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Farmer-friendly technology for mass production of *Trichoderma harzianum* (CPTD28)

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The complexity and intensity of crop diseases have increased with the advent of intensive agriculture. To control these diseases, using an indiscriminate amount of pesticides leads to unforeseen problems like environmental pollution and health hazards worldwide. Now the scenario has changed since technologies are available for quality crop production with increasing awareness of alternative plant protection options such as the use of biocontrol agents. Management of plant diseases using biocontrol agents is increasingly becoming popular, as it has an advantage over chemical pesticides that it doesn't cause any harmful effects to the environment. *Trichoderma* spp. is one of the most extensively used bioagents, well known for their ability to induce mycoparasitism, producing several secondary metabolites inducing resistance, both local and systemic, in plants against invading pathogens and improving nutrient use efficiency (Elad *et al*., 1980; Harman *et al*., 2004; Nidhina *et al*., 2016).

The success of biological control mainly relies on bio-efficacy, shelf life, easy availability of cost-effective substrates and simple preparation procedure and delivery system. An appropriate medium for mass production is essential for the large-scale application of bio-control agents in the field. Different formulations of *Trichoderma* were developed (lignite, lignite and fly ash-based powder formulation, and talc powder) for seed treatment with viability up to nine months at storage at 24 °C (Jayaraj *et al*., 2006). Substrates such as coir pith, vermiculite and neem cake were suggested for mass production of *Trichoderma* (Mustaf *et al*., 2009; Prathibha *et al*., 2015).

Conventionally used costly raw materials, sophisticated laboratory facilities for commercial production of biocontrol agents, and the short shelf life of the products are the major limitations behind the restricted use. Developing a feasible and effective biocontrol agent using locally available agricultural waste is an important component of biocontrol programme and effective utilization of agricultural waste. The arecanut leaf sheath is an easily and freely available agricultural waste in arecanut gardens and is also available as a by-product from plate and bowl production units. The arecanut leaf sheaths can be exploited as a medium for multiplication and transferring the rapidly multiplied biocontrol agents to the soil system. Hence, attempts were made to develop an easy, cost-effective and farmer-friendly technology for mass production of a potential native isolate of *Trichoderma harzianum* (CPTD 28) using an areca leaf sheath with a higher population and extended shelf life.

Preparation of *T. harzianum* (CPTD 28) nucleus culture

Trichoderma strain, *T. harzianum* (CPTD 28 with accession number LC155111), maintained in the culture collection of the Crop Protection Division, ICAR-Central Plantation Crops Research Institute, Kasaragod, was very effective in the

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management of *Ganoderma* wilt and stem bleeding diseases of coconut (Prathibha *et al*., 2020; 2021). *T. harzianum* coir pith cake (TCPC) was also very effective in managing coconut bud rot and cocoa stem canker disease (Prabha and Chandra Mohanan, 2013; Sharadraj, 2013). A potential native *T. harzianum* isolate (CPTD 28) was utilized for the study and was subcultured on a PDA medium to prepare the nucleus culture.

Areca leaf sheaths were collected from the field, washed thoroughly and cut into pieces (2-3 cm size) and soaked in water for one hour in case of fresh and three hours if dried leaf sheaths were used. Excess water was squeezed from areca leaf sheath bits and packed in autoclavable ploy propylene covers. The bags were sealed and autoclaved at 15 p.s.i. for 20 minutes using an autoclave or a pressure cooker (three whistles followed by sim for 20 minutes). The next day, these were inoculated with 5 mm culture discs of five-day-old *T. harzianum* (CPTD 28) grown on a PDA medium and incubated at room temperature (27-29 ^{0}C).

Mass production of *T. harzianum* (CPTD 28) using areca leaf sheaths

For mass production of *T. harzianum* (CPTD 28), the areca leaf sheaths collection and preparation method was the same as described earlier. Autoclaved leaf sheath bits were inoculated with the nucleus culture of *T. harzianum* enriched areca leaf sheath @ 5 bits kg^{-1}. The shelf life of *T. harzianum* enriched areca leaf sheath was calculated by enumerating colony forming units (cfu g^{-1}) at monthly intervals till 24 months after incubation using *Trichoderma*-specific media.

