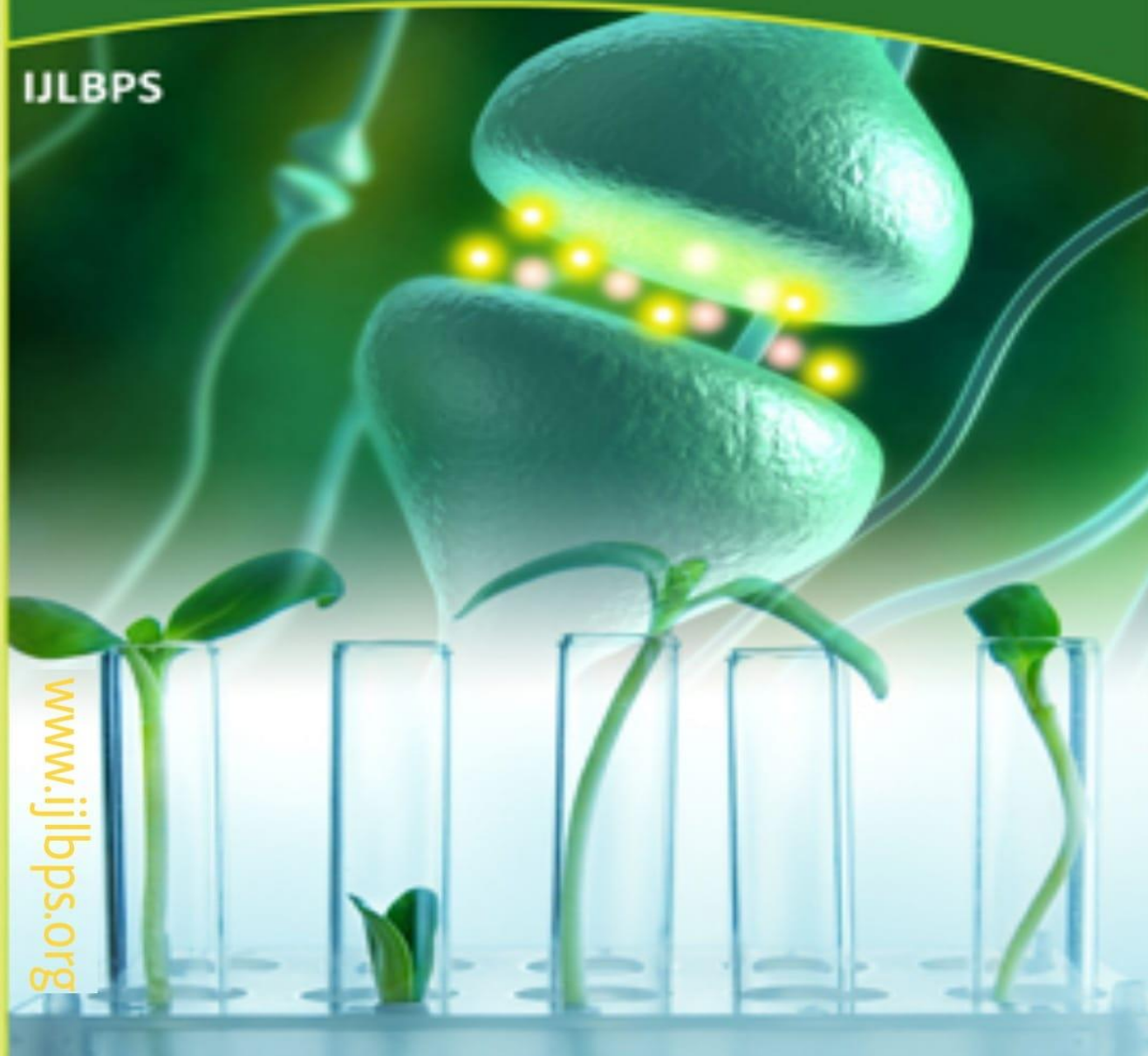




ISSN 2395-650X

International Journal of
Life Sciences Biotechnology Pharma Sciences

IJLBPS



www.ijlbps.org

E-mail: editorijlbps@gmail.com editor@ijlbps.org



The isolating, naming, and mass-producing of the bio-control agent Trichoderma

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Abstract

Overuse of chemical pesticides has polluted the environment, thus there has been a resurgence of interest in integrated pest management, in which bio-pesticides are employed instead of chemical ones. When it comes to combating various plant infections, the fungus *Trichoderma candidum* shows promise as a bio-control agent. Selection of effective strains, creation of cost-efficient procedures, for mass multiplication, effective ways of storage, transportation, and its formulation are the primary issues involved in the industrial production and use of biocontrol agents. Use of home trash, vegetable waste, and other wastes as substrates for mass growth of *Trichoderma candidum* is the focus of the current research, and a practical, readily implementable production process is developed as a result.

Key-Words: *Trichoderma candidum*, Biocontrol, Mass production, Formulation

Introduction

Approximately 10–20% of global food production is lost every year as a direct result of plant diseases, costing billions of dollars. The problem with chemical control is that it may kill out good species along with the bad ones, and it also poses a danger of contamination to humans and the environment. In order to combat soil-borne plant parasite fungus, *Trichoderma candidum* has been shown to be an effective biological control of infections. Fungal infections are mostly responsible for the severe plant yield losses that accompany the development of illnesses on many key field and horticultural crops. The diseases' resistance has increased in part due to the increased use of fungicides, which has led to the accumulation of toxic compounds that might be harmful to humans and the environment. Effective alternatives to chemical control are being used to address these national and global issues. Biological control is a method

that doesn't harm the environment since it employs harmful microorganisms. Antagonistic biological management is a promising nonchemical and environmentally benign method for treating plant diseases¹. The biological/integrated management of soil-borne fungal plant infections using fungal antagonists like *Trichoderma* spp. has received the most attention among several classes of plant diseases. Worldwide, *Trichoderma* is