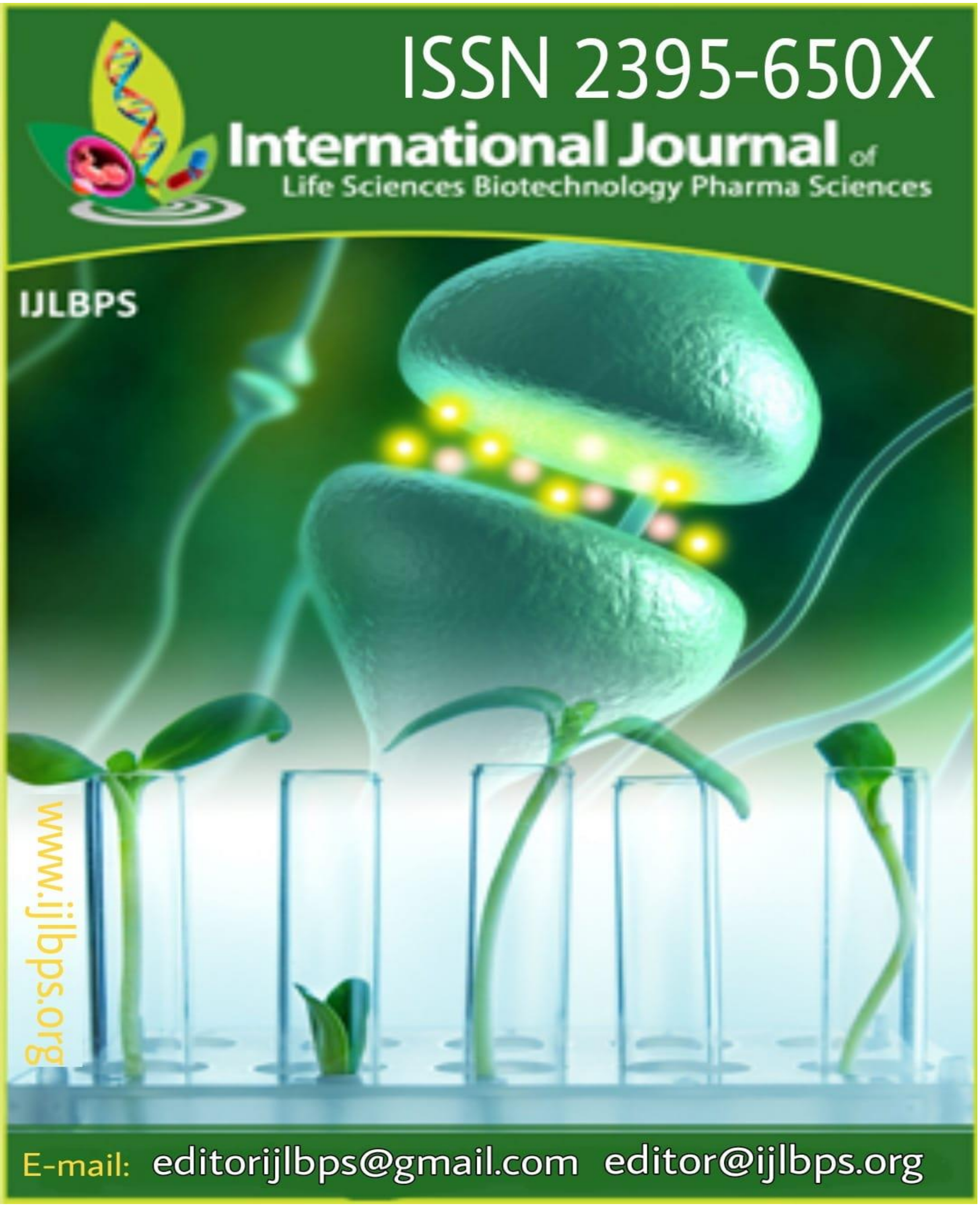
ISSN 2395-650X
International Journal of
Life Sciences Biotechnology Pharma Sciences
IJLBPS
www.ijlbps.org
E-mail: editorijlbps@gmail.com editor@ijlbps.org

International Journal of Life Sciences Biotechnology Pharma Sciences

Volume7 Issue1, Marh2019

Isolation, identification and mass multiplication of Trichoderma- an important bio-control agent

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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. The key difficulty involved in mass production and application of biocontrol agent are selection of effective strains, development cost efficient procedures, for mass multiplication, effective ways for storage, transportation and its formulation. This research investigates the viability of using various types of trash, including food scraps and compost, as substrates for the rapid proliferation of Trichoderma candidum, and results in a practical, adaptable production approach.

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. Chemical control is very generic in its impact, killing beneficial species and it may have adverse health and environmental pollution risk. Biological management of diseases employing Trichoderma candidum is extremely promising technique against soil based plant parasitic fungi. 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.

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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. 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. Development of acceptable conveniently made and cost efficient formulations for distribution should be main 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 AlbicansTypes 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 gram flour and yeast extracts. That blend retained up to 40% of its original moisture content. The medium was autoclaved to ensure sterility. Non- There were experiments done using synthetic solid media, such as vegetable wastes, fruit juice waste, sugarcane baggase, and rotting wheat. 50 g of each solid substance was collected in 500 ml conical flasks, inoculated with 5 mm mycelia mat kept at 28oC incubator for 7-10 days. As was previously noted, a count of spores was performed.

Processes in which ingredients that are soluble in water are utilized to cultivate microorganisms are referred to as liquid state fermentation (LSF). Water should be used in excess in these types of fermentative procedures. Water is present in thin layers and sometimes seeps within the substrates in LSF, making it crucial for microbial development.

Making ready liquid media substrates

Trichoderma candidume's ability to grow and produce spores in liquid medium, such as powdered vegetable wastes, fruit juice wastes, sugarcane baggase, and powdered rotting wheat grains, was tested. At 30oC4, 500-milliliter Erlenmeyer tubes were filled with 200 milliliters of each medium.

Discussion and Results

In the current investigation, we examined the viability of Trichoderma candidum on a variety of commercially accessible solid and liquid substrates for large-scale cultivation. For biological control to be effective, the fungal agent must be successfully mass produced in the laboratory once it has been isolated, characterized, and shown to be harmful. The biocontrol effort relies heavily on the widespread availability of the pathogen. It is important for an IPM program to use agents that can be easily and cheaply mass-produced, such as pathogenic fungus.

Moldy Cereals

The ideal nutritive medium for the bulk proliferation of numerous microorganisms are grains, which are both inexpensive and readily accessible. India relies heavily on wheat as a grain since the country has a large supply stashed away in silos. Because this whole warehouse is now being utilized, spoiled wheat is a distinct possibility. Trichderma candidum6 grew rapidly on a substrate of rotting wheat. On wheat solid medium, the CFU count was found to be 71,908,008. While on liquid wheat water medium 77.1 x 108.

Baggase from sugarcane

The sugarcane baggase is readily or nearly freely accessible substrate for mass proliferation of fungal agent. More of the fungal agent Trichoderma candidum's food source, sugarcane baggase, is present in it. The maximum number of CFU was found on a solid surface. 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 formulated CFU count was 41.5x108.

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