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TRICHODERMA AS A POTENTIAL BIOCONTROL AGENT

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ABSTRACT

Plant pathogens are the most important factors affecting the production of crops inflicting severe losses to agricultural products every year. Utilization of bioagents to control the plant diseases has been considered a viable alternative method to chemical control. Use of biopesticides is an ecofriendly method of disease control. The genus Trichoderma comprises a number of fungal strains that act as biological control agents, the antagonistic properties of which are based on the activation of multiple mechanisms. The antagonistic activity of Trichoderma sp showed that it is parasitic on many soil-borne and foliage pathogens. The fungus is additionally a decomposer of cellulosic waste materials. Different mechanisms of action for controlling phtopathogens by Trichoderma sp entails competing for nutrients and space, mycoparasitism, promoting plant growth and inducing the defensive responses in plants. A number of successful products based on different species of Trichoderma have been commercialized in India. The potential Trichoderma isolates are formulated using different organic and inorganic carriers either through solid or liquid fermentation technologies. They are delivered either through seed treatment, bio-priming, soil application, and foliar spray. A significant improvement has been made in different aspects of biological control of plant diseases. For more effective biological control strategies it is necessary to carry out more research on less developed aspects of biocontrol together with improvement of novel formulations, understanding the impact of environmental factors on biocontrol agents, their mass production and utilization of biotechnology and nanotechnology in improvement of biocontrol mechanisms and strategies.

Key words:Trichoderma sp, Biocontrol Agent, Mechanism of Action, Formulation.

I.INTRODUCTION

With the advent of agriculture, plant diseases became a problem. Thousands ofinsects, fungi, viruses, bacteria, nematodes and other living forms are known to cause potential hazards toagricultural crops. Distinctive methodologies may be utilized to prevent, mitigate or control plant diseases.Theuse of chemical pesticides has increased dramatically amid the last few decades. Control of pests with synthetic chemicals consequences in numerous problems. They can also be harmful to humans and domestic animals. In the environmentally conscious world consumption of food material treated with pesticides is disregarded, and the food materials produced through organic farming are gaining preference [1].Other environmental concern is the contamination of ground water[2].Consequently, the development of non-chemical and eco-friendly alternative to govern plant diseases is the need of the hour. Biological control of plant diseases has been considered as a viable alternative to manage plant diseases [3].Biological control is the purposeful utilization of introduced or resident living organisms, other than disease resistant host plants, to suppress the activities and populations of one or more plant pathogens [4].

Trichoderma spis most commonly used biological control agent and have long been known as effective antagonistic against plant pathogenic fungi [5, 6, 7, 8].*Trichoderma* spas a potent fungal biocontrol agent against a range of plant pathogens has attracted considerable scientific attention during last few decades [9, 10].The success of *Trichoderma* sp. as biocontrol agents (BCAs) is due to their high reproductive potential, ability to survive under very unfavourable conditions, efficiency to utilize nutrients, potential to alter the rhizosphere, sturdy aggressiveness against phytopathogenic fungi and efficiency in promoting plant growth and defense mechanisms. These properties have made *Trichoderma* spa ubiquitous genus found in any habitat and at excessive population density [11].

II.TRICHODERMA AS BIOPESTICIDES

Biopesticides are living organisms (naturalenemies) or their products (phytochemicals, microbial products) or byproducts which may be utilized for the management of pests that are injurious toplants [12].The biopesticide based on these microorganisms have also successfully controlled these diseases [13, 14].The potential benefits to agriculture and public health programmes using biopesticides are considerable and the advantages associated with their use are tremendous. As they pose less threat to the environment and to human health.Primarily based on pathogenic microorganisms specific to a target pest, or, in some cases, a few target organisms, biopesticides offer an ecologically sound and effective solution to pest problems. Not only this they are effective in very small quantities and often decompose rapidly, thus resulting in lower exposures and largely averting the pollution problems and whilst used as a component of Integrated Pest Management (IPM) programs, biopesticides can contribute significantly.The most commonly used biopesticides are living organisms, which are pathogenic for the pest of interest. These include biofungicides (*Trichoderma*), bioherbicides (*Phytopthora*) and bioinsecticides (*Bacillus thuringiensis*). *Trichoderma* species and *Pseudomonas fluorescens* have been found effective in suppressing the soil borne plant pathogens [15, 16].