employed as a fungal biocontrol agent for the effective control of a broad range of foliar and soil-borne plant pathogens². It has been widely reported that using biocontrol agents like *Trichoderma* spp. may mitigate the negative effects of pesticides without breaking the bank. Therefore, these biocontrol agents have recently been found to work against a wide variety of significant soil-borne plant infections that may result in devastating crop losses.

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In light of the high price and potential dangers of chemical pesticides, biological management of plant diseases looks to be a viable alternative. As an integral part of an IPM program, biological control strategies have shown to be extremely compatible with sustainable agriculture. The effectiveness of biological control depends on mass manufacturing, stability, and the ability to establish bioagents in the desired niche. Concerns with augmentative biological control center on large-scale, cost-effective manufacturing, formulation stability, bioagent establishment in the specified niche, and consistency in disease control. The biocontrol arsenal requires research on how technology may be adapted. The creation of viable, quickly prepared, and economically sound delivery formulas need to be a primary focus. Solid state fermentation technique requires a massive amount of spore biomass for bioagent mass multiplication on a large scale. Baggase from sugarcane, fruit juice, and other substrates *Trichoderma candidum* is being mass-produced using a wide variety of waste products, vegetable waste, spoiled wheat grains, etc., with varying degrees of success³. Wastes from sugarcane baggase, fruit juice, vegetable, and spoiled wheat grains add to the already serious pollution and disposal issues. For this reason, the current study is being conducted to investigate locally accessible inexpensive substrates for bulk proliferation of *Trichoderma candidum* for sustainable environment and sustainable agriculture. Compared to submerged (liquid state) fermentation, solid state fermentation provides benefits including higher volumetric productivity, lower equipment costs, higher product yields, lower waste creation, and shorter processing times.⁴.

Substances and Techniques

Detection and Isolation of *Candida Albicans* Types of Fungi The soil fungus *Trichoderma candidum* was cultured on potato dextrose agar (PDA) medium. Multiple tube dilution method (MTDT) inoculations were performed over plates, and the plates were then incubated at 30 degrees Celsius for four days.

