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Manufacturing of Biofertilizers and Bio-control Agents for Disease Management

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Abstract:
Increased use of chemical fertilizers and pesticides in agriculture for achieving self-sufficiency in food production has resulted in environmental pollution and deterioration of soil health. To overcome high cost of fertilizers and pesticides have necessitated the development of novel ways of sustainable agriculture by production of biofertilizers and biocontrol agents in an eco-friendly manner for plant growth. In present work, isolation and morphological identification of organisms have been done in laboratory. The isolated and purified organisms are Azatobacter and Azospirillum (nitrogen fixers), phosphate solubilizing bacteria (PSB), potassium solubilizing bacteria (KSB) and biocontrol agents like Pseudomonas, Trichoderma and Beauveria. The nitrogen fixers, PSB and KSB were tested on the pomegranate plant with nutrient deficiency and biocontrol agents on cucumber and paddy crops. Our results indicate the recovery of nutrients in pomegranate by using nitrogen fixers and Tricoderma used as antagonist for cucumber downy mildew, pseudomonas for leaf blast of paddy and beauveria for cucumber plant against fruit borer.

Keywords: phosphate solubilizing bacteria (PSB), potassium solubilizing bacteria (KSB),Pseudomonas,Trichoderma, Beauveria, antagonistic, horticulture crop diseases, bio-control agent.

INTRODUCTION

India with diverse soil and climate comprising several agro-ecological regions provides ample opportunity to grow a variety of horticulture and food crops. Cultivation of these crops is labor intensive and as such they generate lot of employment opportunities for the rural population. Fruits and vegetables are also rich source of vitamins, minerals, proteins, and carbohydrates etc. which are essential in human nutrition. Hence, these are referred to as protective foods and assumed great importance as nutritional security of the people. India is the second largest vegetable producer in the world, next only to China with an annual production of 81 million tons from 5.1 million hectares of land. However, the major factors responsible for low production of fruits, cucurbitaceous, brassicaceous and solanaceous vegetables are the diseases caused by Pathogenic fungi. The disease development is so fast that whole crop is lost in a few days. Therefore, the problem deserves immediate and effective measures of control (Neeraj, *et.al*.2010).

Fruit plant diseases often are the worst natural hazards in horticulture and food crops. The most startling aspect of fruit plant diseases is that their control cost us a huge sum every year with serious environmental consequences. Therefore, integrated disease management practices need to be adopted to reduce the losses Fruits and vegetables are highly perishable products, especially during the postharvest phase, when considerable. Losses due to microbiological diseases, disorders, transpiration and senescence can occur. A number of microorganisms, which effectively control postharvest pathogens, have been identified for post-harvest control (Wilson and Wisniewski, 1989).

Azotobacter species are free-living, nitrogen-fixing bacteria; in contrast to Rhizobium species, they normally fix molecular nitrogen from the atmosphere without symbiotic relations with plants, although some *Azotobacter* species are associated with plants. *Azospirillum* is a Gram- negative, microaerophilic, non-fermentative and nitrogen fixing bacteria. It also promotes plant growth similar to azotobacter by producing nitrogenase enzyme and phytoharmones. Phosphate solubilizing bacteria (PSB) are beneficial bacteria capable of solubilizing inorganic phosphorous from insoluble compounds. P-solubilization ability of rhizosphere micro-organisms is considered to be one of the most important traits associated with plant phosphate nutrition. Potassium solubilizing bacteria (KSB) the special focus on K solubilizer was due to the fact that potassium is one of the major nutrients required by all crops.

Pseudomonas species with biocontrol properties, which produces a phenazine-type antibiotic active agent against certain fungal plant pathogens and antibiotically active against Gram-positive organisms. *Trichoderma* have been developed as biocontrol agents against fungal diseases of plants. The various mechanisms include antibiosis, parasitism, inducing host-plant resistance, and competition. *Beauveria bassiana* can be used as a biological insecticide to control a number of pests such as termites, whiteflies, stem borers, fruit borers and many other insects.