*Trichoderma*as a genus was first proposed by[17]in Germany. He described it as fungi having mealy powder like appearance enclosed by a hairy covering. The four species proposed by Persoon were,*T. viride, T. nigroscens, T.aureum* and *T. roseum*). A huge diversity is found among the species of *Trichoderma*. They are easy to isolate and culture, multiply rapidly on different substrates, act as mycoparasite,producers of spores and effective antibiotics, antifungal compounds, secondary metabolites and enzymes. They occur worldwide and are typically associated with roots,temperate and tropical soils and plant debris [18].

2.1Mode of action

The potential value of the genus *Trichoderma* as bioagents was first reported by [19]. Laboratory and field trials in Viet Nam has proved that *Trichoderma* species has potential to suppress growth of fungal plant pathogens and enhance plant growth and development. Experiments performed on several crops including peanut, tomato, cucumber and durian suggest that *Trichoderma* strains canbe effectively used to control diseases as a result of fungal pathogens consisting of: *Phytophthora palmivora*, *Rhizoctonia solani*, *Fusarium* sp, *Sclerotiumrolfsii* and *Pythium* sp. [20, 21].*Trichoderma* sp. are biocontrol agent effective against fungal phytopathogens. They can act indirectly, by competing for nutrients and space. Biocontrol may additionally result from an immediate interactionamong the pathogen itself and the biocontrol agent, as in mycoparasitism.*Trichoderma* biocontrol agents can promote plant growth and stimulate the plant defence mechanisms [6, 18, 22].Weindling (1932) [19]was the first to report mycoparasitic activity by *Trichoderma* sp. Since then several studies on the suppression of soil borne fungal diseases, with the application of *Trichoderma* sp. have been made in many countries [6].The mechanisms can be described as below.

1.1.1 Competition

Biocontrol agents and the pathogens compete with one another for the nutrients and space to get established in the environment. This process of competition is considered to be an indirect interaction between the pathogen and the bio control agent wherein the pathogens are excluded by the way of depletion of food base and via physical occupation of site [23].The most common cause of death of microorganisms is starvation, thus competition for limited nutrients isparticularlyvital in the biocontrol of phytopathogens.Iron uptake is essential for filamentous fungi and underiron starvation; fungi excrete low-molecular weight ferriciron-specific chelators, termed siderophores. *Trichoderma*sp. producesextraordinarilyefficient siderophores that chelate ironand prevent growth of other fungi [24].Therefore, soil characteristics affect*Trichoderma* as abiocontrol agent. *T. harzianum* T35 has been proved to manipulate*Fusariumoxysporum* through competing for both rhizosphere colonization and nutrients, with biocontrol becoming more effective as thenutrient concentration decreases [25].

Trichoderma has an advanced potential to mobilize and take up soil nutrients compared to other organisms due to its capacity to gain ATP from the metabolism of different sugars, cellulose, glucan and chitin which are widespread in fungal environment, all of them rendering glucose [26].The key components of glucose metabolism encompass assimilation enzymes and permeases, together with proteins concerned in membrane and cellwall modifications.

1.1.2 Mycoparasitism

Mycoparasitism is direct interactionbetween *Trichoderma* and pathogen. It is a complex process which includes chemotropism and recognition; attachment andcoiling; cell wall penetration; and digestion of host cell. Initially, the mycoparasite grows directly towards its host and coils around it or attaches to it with the aid of forming hook-like structures and apressoria. Following these interactions, *Trichoderma* sp. sometimes penetrate the host mycelium, apparently by partially degrading its cell walls [27]. Subsequently, it is far assumed that *Trichoderma* spp. utilize the intracellular contents of the host.The key enzymes suggested in the lysis of phytopathogenic fungal cell walls during mycoparasitic action are Chitinases and b-1,3-glucanases proteins secreted by *Trichoderma* sp [28, 29].Mycoparasitic *Trichoderma* spare used commercially as biological control agents against plant-pathogenic fungi inclusive of *Rhizoctonia solani*, *Botrytis cinerea*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Pythium* sp., and *Fusarium* sp. in, amongst others, the United States, India, Israel, New Zealand, and Sweden as a promising alternative to chemical pesticides [18].

