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Production of Biopesticides Namely *Trichoderma viride* and *Beauveria bassian*

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Abstract

Objectives: To highlight the barriers to the effective usage of biopesticides and solutions to these barriers for biopesticides produced from fungi *Trichoderma viride* and *Beauveria bassiana.* **Methods and Statistical Analysis:** Recent paper in last decade on production techniques and method of biopesticides from environmental friendly fungi namely *T. viride* and *B. bassiana* have been reviewed and analyzed. Findings: Optimized conditions of temperature and pressure can maintain the pH at about 7 which will result in an enhancement in the shelf life of the biopesticides from 2-3 months to about 1 or 1.5 years. Effective selection of carrier can benefit in the stability of the biopesticides characteristics and also decrease the cost of the products. The usage of metabolites and additives can highly affect the performance of the bio products and can increase their potential. **Application/Improvements:** Active packaging could greatly enhance/increase the shelf life of the biopesticides and thereby making them viable alternative cheap ecofriendly biopesticides for organic farming.

Keywords: *Beauveria bassiana,* Biopesticides, Organic Farming, *Trichoderma viride*

1. Introduction

Biopesticides are powerful tools for sustainable agriculture products as these are the alternatives to chemical pesticides as they offer a solution to problems such as pest resistance. Biopesticides are certain types of pesticides which are derivative of naturally occurring substances like bacteria, plants, animals and certain minerals. Biopesticides also consist of active creatures that have the ability to repel the agricultural pests. They are quite less harmful as compared to the chemical pesticides. Biopesticides are generally host specific and do not harm the pests that are beneficial for soil health whereas, the chemical pesticides may also mark the organisms which are desired such as mammals, birds, and insects.

2. Fungal Biopesticides

In the growth of diseases on many agricultural crops, the fungal pathogens show a key part causing serious losses in the productivity of crops[1]. Fungal biopesticides can control insects and plant diseases and their method of operation varies and depends on the pest and pesticidal

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fungus. Two of the most common commercial fungal biopesticides are *Trichoderma viride* and *Beauveria bassiana*. In this review, biopesticides are produced using *T. viride and B. bassiana*.

2.1 Trichoderma Viride

T. viride is a fungal antagonist present in almost all farming soils. For multiplying its own spores, Trichoderma rise in the direction of the mycelium of disease producing fungi and then discharge enzymes. Many crop diseases are controlled by *Trichoderma* which consists of phaseolin, fusarium wilt, root rot and many seed plant diseases. Banana, brinjal, cardio, tomato, ginger, guava, tobacco, potato, coffee, soybean, chickpea, tea, black gram, cotton, turmeric, pea, groundnut, citrus, sunflower, black pepper, sorghum, sugarcane and sesame are some of field and vegetable crops in which Trichoderma can be used. This can be also used for regulating various fungal diseases which are soil borne. The variety of plant pathogenic fungi for which this biocontrol agent is effective consists of *Botrytis cinerea, Pythium spp., Sclerotium rolfsii, Rhizocctonia solani, Slerotinia homoeocarpa and Fusarium spp.*

2.2 Beauveria Bassiana

Beauveria is a type of fungus which is occurring naturally in soils and has been tested for using against soil-borne insects. A number of soil insects have natural tendency to resist these pathogens. Some of the soil insects such as foliar pests do not exhibit this characteristic. Therefore, the development of this fungus is being targeted to resist the foliar feeding pests. *B. bassiana* is responsible for many diseases such as muscadine disease in insects. The *B. bassiana* spores start growing in the internal body of the masses as it came in contact with the cuticle of the specified insects. The fungus starts producing toxins as it gets multiplied through the body of the insect and drains out the nutrients from the insect, ultimately slaying them. The fungal pathogens are not desired to be eaten up by masses in order to be effective, unlike bacterial and viral pathogens. As a white mould, the fungus raises over the cuticle after the death of host and discharge out millions of fresh infectious spores. The rate of killing a host of Beauveria spores is temperature dependent, for example, the plant lice are killed in 3 to 5 days at a constant temperature of 72^0 F. It can be used against number of insects such as aphids, weevils, whitefly, thrips, mealybugs and psyllids. This can be also useful for plantation crops comprising ornamentals, vegetables, turf, fruits and berries, forestry, and herbs and species.

3. Existing Methods

In[2] studied the effects of various parameters on the production process. Solid State Fermentation (SSF) is a better option as compared to submerged liquid fermentation due to its applications in the production of Biopesticides, enzymes, biopharmaceuticals, aroma compounds etc. and also due to the microorganism's growth similar to their natural habitat. The selection of the substrate and suitable microorganisms, isolation, optimised process parameters are some important factors to be considered. Substrate particle size should be between optimum as it affects the process at large rate and should between 180 µm to 1.4 mm. The degree of polymerization and crystallinity are the chemical factors that affect solid substrate utilisation. The important parameters to be controlled are temperature, pH aeration and agitation. The temperature varies largely around the trays. pH, control is very difficult in SSF process but pH correcting solutions can help in maintaining it during the process. Water activity is also an important factor which affects the product formation. Better knowledge of the design and process parameters can be helpful in the successful implementation of the SSF process.

