8 Incubate the plates for 5 to 7 days at room temperature .

9 Transfer representative of each fungal colony to fresh potato dextrose agar meia plate to establish pure culture .

***Azolla***

1 Dug the pits of length 10 m X 4 m .

2 Levealed up the 4 pits .

3 Applied polythene on it .

4 Applied the sieved fine soil and cow dung slurry in it in ratio of 10 : 4 in every pit .

5 Added water .

6 After 24 hours added the seeds of azolla .

7 After 20 days 1st harvesting was done

8 Two to three harvesting has been done between 12-15 days interval .

2.6 Morphology

1] Trichoderma

1. Shape and colour – The shape and colour was observed on potato dextrose agar media .

2. Shape and colonies – These fungi are multibranched with pyramidal appearance and cluster .

3 Elevation – Asymetrical bent , flask shaped

4 . colour - sr no Isolate colony shaped

1. GRT Dark green

2. GRT Dull green to bluish green

3 GRT white

4 GRT Pale yellow green

**Azolla**

1] Shape and colour – The stem bear many rounded or angular overlapping

Leaves each 1 or 2 mm long

2] colour – Red

3] Shap and colonies – Triangular stem measuring up to 2.5 centimeter .

**CHAPTER – III**

**RESULT AND DISCUSSION**

**CHAPTER – III**

**RESULT AND DISCUSSION**

The module work on “Biofertilizer production of trichoderma asperellum and azolla “ was carried out during 2021-2022.

The result and its interpretation of the present studies given in this chapter .

Trichoderma culture were collected from the rhizosperic soil on which rabi crops were grown from different location of field of college of agriculture Umarkhed .These sample were processed in the laboratory for isolation f trichoderma aperellum on potato dextrose agar medium by serial dilution method.

Sr no crop location district

1 cowpea field of college of agriculture, Yavatmal

Umarkhed

After 7 days of inubation , dark green colour colonies were found on potato dextrose agar medium which were later picked and streak on fresh agar medium for pure culture and used for further studies .

In 1927 Gilman and Abbott recognized four species of trichoderma based on conidia and colony appearance .

Azolla generally inoculate and grown as a cover for incorporating into the soil as a top dressing in rice cultivation but in this module work we use azolla as a cheap cost of fodder to the animals

Azolla expansion was heavily promoted in the early 1960s in China .

**CHAPTER – IV**

**SUMMARY AND CONCLUSION**

**CHAPTER - IV**

**SUMMARY AND CONCLUSON**

Trichoderma is a fungus extremely well adapted to different ecological niches . This is because it has a remarkably diverse metabolism capable of catabolizing a wide variety of substrate as well as producing awide variety of secondary metabolites .

Trichoderma genome has revealed numerous gene cluster , although a deeper understanding of metabolite pathway .Additionally , at low concentration, some trichoderma have displayed beneficial effect to plants , increasing plant growth and development . A group of trichoderma species within the genus describe which share interesting feature , including adaptation to different environmental condition , metabolism of different environmental condition , metabolism of different biomolecule compound and fast growth .

The Trichoderma genus include several species that have been widely studied regarding their beneficial characteristics and application in agriculture . These fungi have ability to produce antibiotics , enzymes , as well as hormone that regulate root architecture and promote growth of plant . Trichoderma can confer protection against a wide range of foliar pathogen.It is known that trichoderma based biocontrol , production of antibiotic as well as induce plant resistance

Azolla is a water ferm algae which fixes atmospheric nitrogen , in this module work we harvest it for animal feeding .

In this chapter we summarize recent advance made understanding the role of trichoderma in modulating plant defense and genetic involved in trichoderma include immunity.