1.1.3 Promotes plant growth

Apart from control of pathogens*Trichoderma* sppromotes plant growth and root development,crop productivity, resistance to abiotic stresses and the uptake and use of nutrients [30].Species of *Trichoderma* were shown to set up robust and long-lasting colonisations of root surfaces via penetrating into the epidermis and produce compounds that set off localized or systemic plant resistance responses. Plants react against fungal invasion by synthesizing and accumulating phytoalexins, flavonoids and terpenoids, phenolic derivatives, aglycones and other antimicrobial compounds.*Trichoderma* strains are commonly extra resistant to thesecompounds than other fungi. Their ability to colonizeplant roots strongly relies upon the ability of eachstrain to tolerate them. This resistance, considered anessential requirement for plant colonization, has been related with the presence of ABC transport systems. Several studies imply that the fungi enhances tolerance to abiotic stresses during plant growth [31], due to improved root growth, improvement in water-holding capacity of plants, or enhancement in nutrient uptake (i.e., potassium); whereas, in the absence of stress, plant growth may or may not be enhanced. *Trichoderma* sp. have been shown to improve growth of lettuce, tomato and pepper plants [32].Earlier reported that the secondary metabolites produced by *Trichoderma koningii* (koniningin A) and *Trichoderma harzianum* (6-pentylalphapryone) act as plant growth regulators [33].

1.1.4 Induces Plant defence

Numerous studies have reported that pre-treatment of plants with*Trichoderma* sp. accelerated plant resistance to pathogenattack [30]. They stimulate the induction of resistance mechanisms much like the hypersensitive response (HR), systemic acquired resistance (SAR), and induced systemic resistance (ISR) in plants [24, 22]. In a study of cucumber plants, *T.asperellum* induced a systemic response of two defencegenes encoding phenylalanine and hydroperoxidase lyaseand systemic accumulation of phytoalexins against*Pseudomonas syringae* pv. *Lachrymans* [35]. [30] Reported that the induction oflocalized or systemic resistance is a critical issuefor plant disease control by *Trichoderma* sp. As a consequence diseasecontrol through root-colonizing *Trichoderma* sp. entails acomplex interaction among the host plant, the pathogen,the biocontrol agent and numerous environmental factors[30, 36, 37].

2.1.5 Production of antibiotics and secondary compounds

Most *Trichoderma* sp.producesseveral secondary compounds, consisting of antibacterial and antifungal antibiotics such as polyketides, pyrones, and terpenes. Secondary metabolites play essential roles in the defence response, symbiosis, metal transport, differentiation, and stimulating or inhibiting spore formation and germination [38, 22], secondary metabolites produced by *Trichoderma* sp., can be volatile compounds (e.g., 6-pentyl-alpha-pyrone), water-soluble compounds (e.g., heptelidic acid), and peptaibol compounds, which are linear oligopeptides composed of 12-22 amino acids that are rich in alpha-aminoisobutyrate, N-acetylated at the Nterminus and have an amino alcohol institution at the C-terminus. Antibiotics are frequently associated with biocontrol activity [39]. *T. harzianum* has been reported to exhibit biocontrol activity against Ganumannomyces graminis due to the production of pyrone like antibiotics [40].

III. MASS PRODUCTION OF TRICHODERMA

There are two methods utilized for mass production of Trichoderma, (a)Solid state fermentation, (b) Liquid state fermentation.