The present review expects to formulate biofertilizers and biocontrol agents for disease management and to get high yield with organic nature. The bio control agents response was analyzed by field application on horticulture and food crops and was utilized as a part of the recreation. Advance enhancement of active parameters for forecast of trial information in the production process was done.

II. MATERIALS AND METHODS

(A). ISOLATION AND PURE CULTURING

Inoculation of organisms from competent products are pure cultured on nutrient broth, PDA broth and plates in the laminar air flow. For preparation of 500 ml Synthetic PDA medium 20g of PDA powder, nutrient broth 13g and nutrient

agar 14g for 500ml synthetic media (Himedia laboratories Pvt. Ltd.) After autoclave at 121 °C for 15–20 minutes, cool the media up to tolerable temperature then pouring was done into sterile petridish under the flaming area in the laminar air flow. The liquid media plates were successfully prepared.

The inoculated plates were incubated in BOD at 26º C for 72 hrs. Pure colonies are isolated from inoculated petri plates separately in aseptic condition. Pure cultures are made with the help of sterilized inoculation loop on media plate. The isolated pure cultures on separated media plates are then kept on BOD incubator for incubation at 26º C for 72 hrs. Observation was taken daily to record the growth of microbes.

Fig 1. PDB containing *Tricoderma* after incubation

Fig 2. NB containing *Azospirillum, Azotobacter,* PSB &KSB.

Fig 3. NB containing *Pseudomonas* after incubation

Fig 4. Potato dextrose broth containing *beauveria*

A serial dilution is the stepwise dilution of substance in the solution, usually the dilution factor at each step is constant, resulting in geometric progress of the concentration in a logarithmic fashion. Label the test tubes up to 10^ (-8) dilutions and keep ready the agar petri-plates labelled. Using sterile pipette transfer 1ml of liquid from the source culture and add to the test tube 1.Transfer 1ml liquid from test tube 1 to test tube 2. Repeat the same steps, 6 or 8 times moving along the chain shown in figure below. Plate the solution of 0.1ml from each dilution and spread on agar plates aseptically. Incubate the plates overnight. The growth of colonies seen in plates, which is used for cell count method. Pure colonies are isolated from inoculated petri plates separately in aseptic condition. Pure cultures are made with the help of sterilized inoculation loop on media plate. The isolated pure cultures on separated plates are then kept on BOD incubator for incubation at 26º C for 72 hrs. Observation was taken daily to record the growth of microbes.

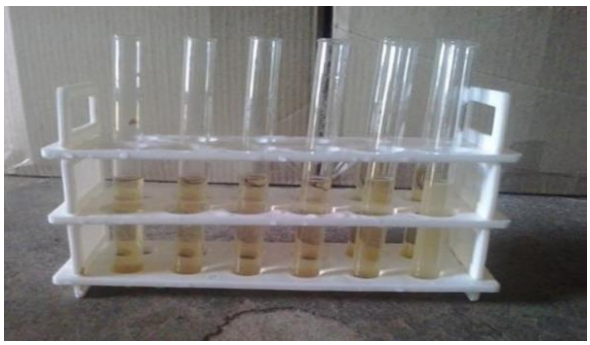


Fig 5. Serial dilution carried up to 10^{-8} dilution

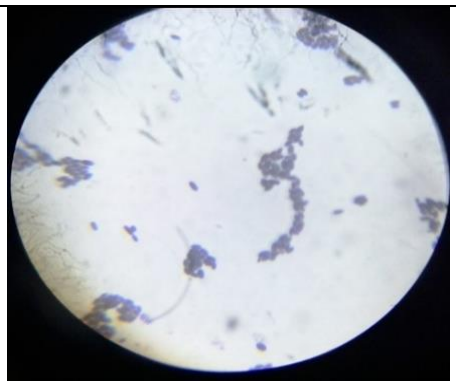
Fig 6. Growth on plates after serial dilution.

Fig 7. Beauveria colonies grown after serial dilution.

