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Biocontrol of Fungal Diseases in Large Cardamom Using *Pseudomonas fluorescens*

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Pseudomonas fluorescens is widely used as a bioagent for disease management in large cardamom in Sikkim. The mass multiplication technique and field application schedule were worked out at Indian Cardamom Research Institute, Regional Station, Spices Board, Tadong, Gangtok, Sikkim for large cardamom cultivation. The bioagents, by virtue of their properties such as quick growth, fast multiplication capacity, antagonism to disease causal organism, hyperparasitsm and competition with other microbes, etc., suppresses the pathogenic fungi and promote growth and protect the plants from various soil borne fungal diseases of large cardamom.

Introduction

Large cardamom (*Amomum subulatum* Roxb.) is cultivated in the Sub-Himalayan state of Sikkim, Darjeeling district of West Bengal and in some other North-Eastern Hill states like Arunachal Pradesh, Nagaland, Mizoram, Manipur, Meghalaya and Assam. Sikkim is the largest producer of large cardamom. Sikkim being an organic state only eco-friendly and non chemical measures are to be adopted. Therefore, bioagents are now being used for managing various fungal diseases of large cardamom replacing the use of chemical fungicides. Biocontrol can make an important contribution to sustainable organic cultivation of large cardamom in Sikkim.

Bacterial Bioagent- *Pseudomonas fluorescens*

Pseudomonas fluorescens is a plant growth promoting rhizobacteria colonizing in the root zones (rhizosphere) of plants. These can grow, multiply and produce a number of hormones on organic matters, which promote plants growth and vigour. This also produces various types of substances which are antibiotic in nature and are helpful in protecting the plants from various diseases. The bacteria are both growth promoters as well as bio-control agents. In large cardamom cultivation the bio-control agent *Pseudomonas fluorescens* was found to be effective as a protectant against major fungal pathogens and thus, offer disease control. This bio control agent was best used as a prophylactic agent to prevent the occurrence of diseases in large cardamom.

Medium for Culture of *Pseudomonas fluorescens*

Kings B medium ('KB' medium)
Peptone- 20 g
Dipotassium hydrogen phosphate (K_2HPO_4) - 1.5g
Magnesium sulphate ($MgSO_47H_2O$) - 1.5 g
Glycerin (Glycerol) - 10 ml
Water - 1000 ml

Preparation of Culture Medium

Liquid culture of *Pseudomonas fluorescens* was prepared in liquid kings B medium (broth culture) as follows:

For 1 liter medium, weigh out 20 grams of Peptone powder (Bacteriological peptone), 1.5 grams of Magnesium sulphate ($MgSO_4$) and 1.5 grams of Dipotassium hydrogen phosphate (K_2HPO_4). Dissolve these in 1 liter of pure water. Stir well until these mix with the water. Then add 10 ml. Glycerin (Glycerol) to it and again stir well. Adjust the pH of the medium to 7.2 using a pH meter or pH indicator papers and dilute solution of Potassium di-hydrogen phosphate (KH_2PO_4). Pour out this solution into a clean flask. (Conical flasks of 500 ml capacity or Raux Bottles of 1 liter capacity are ideal). Plug the mouth of the bottle with rolled absorbent cotton tightly. Cover this with a piece of paper and tighten with a rubber band. This has to be sterilized in an Autoclave or Pressure cooker for 1 hr. After one hr, the cooker is removed from the flame and allowed to cool. After cooling, the lid of the cooker is gently removed and the flasks are taken out and kept ready for inoculation.

Fig.1 Mass multiplication of *Pseudomonas fluorescens* in liquid medium

Mass Multiplication of *Pseudomonas fluorescens*

For effective disease management, it is necessary that this bio-agent is to be multiplied artificially in the laboratory and applied to the plants either as spray or soil drench before the appearance of diseases. Two types of formulations namely solid and liquid forms are available. The liquid form is always better as it can provide continuous nutrient supply for bacterial multiplication. Also in liquid culture, the bacterium continues to produce hormones and antibiotics and make them easily available. The solid form is made in powder form with talcum powder. In talc, this bacterium does not multiply and grow but, remains alive up to three months.

Ingredients for mass preparation of different volumes of liquid medium are given below (Table1). The ingredients have to be carefully weighed out or measured and prepared under aseptic conditions using either glass wares or stainless steel vessels.