Colonies of the fungus were collected, then separated by streaking, before being cultured at 30 degrees Celsius for seven to eight days. Fungal entities with green conidia were chosen for microscopic examination, and the fungus *Trichoderma candidum* was positively identified. The PDA slants were used to keep the culture alive during solid state fermentation (SSF). Microbial growth on insoluble materials in water is referred to as "solid state fermentation" (SSF). In these types of fermentative processes, the water used must not be enough to completely saturate the solid substrate on which the microorganisms (fungi) are growing. Thin layers of water, and sometimes water that has been absorbed into the substrates themselves, are found in SSF and are needed for microbial development. In the West, the SmF and SLF4 have received more attention than the SSF.

Substrate preparation

Substrates for mass multiplication of *Trichoderma candidum* were evaluated, including vegetable waste, fruit juice waste, sugarcane baggase, rotten wheat grains, and certain combinations thereof, with or without additives like gram flour and yeast extracts, and having varying moisture levels.

Solid-media substrate preparation

Pieces of the waste substrate are cut off and air dried in the shade. To the dry substrate, we add nutrient support ingredients such as gram flour and yeast extracts. That blend retained up to 40% of its original moisture content. The medium was autoclaved to ensure sterility. Non-synthetic solid media i.e. vegetable wastes, fruit juice waste, sugarcane baggase, rotten wheat were tested. 50 g of each solid material was taken in 500 ml conical flasks, inoculated with 5 mm mycelia mat incubated at 28°C incubator for 7-10 days. The spore count was made as mentioned earlier.

Liquid state fermentation

Term liquid state fermentation (LSF) is applied for the processes in which soluble materials in water is used for the microbial growth. In fermentative processes of this type, the quantity of water should exceed. Water essential for the microbial growth and in LSF and it is present in



thick layers and in occasions absorbs inside the substrates.

Preparation of Liquid substrates media

Liquid media; vegetable wastes powder, fruit juice waste powder, sugarcane baggase powder, rotten wheatgrains powder were evaluated for the growth and sporulation of *Trichoderma candidum*. 200 ml of each medium was poured in 500 ml capacity Erlenmeyer days at 30°C⁴.

Results and Discussion

In the present study, several naturally available substrates of both solid & liquid media were tested for mass multiplication of *Trichoderma candidum*. The success of biological control depends not only the isolation, characterization & pathogenicity, but also on the successful mass production of the fungal agent in laboratory. Large scale availability of the pathogen is a primary requirement in the biocontrol programmed. For a successful integrated pest management program me, the agents like the pathogenic fungi should be amenable to easy & cheap mass multiplication.

Rotten Grains

Grains are cheap, easily available & act as best nutritive media for the mass multiplication of many micro-organisms. In India the wheat Grains mostly used, that's because there are huge storage of wheat in godowns. This whole storage is in used therefore possibilities of rotten wheat. The Rotten wheat used of substrate for mass multiplication of *Trichoderma candidum*⁶. The CFU count recorded on solid media of wheat was 71.9 x 10⁸. While on liquid wheat water media 77.1 x 10⁸.

Sugarcane baggase

The sugarcane baggase is easily or almost freely available substrate for mass multiplication of fungal agent. The sugarcane baggase contains much more amount nutrition for growth of fungal agent *Trichoderma candidum*. The highest CFU count was recorded on solid Vegetative waste and fruit juice waste

Vegetable waste & fruit juice waste are recorded for the maximum spore production. Vegetable wastes are support nutrition for maximum spore production than fruit juice waste. CFU count recorded on solid media of vegetable waste is 41.2 x 10⁸ & liquid media 55.5 x 10⁸. While the CFU count recorded on solid medium of fruit juice waste 34.7 x 10⁸ & on liquid medium 46 x 2

x 10⁸. The vegetable waste & fruit juice waste was found to be the cheapest & best suitable media for

the large-scale production of fungi.

Conclusion

The results showed that all of the substrates tested could support *Trichoderma candidum* growth. Sugarcane baggase is the substrate that produced the highest mycelia and spore yields, with a CFU count of 101.3 x 10⁸ on solid medium and 94.7 x 10⁸ in liquid. *Trichoderma candidum* production has been confirmed in both the solid and liquid states. The sugarcane baggase medium has a high spore count. With sodium silicate as an inert carrier, the calculated CFU count was 41.5x10⁸.

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