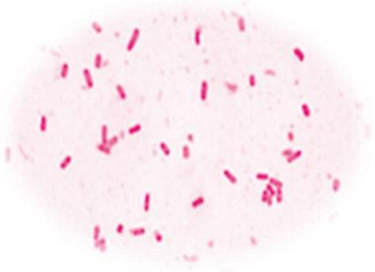
(B). MICROSCOPIC IDENTIFICATION

The pure colonies were picked and identified under Light microscope.

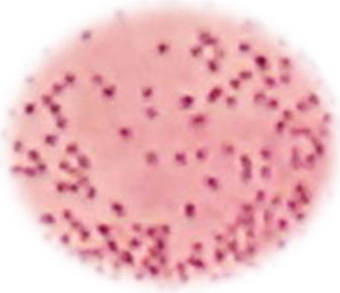
OBSERVATION



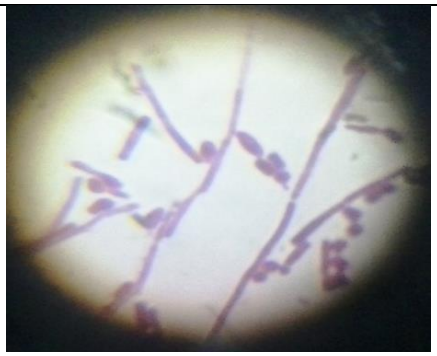
Azospirillum



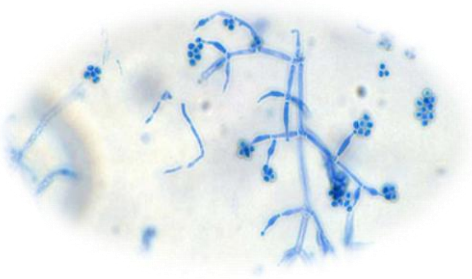
Acetobacter



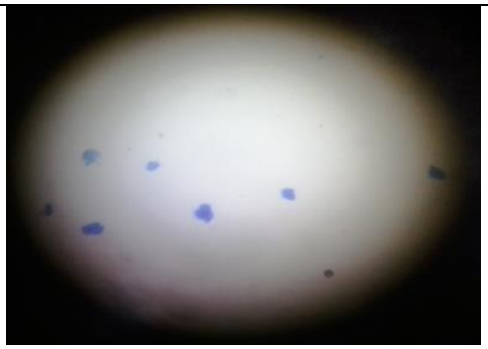
Phosphatesolubilizing bacteria



Pseudomonas



Tricoderma



Beauveria

Fig 8. Microscopic observation of organisms

III. SCALE UP PROCESS

(a).Large scale production and carrier preparation

After isolation and identification of Biocontrol agents, the large scale production is carried out. Preparation of appropriate media for specific to the bacterial and fungal inoculum in fermentor. Media prepared in large quantities, pH is adjusted to 6.5 - 7.0. Continuous aeration is done by forcing sterile air through sparger. Inoculum should be added @ 5%. Incubate culture till the bacterial population reaches 10^8 cells/ml. The broth is checked for the population of inoculated organism.

The use of ideal carrier material is necessary in the production of good quality of Biocontrol agents. Different carrier materials viz., peat lignite, compost, leaf manures, cellulose powder, charcoal powder, press mud etc are extensively used carrier for inoculum preparation. The lignite is used as carrier material in Biocontrol agent manufacturing.

Fig 9. (a) Press mud (b) Lignite (c) Charcoal (d) Cellulose powder (e) Leaf manure (f) Peat.

Carrier is sun-dried up to a moisture level of 5 %. The carrier is ground to pass through a 100-200 mesh sieves. The carriers are mixed with calcium carbonate to neutralize pH (6.5-7.0). Carrier is mixed with 10 % water before sterilization. The carrier material is sterilized and spread on a clean, dry, sterile metallic tray. Carriers are sterilized at 121°C for 3-4 h continuously or 1h each for three successive days. Grow culture in fermenter till population reaches to 10^8 cells /ml. Blend inoculum broth with the finely powdered and sterilized carrier. Add broth @ 1/3 of the water holding capacity of the carrier. Thoroughly mix the broth culture with sterilized carrier by manual mixing in trays aseptically. Curing is done by spreading the blended carrier on a clean polythene sheet for 24 h to ensure acclimatization of bacteria with the carrier.