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Estimation of *Trichoderma*

9650

|  |  |  |  |
| --- | --- | --- | --- |
| Sr.No | Raw Materials Required | Quantity | Cost(Rs.) |
| 1 | Culture | 1plt | 2500 |
| 2 | Carb.methyl cellulose sod. Salt | 500g X 2 | 1000 |
| 3 | D- Glucose | 1 Kg | 2000 |
| 4 | Spirit | 1Lit | 250 |
| 5 | Cotton Bundle | 3 | 900 |
| 6 | Aluminium Foil | 4 | 280 |
| 7 | Potato | 20 Kg | 400 |
| 8 | Agar | 100 Gm | 700 |
| 9 | Talc Powder | 1 Q | 1300 |
| 10 | Polythene Bags | 2 Kg | 300 |
| 11 | Rubber band | 1 Packet | 20 |

Other Expenses=Labor(2)=500 Rs

Total Raw Material Cost=9650 Rs

Total Cost Of Production=Cost Of Raw Material + Other Expenses

=9650+500

=10150

Net Product Weight=100Kg

Packaging=1Kg/Packet

Total Packet=100

Cost Of Packet=200

Income from Selling Of Product=200X100

=20000 Rs.

Net Profit = Total income - cost of production

=2000 – 10,150

=9850

Benefit Cost Ratio=Total Income/Cost of Production

=20000/10150

=1.9

B:C Ratio is more than 1,Hence the product is profitable.

Estimation of Azolla

|  |  |  |  |
| --- | --- | --- | --- |
| Sr. No. | Raw material required | Quantity | Cost(in Rs) |
| 1 | Azolla culture | 2kg | 400Rs |
| 2 | Sheet | 10m-15m | 1900Rs |

Other Expenses= labour(2 days)= 400Rs

Total raw material cost= 1900Rs

Total cost of production= Cost of raw material+ other expenses

=1900+400

=2300Rs

Cost of 1 kg azolla = 230Rs

Total income from selling the product= 230 X 24= 5520Rs

Net profit= Total income-cost of production

= 5520-2300

= 3220Rs

Benefit cost ratio= total income

Cost of production

= 5520

2300

= 2.4

B:C ratio is more than 1, hence he product is profitable.

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**AGRIULTURAL ENTOMOLOGY**

**ENTOMOPATHOGENIC FUNGUS**

An entomopathogenic fungus is a fungus that can act as a parasite of insect and kill or seriously disable them ,since they are considered natural ortality agent and environmentally safe there is world wide interest in the use and manupultion of entomopathogenic fungi before biological control insect and other arthropode pest

In particular , the asexual phases of ascomycota are under intense scrutiny due to the trait favouring their use as biological insecticide .

1] *Beauveria bassiana*

Beauveria bassiana is a fungus that grow naturally in soil through out the world and act as a parasite on vaiou arthropode species causing white muscardine diseas , it thus belong to entomopathogenic fungi .

Family - cordycipitaceae

Order - hypocreales

Class - sordariomycetes

Species - Beauveria bassiana

Genus - Beauveria

Kingdom - Fungi

Mode of Action

* These fungi usually attach to external body surface of insect in the form of microscopic spore.

Under permissive condition of temperature and moisture, these spore germinate grow as hyphae and colonize the insect cuticle, eventually they bore through it and reach the insect body cavity.

Then the fungal cells proliferate in the body cavity usually as walled hyphae or on the form of wall-less protoplast.

After some time the insect usually killed and new propagules (spore) arev formed, on/in the insect if environmental condition are again permissive usually high humidity is required.

**MASS MULTIPLICATION**

Material required

1) Sorghum

2) Water

3) Chalk powder

4) Autoclave

**Methodology**

1. Sorghum in water for 48 hours.
2. Replace water after 24 hours, after 48 hours rinse water completely.
3. Separate equally in 10-15 flask and plug with hard cotton cushion and wrap double Aluminium foil.
4. Sterilize for 40 min with 21 Psi. Inoculate each flask containing the jowar with 2-3 drops of nucleus culture after cooling.
5. Beauveria culture will grow fully after 20-25 days.
6. Mix 2kg of chalk powder in Beauveria culture and dry in shade.