In[3] studied a vast variety of compounds comprising fungicides, herbicides, insecticides, rodenticides, nematicides. Plant growth regulators and others are covered by the term pesticides. Insecticides named as Organochlorine (OC) have been successfully used for monitoring many diseases, for example, typhus and malaria. The pesticides

are preferred by the farmers due to their fast, simple and cheap resolution for monitoring the pest problems. Due to these advantages, their extensive use leads to their accumulation in the environment in a significant amount. The deposits of the pesticides have been detected in ground and surface water as well as in air and soil throughout the nations and ushered serious issues. Even the herbicides can contribute to the destruction of the environment. Due to the maximum usage of weed killers, they can also cause severe problems. So this paper concluded that in order to minimize the accumulation of pesticides in the environment and to prevent long-term impacts, the usage of synthetic pesticides should be decreased by using eco-friendly solutions to the problems.

In[4] studied that the biopesticides are derived from the microbes and other natural resources. The processes of biopesticides production consist of the genetic integration of DNA which deliberate a shield against damage caused by pests. The acceptance of biopesticides by the farmers can be enhanced by the implementation of organic farming and forcing the use of products which should be chemically free. The paper concluded that in order to register a product in India, the registration committee needs information on the efficiency of the product, its chemistry, poisonousness and labeling and packaging. A few of biopesticides such as *Bacillus thuringiensis* var. *galleriae*, *Bacillus sphaericus*, *Trichoderma viride*, *T. harzianum* and neem-based pesticides are previously registered in India and also being used.

In[5] studied the production of biopesticide from *Bacillus thuringiensis* using commercial lab media. Accordingly, medium 1 showed the maximum spore concentration during the period of fermentation.

In[6] studied the effects of modified atmosphere packaging on the *Beauveria bassiana*'s shelf life at high temperatures. Shelf lives are mostly tested under refrigerated storage conditions but its determination under non-refrigerated conditions is of vital importance particularly under high temperatures. They shelf life of *B. bassiana* can be increased using optimal conditions. The improvement in the shelf lives of biopesticides under non-refrigerated conditions is very important. Active packaging should be broadly adopted to enhance the shelf life of *B. Bassiana*.

In[7] studied the influence of sources of carbon and nitrogen on *B. bassi*ana and *T. viride*. The study of the mycelium growth and sporulation of both the fungi was done. With the availability of the Czepeck Dox Broth media, the effects of nitrogen and carbon sources were determined. The *T. viride* is known for its biofungicide whereas the *B. bassiana* is entomopathogenic fungi. It was concluded that these fungi represent more growth rate on all the nutrient sources considered for the study. In the Czepeck Dox media, the sources of nutrients are potassium nitrate, ammonium sulphate and sodium nitrate. The *T. viride* showed maximum biomass product in ammonium sulphate whereas *B. bassiana* showed in potassium nitrate.

In[8] studied *B. bassiana's* mass production on solid and liquid phase for its conidiosopre and clamidospore production. The paper concluded that the various extracts of plants used as solid and liquid media were different at 1% probability. Among the liquid media, potato culture media gives lowest spore production of 12 spores/ml whereas sugar cane molasses gives the highest production of 2.4 × 108 spores/ml. Among the solid media, the carrot was concluded as the cheapest and most suitable media and maximum spore production was supported by rice waste. So the maximum production, as well as the optimised economic performance, was given by the sugar cane molasses and rice to produce conidiospores and conidia of *B. basssiana*.

In[9] studied mass production, quality control and formulation of *T. viride*. In the study, it was concluded that as compared to the synthetic pesticides, biopesticide universalization is very slow. Trichoderma is the most used biopesticide and is very successful. In India, *T. viride* and *T. harzianum* got the positions of significant organic con-

trol agents for reducing the crop diseases. Depending on the various species of *Trichoderma*, many effective products have been obtained. The isolates of *Trichoderma* are formed using various inorganic and organic carriers. This can be done by using either liquid or solid fermentation technologies. The distribution of the end product can be done through bio-priming, seed treatment, soil application, foliar spray and seedling dip. For managing the pest and crop diseases and also for promoting the growth of plants, the usage of formulations of *Trichoderma* along with mixtures of strain accomplish better as compared to individual strains. The paper concluded that the application of biopesticides is basically slowed down due to the poor shelf life of these products. Thus, the studies should be done in order to enhance the shelf life. The shelf life can be increased by the development of strains that are superior or in other words by developing the organic formulations with less contaminant that can support the higher shelf life.