Table 1. Kings B medium for *Pseudomonas* (Weight in grams/volume in ml) (pH 7.2)

Volume	1L	5L	10L	15L	20 L	25L
Ingredients						
Peptone	20	100	200	300	400	500
Glycerol	10	50	100	150	200	250
Magnesium sulphate	1.5	7.5	15	22.5	30	37.5
DiPotassium hydrogen phosphate	1.5	7.5	15	22.5	30	37.5
Water	IL	5 L	10 L	15 L	20 L	25L

Inoculation

Inoculation is to be done very carefully. It is done in a Laminar Air Flow (LAF chamber) or

in a sterilized small chamber or room. The chamber has to be disinfected 30 minutes before starting the inoculation. The materials used for inoculation should be cleaned with rectified spirit to remove all contaminating microbes. *Pseudomonas* mother culture is to be procured from Indian Cardamom Research Institute, Regional Station, Spices Board, Tadong, Gangtok, Sikkim. Now, gently open the cotton plug of the sterilized bottle containing the prepared liquid medium under aseptic conditions. From the test tube or conical flask, pour out carefully about 1 ml. of liquid *Pseudomonas* mother culture into the liquid medium and immediately close with the cotton plug. Remove the inoculated flask and keep it for multiplication (incubation at room temperature) for about 48 hours. After 24 hours the clear medium turned turbid and as days passes on, the turbidity of the medium increases showing high concentration of the bacteria multiplying in it. The growth proceeds in full swing up to about 5 to 6 days. At this stage, the concentration is about 10^{12} cfu per ml. The liquid can be used directly for spraying or drenching in the field after dilution with water (3 to 5 liter in 100 liter of water). The solid preparation is made by mixing the *Pseudomonas* liquid with talc powder at 25 to 30% concentration.

Carrier Medium

Pseudomonas fluorescens has a very good potential for management of fungal diseases of large cardamom under field conditions. The multiplied cells suspensions in liquid culture should be prepared as formulations for easy application, storage, commercialization and field use. Carriers must be cheap and readily available for formulation development. Carriers used should support the survival of bacteria for a considerable length of time. Organic carriers used are peat, talc, turf, lignite, kaolinite, pyrophyllite, zeolite, pressmud, sawdust, coffehusk, vermiculite, etc. Formulations of *Pseudomonas fluorescens* were developed through liquid fermentation technology. The fermented biomass was mixed with different carrier materials and stickers. Carboxy Methyl Cellulose (CMC) is added as a sticker at 1:4 ratios to talc and blended with bacterial suspensions at a concentration of 10^{12} cfu/ml. The solid preparation is made by mixing the *Pseudomonas* liquid with talc powder/farm yard manure or vermicompost at 25 to 30% concentration.

Storage

The multiplied *Pseudomonas* liquid culture was poured in thick polythene bags and sealed using a sealing machine. The liquid preparation can be stored under refrigerated condition up to two weeks. However, it is better to use the liquid within a week as long storage in the liquid may develop a foul smell due to fermentation and production of various metabolites by the bacteria. Use of liquid preparations has been found more effective than the talc formulation (Solid formulation). This is due to the presence of actively growing cells which continue to produce antibiotics in the medium. In talc, *Pseudomonas* does not undergo multiplication but remains inactive. The shelf life period of solid preparation in talc is up to 3 months after which the population declines.

Field Application to Large Cardamom Nursery and Plantations

Large cardamom plants in the nursery and main plantation is often subjected to the attack by various soil borne pathogenic fungi and cause considerable crop loss. The fungal diseases of large cardamom are *Colletotrichum* blight caused by *Colletotrichum gloeosporioides*, Phoma leaf spot caused by *Phoma sp.*, Leaf streak caused by *Pestalotiopsis royenae* and plant wilt caused by *Fusarium oxysporum.*

Fig.2 Treatment of large cardamom suckers with *Pseudomonas fluorescens* in the field before planting

Pseudomonas fluorescens can be used for boosting the growth and controlling various fungal diseases of large cardamom. Liquid preparation of *Pseudomonas fluorescens* at a concentration of 10^8 cfu per ml. is used as sprays/ or drenching and also for pre treatment of large cardamom suckers before planting in the field (The concentrated liquid of *Pseudomonas* is diluted at 3: 100 i.e. three liters solution in 100 liter water).

The schedule recommended for large cardamom in the field is as follows:

During last week of April or first week of May carry out phytosanitation (by removing and burning infected plants/ plant parts) in the large cardamom nursery and plantations. *Pseudomonas fluorescens* can be sprayed per plant (3- 5 lit. conc. liquid in 100 lit. water or 3-5 Kg talc powder in 100 lit. water) depending on disease pressure in the field. During August and October the application of these bio agents can be repeated. Care should be taken not to use fungicides after application of bioagents.

Fig.3 Large cardamom plants applied with bioagent in the field

Conclusion

Fungal diseases such as *Colletotrichum* blight caused by *Colletotrichum gloeosporioides*, Phoma leaf spot caused by *Phoma sp.* and Leaf streak caused by *Pestalotiopsis royenae* of large cardamom in Sikkim can alternatively be controlled by using the bioagent *Pseudomonas fluorescens* in the field. This method is cost effective and environment friendly. This technology is easily adoptable to the farming community of large cardamom in Sikkim and other North-Eastern hill states of our country.