3.1 Solid state fermentation

Solid state fermentation is a common method for large scale production of *Trichoderma*. It hasgained renewed interest as it exhibits certain product recovery, and reduced energy requirements. Solid state fermentation system includes cultivation on natural substrates or on an inert support impregnated with a liquid medium relying on the nature of solid phase used. Various cheap cereal grains like, sorghum, millets, ragi are utilized as substrates [41]. The grains are moistened, sterilized and inoculated with*Trichoderma* and incubated for 10 to 15 days. A dark green spore coating is delivered by Trichoderma on the grains.These grains can be powdered finely and used as seed treatment or the grains can be utilized as it is for soil application.This strategy is appropriate for smallscale production in cottage industries or at individual farmer level.

3.2 Liquid state fermentation

In this method *Trichoderma* is grown in liquid media in stationary/shaker/fermenter cultures formulated and utilized for field application. Potato dextrose broth, V-8 juice, Molasses-yeast medium and Wheat bran are usually utilized for the large scale production of *Trichoderma* sp.[42, 43, 44]. Maximum biomass of *Trichoderma* sp. can be produced in short-time by utilizing appropriate medium in a fermentor with aeration, agitation,temperature, pH and antifoam controls than in shake-flask cultures and are more suitable for industrial production of *Trichoderma* sp.

IV. METHODS OF APPLICATION

The most common methods of application of *Trichoderma* are described as:

4.1 Seed treatment

It involves coating of seeds with dry powder or dusts of *Trichoderma* just before sowing. Propagules of biocontrol agentsgerminate on the seed surface and colonize rootsof germinated seedlings and rhizosphere [45].

4.2 Seed biopriming

In this method seeds are treated with *Trichoderma* and incubated under warm and moist conditions until just prior to radical emergence resulting in rapid and uniform seedling emergence. On germination *Trichoderma* forms a layer around bioprimed seeds which increases the tolerance of adverse soil conditions. Seed biopriming was successfully used in tomato, brinjal, soybean and chickpea in Tarai region of Uttaranchal[46].

4.3 Root treatment

This method is usually used for the vegetable crops, rice where transplanting is practiced. It involves treatment of seedling roots with spore or cell suspension of antagonists either by drenching the bioagent in nursery beds or by dipping roots in bioagent suspensionbefore transplanting. Root dipping in antagonist's suspension not only reduces disease severity but also enhances seedling growth in rice, tomato, brinjal, chili and capsicum [47]

4.4 Soil treatment

It involves application of biocontrol agents to the soil and other growing media before or at the time of planting for control of wide range of soil borne fungal pathogens [48]. This method ismostly suited for greenhouse and nursery.

4.5 Aerial spraying

Trichoderma has been successfully applied to aerial plant parts for the biocontrol of decayfungi in wounds on shrubs and trees [6].

V. FUTURE PROSPECTUS

Being plant symbionts and effective mycoparasites, numerous species of the genus Trichoderma have the potential to become commercial biofungicides. The challenge in this field of research can be the development of reliable screening techniques, which allow for prediction of the biocontrol efficiency of a given isolate by determination of the key factors for this process. Nonetheless, also the ecological effects of widespread application of a single (or few) fungal species in agriculture remain to be investigated in order toensure a truly beneficial effect for the environment.Biofuel production is now being pushed to decrease the requirement for fossil fuels. In this respect, production of the so-called second generation biofuels from agricultural waste products by the aid of cellulases and hemicellulases produced for example by *T. reesei* and fermentation of the resulting oligosaccharides by yeastprovides an alternative strategy. Besides theforemost applications of *Trichoderma* sp., the fields of green and white biotechnology becomeincreasingly important for environmentally safe production of enzymes and antibiotics. These industrial applications can also benefit from studies on molecular physiology and regulatory processes, which continuously display novel and valuable metabolites and enzymes in addition to components to be modified or adjusted for cost effective high yield production.The extensive research on various physiological traits available and nonetheless progressing for *Trichoderma* make these fungi versatile model organisms for research on industrial fermentations as well as natural phenomena.

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