In[10] studied that the eco-friendliness and easy decomposition are the salient features of the biopesticides. These features contribute in decreasing the pollution problems that are caused by the usage of synthetic pesticides. Moreover, as the biopesticides are giving the same level of productivity as the chemical pesticides, their demand is increasing day by day. In order to use the biopesticides efficiently, the farmers should be made more aware about pest management. According to the paper, though the biopesticides are replacing chemical pesticides due to advantages such as less research expenses, quicker product advancement and also their simple process for registration but still there are many factors like low reliability, target specificity, slow in action and shorter shelf life which have restricted the growth of biopesticides and need to be improved.

In[11] gives a review on the mass production and commercialization of the *Trichoderma*. The various methods for the formulation of *Trichoderma* are talc based, triticum-wheat bran based, pesta granules based, coffee based formulations in an organic food base has a longer shelf life in contrast to the organic food bases. The efficacy of the formulations can be improved by molecular approaches, genetic recombination, mutation and developing compatible consortia. The usage of *Trichoderma* along with the mixtures of strain accomplish better as compared to the individual strain. *Trichoderma*'s performance in the formulations can be enhanced using oils, water-soluble adjuvants, emulsions and stickers but a good knowledge is essential for their usage due to certain demerits associated with them which can alter the efficacy. Seed bio-priming gives optimum results as such seeds can tolerate the adverse conditions of the soil more effectively. Particularly for managing the soil-borne diseases, soil treatment is the most effective application method. Basically, poor shelf life is the biggest constraint in the commercialization of the biopesticides. Researches should be carried out to optimise the solid or liquid fermentation methods for mass production with a suitable medium. All the problems related to the Trichoderma products can be sorted out by using mixtures of beneficial organisms, increasing biological control by manipulating the surrounding or by identifying the accurate strain using molecular approach.

In[12] used the naturally arising substances such as plants and microbes, to form the environment-friendly pesticides known as biopesticides. The paper concluded that India has a wide probability of biopesticides. The some of the biopesticides which are presently being used in the country can be a brilliant solution for replacing synthetic pesticides. As the biopesticides are host specific so they are assumed to be safer for the soil beneficial pests and human beings. Though, India is already using many biopesticides such as NPV, BT, *Trichoderma* and neem based pesticides etc. Moreover, there are number of locally existing plants in India which can be simply treated such as garlic, neem, triphala etc. and can enhance the consumption of biopesticides

Table 1. Production of biopesticides namely Trichoderma viride and *Beauveria bassiana*

Serial no.	Author/ Reference	Type of fungi/ bacteria	Major Finding
1.	2	Fungi	Better knowledge of the design and process parameters can be helpful in the successful implementation of the SSF process
2.	3	Various Pesticide	Pesticide impact on the Environment. So the usage of synthetic pesticides should be decreased by using eco-friendly
3.	4	Micros Pesticide	Biopesticides production consist of the genetic integration of DNA.
4.	5	Bacillus thuringiens	Testing of different media for growing B.thuringiensis was investigated in Commercial lab medium.
5.	6	Beauveria Bassiana	The shelf life of Beauveria Bassiana can be increased using optimal conditions of pressure and temperature parameters.
6.	7	Trichoderma viride and B. Bassiana	The Trichoderma viride showed maximum biomass product in ammonium sulphate whereas B.bassiana showed in potassium nitrate.
7.	8	Trichoderma Viride	Mass production, quality control and formulation of Trichoderma viride by using mixtures of beneficial organisms, increasing biological control.
8.	10	Beauveria bassiana	Suitable medium for Beauveria Bassiana's production in solid and liquid phase.

Table 1 Continued

9.	11	Trichoderma	The usage of Trichoderma along with the mixtures of strain accomplish better as compared to the individual strain.
10.	12	Biopesticides	Biopesticides are host specific and India have wide market to access.
11.	13	Bioformulation	Protectants, attractants, additives, and stimulants are the various forms of the metabolites which can be used in the bioformulations and aid the microbial growth.

In[13] studied the role of metabolites and additives in bioformulations. The biopesticides are not still able to take over the chemical pesticides in the market due to certain limitations such as low organic carbon in the soil, unstable performance in the fields, poor quality, less mass production and poor shelf life. The metabolites and additives can improve the effectiveness of the product. The biomolecules such as Flavanoids and lipochitooligosaccharides can be helpful in increasing the ability to tolerate the environmental stresses on the crop and will thus increase the yield. Protectants, attractants, additives and stimulants are the various forms of the metabolites which can be used in the bioformulations and aid the microbial growth. Table 1 shows the production of biopesticides namely *T. viride* and *B. bassian*.

4. Conclusions

From the literature reviewed these following conclusions can be drawn:-

- The biopesticides are completely biodegradable hence these are environmental compatible.
- The biopesticides can help in reducing the Carcinoma as Chemical pesticides are seen as one major root cause of cancer.
- The proper selection of carrier can benefit in the stability of the biopesticides characteristics and also decrease the cost of the biopesticides.
- Shelf life can be increased by the development of novel strains.
- Active packaging can be helpful in increasing the shelf life of the biopesticides.

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