***2] METARIZIUM ANISOPLIAE***

- Metarizium anisopliae shift to saprophytic phase and remain alive within the soil in absence of susceptible host. As a shortcut we can transfer the fungus from soil to lab by culturing soil suspension. One hundred cultural soil suspension from different region of Iran were tested to find the M. isolate.

Order -- hypocreales

Family – clavicipitaces

Class – sordariamycetes

Species – Metarhizium anisopliae

Genus - Metarhizium

**Mode of Action**

- Fungi have virulent spore absorbed to the carrier/neutral material that remain ether dormant or active on the carrier particle and start multiplying when congenial environmental condition are met with.

The soil borne insect like termite comes in contact with the fungus start growing on the body termite and start feeding on the body fluid on insect and result in disease and death of the insect pest.

**Mass multiplication**

Material required

1) Coconut Water

2) Flat glass bottle

3) Cotton plug

4) Pressure cooker

5) Injection syringe

6) Bunsen burner

7) Laminar air flow chamber

8) Mixer grinder

**Methodology**

1. Containing 40ml of coconut water
2. Plug these bottle with cotton plug and sterilize in autoclave for 20 min at 15 Psi.
3. The bottle are inoculate with 1 ml suspension containing the spores of fungus with the help of a sterile injection syringe.
4. Sterilize the bottle with the help of the burner.
5. Sore are inoculated in the bottle with the help of syringe in a laminar flow chamber.
6. Keep inoculated bottle till the surface of medium is fully covered by the olive green sporulated fungus.
7. Whole culture is grinded thoroughly in the mixer grinder.
8. Keep culture in cool and dry place in packes.

***3) Verticillum lecanni***

Verticillun lecanni is now an approved name of entomopathogenic fungus , species . That was previously widely known as verticillum lacanii.

Vigas but now understood to be an anomorphic from in the cordyceps

Group of genera.

Order - Hypocreales

Scientific Name *– Lccanicillumm lecanii*

Family - Cordycipitacea

Kingdom – Fungi

**Mode of Action**

Fungi have any virulent spore adsorbed to the carrier . It infect all stages of insect . The spore of verticillim lecanii get attached to the cuticle of insect on contact . The fungus gains entry through the cuticle by enzyme .

It infect insect by producing hyphae from germinating spore that penetrate the insect cuticle

**Mass multiplication** .

1] 200 gm grains in 250 gm polypropalene bag or bottle with 190 ml of water

2] Sterilization at 1200 C for 45 min .

3] Cool and inoculate with 5 ml fungas spore suspension.

4] Bag incubate at 250 c for 25 days .

5] Air dried under laminar air flow 3 days at 40 o C

6] Ground to fine texture

dwdnjddeghgguihuhuhiojiojudnjf

**ENTOMOPATHOGENIC BACTERIA**

Entomopathogenic bacteria are unicellular prokaryotic organism having size ranging from less than 1 micrometer to several in length . Bacteria with rigid cell wall are cocci, rod shape and spiral while bacteria without cell wall are pleomorphic

1] ***Bacillus thuringensis***

Bacillus thuringinensis is a species of bacteria that lives in soil . It make protein that are toxic to some insect when eaten , but not other . Bt is not toxic to non – targate species

Species *- Bacillus thurenginensis*

Order - bacillus

Class - Bacilli

Family - Bacillaceae

Genus - Bacillus

Domain - Bacteria

**Mode of Action**

The sporulated Bt with ICP or spore ICP complex must be ingested by susceptible insect larvae folled by solubilization and rocessing from a protoxin to an activated toxic core in the insect digestive fluid .

The toxic core travel across the pentrohic matrix and the terminal region bind to specific receptor called cadhenins on the brush border membrane of the gut cell , resulting in pore formation by the N – terminal domain .

**Mass multiplication**

The seed culture (strain 344 B . Tringenensis tolworthi ) was produce using shake flak and grow in LB medium plus salt during 18 hours , incubate on a rotary shaker at 200 rpm at 30 0 c fo 96 hour .