Fig 10. Curing

(b). Packing and storage

After curing, the inoculant is ready to be packed. Select 50-75 micron polythene bags (6 x 10 inches). Dispense 200 g of inoculant in each bag manually or with automatic dispenser. Seal the polythene bags leaving 2/3 vacant spaces. Pin bags on few places for aeration. Keep inoculants for a week at room temperature (25-30°C). The packets should be stored in a cool place away from the heat or direct sunlight. The packets are stored at room temperature or cold storage conditions in lots in plastic crates and dispatch.

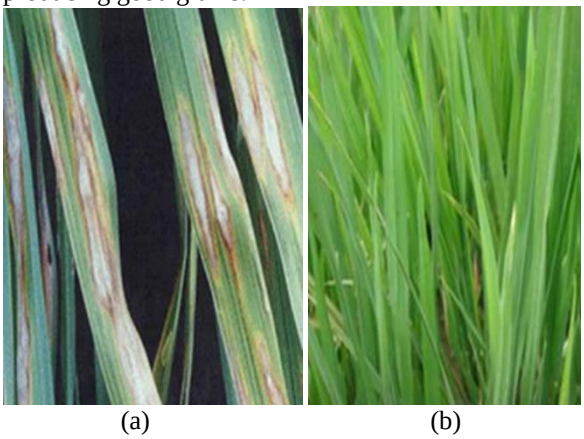
IV. RESULTS AND DSCUSSION

The biocontrol agent *tricoderma* was used against downy mildew of cucumber crop.The biocontrol agent was mixed with water and applied. The disease was controlled within 3 days of application and result showed that the further spreading of disease was controlled.

(a) (b)

Fig 11. (a) Cucumber with downy mildew (b) After *tricoderma* application.

The *pseudomonas* was used for neck blast, leaf blast and sheath blast of paddy as biocontrol agent. After application of *pseudomonas* the crops recoverd by blast and resulted in producing good grains.



(a) (b)

Fig 12. (a) Leaf blast of paddy (b) After application of *pseudomonas*

Beauveria was used as biocontrol agent for cucumber. The usage of beauveria prevented fruit fly, fruit borer, stem borer, root grubs etc..,

(a) (b)

Fig 13. (a) Cucumber affected with Fruit borer (b) After application of *beauveria.*

Nitrogen fixers *(azospirillum, azotobacter*) Psb and Ksb are used as biofertilizers for all type of crops. The usage of these organisms increased the yield upto 25% compared to normal yield

(a) (b)

Fig 14. (a) Nutrient deficient plant (b) recovery after application of NPK.

V. CONCLUSION

In this paper we have proposed the bio control agents and biofertilizer to get good yield with disease management. The isolation and identification of micro-organisms from competent products were successfully done by serial dilution and gram staining methods. Large scale production by fermentor and packaging of Biocontrol agent was successfully done. The application of *pseudomonas* to paddy crop affected by blast (sheath, neck, leaf) was successful, the disease spreading to other parts was controlled and infected parts gets dried. The application of *tricoderma* for cucumber crop showed result by controlled spread of Downy mildew to other parts of plant. The application of nitrogen fixers (azospirillum, *azotobacter*), psb and ksb resulted in improved growth of plant and increases yield up to 25%. The application of *beauveria* for control of pests was successful. The fruit infected by fruit borer in cucumber was controlled.

NOMENCLATURE

Symbols

PSB- Phosphate solubilizing bacteria
KBS- Potassium solubilizing bacteria

NPK–Nitrogen, phosphate, potassium
hrs- Hours
PDA–Potato dextrose agar
PDB-Potato dextrose broth
NB- Nutrient broth
NA–Nutrient agar

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