Strain 344 was cultured in medium composed of luri Bertani enrich with salt and 2 g glucose and ph adjust to 7.5 after sub culturing strain was streaked on sporulating mediu containing the sme salt plus 12 g bacto agentb 8.0 of nutrient broth and incubate for 24 hour at 30 degree c maintain at 4 degree c for future used .

**ENTOMOPATHOGENIC VIRUS**

Entomopathogenic viruses are obligate intracellular parasite having either DNA or RNA encapsulated in to protein coat known as capsid ton form thev virons or nucleocapsid .

**1*] HaNPV***

Nuclear polyhedrosis virus is the host specific for spodoptora litura and helicoverp arnigera NPV in a stomach poison NPV is effective against lepidopteron larvae .

**Mode of Action**

Occluision bodies are ingested by insect larvae . in highly alkaline ph of the midgutv , thecocdusion body protein dilved and degraded by host alkaline protease .

The virus particle are released from polyhedr and subsequently attach to the peritropic membrane lining to midgut . The lipoprotein membrane surround the virus , fuses plasma membrane

**Mass multiplication**

**Material**

1] Agar agar

2] Gram flour

3] Methyl parahydroxyl benzoate

4] Yeast powder

5] Ascorbic acid

6] sorbic acid

7] Streptomycin

8] Multivitamin

9] Vitamin E

10] Distilled water

11] Centrifugal force

12] Glass vials

13] Muslin cloth

**Mass Multiplication**

1. The NPV of pest can be produced by used of both natural as well as artificial diet.
2. The third instar larvae of these pest either collected from field or reared in the laboratory are allowed to feed the diet.
3. Larvae gets infected with virus within 3-4 days and hang themselves upside down in tubes.
4. The infected larvae, are collected in a beaker containing water for 3-4 days for purification.
5. The purified larvae are crushed with help of mixer cum grinder by adding some water.
6. This solution is filtered with the help of muslin cloth and more water is required.
7. The filter extract solution in centrifugal to separate the polyhedral of NPV from solution.
8. It is ready to use now.

**Agricultural Extension**

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4.Member - Shri A.S.Raut

5.External Member

**PACKAGING**

Packaging is the science , art and technology of enclosing or protecting product for distribution , storage , sale and use .

Packaging also refers to the process of desgning , evaluation and producing packages .

**Importance of Packaging**

1] TO protect its content from any damage that could happen during transport .

2] Handling and storage

3] Packaging retain the product intact through out its logistics chain from manufacturer to the end user

4] It protect the product from humidity , light , heat and other external factor

APPLICATION / USAGES - Agiculture

GRADE - Agriculture grade

PACKAGING SIZE - Liquid formulation – 1 lit

Powdered formulation – 1 kg

PACKAGING TYPE - Bottle and polythene (packet)

FORM - Liquid and solid both

MANUFACTURED BY - Department of plant pathology ,

College of Agricuture ,Umarkhed .

**MARKETING**

Marketing refer to activities to undertake to promote buying o selling of product or service.

Marketing includes advertising , selling and delivering product to consumer or other .

**PURPOSE**

The purpose of marketing to generate revenue for a product ,brand and to reach target audience and communicate the benefits of your product or service , so you can successfully acquire , keep and grow customers .

So your marketing goal must relate to the specific objectives your team wants to achieve

The global Trichoderma asperellum is segmented based on formulation ,crop type pest . In order to establish the fungus in and the plant root . Trichoderma is effectively applied during seedling .

Trichoderma is directly sold by the students of college of Agriculture Umarkhed , to the used of biofungicide i.e Trichoderma asperellum and how to use it , They convensed the farmer to used the biofungicide by telling them positive side of it .

**Life cycle of Trichoderma**

Trichoderma is stored by a long term storage method in envelops kept in container with a small amount of desiccant at -20C.

**Types Of Advertising**

* Display Advertising
* Video Advertising
* Mobile Advertising
* Native Advertising

***Thank You***