White mycelial growth was observed on the inoculated areca leaf sheath bits on the third day after inoculation. Later, it turned to a luxuriant green mat of mycelial growth both on the upper and underside of the bits after seven days of incubation (Fig. 1 and 2). The mean population of *T. harzianum* (CPTD 28) differs in different dilutions. It was 235.5×10^7 cfu g^{-1} during the first month, gradually increased and recorded the highest cfu g^{-1} (600×10^7) in the seventh month of incubation. Talc-based

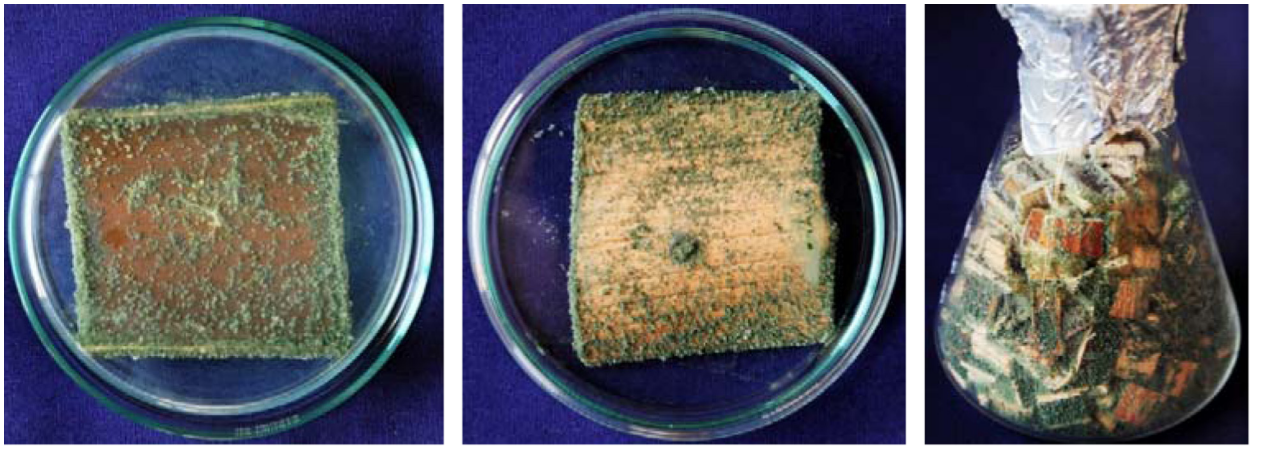

Fig. 1. Close-up view of *T. harzianum* (CPTD 28) growth on the upper and underside of the areca leaf sheath bits

Uninoculated leaf sheath bits | **Inoculated leaf sheath bits (First month)** | **Inoculated leaf sheath bits (Seventh month)**

Fig. 2. Luxuriant growth of *T. harzianum* (CPTD 28) in inoculated areca leaf sheath bits

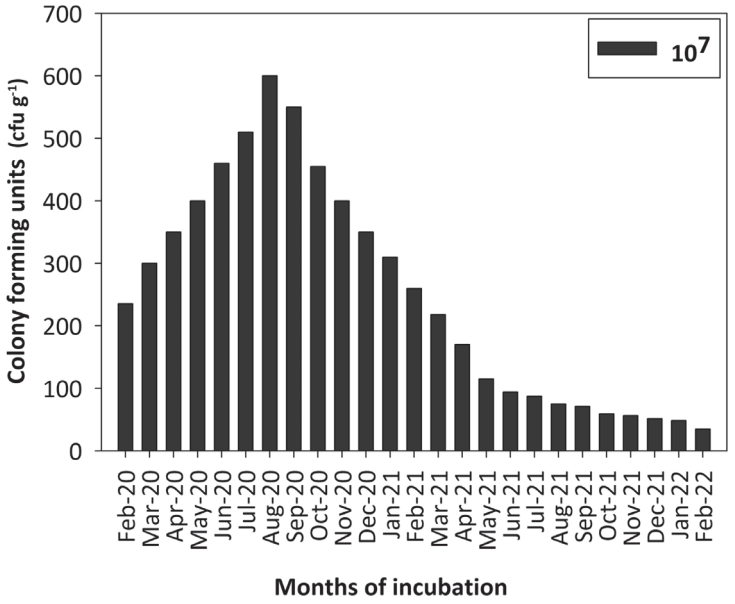

Fig. 3. Assessment of shelf life of *T. harzianum* (CPTD 28) enriched areca leaf sheath

Trichoderma formulations are extensively used as dry powder formulations (Nakkeeran *et al*., 2002; Warrior *et al*., 2002). Many disadvantages of using dry formulations, *viz*., dehydration, shorter shelf life and chances of losing viability (Mukhopadhyay, 1994), badly impact its effectiveness and market value (Singh *et al*., 2000).

In the present study, a significant increase in colony forming units (cfu g^{-1}) was recorded till seven months of incubation and then slowly declined each month while cfu (34.7×10^7) and viability of *T. harzianum* (CPTD28) were detected up to 24 months of incubation (Fig. 3). Areca leaf sheath basically consisted of different layers and due to high moisture holding capacity might have contributed for enhanced faster growth and the spread of *Trichoderma* in between the layers. The high nutritional composition of the areca sheath (Gowda *et al*., 2012) also supports rapid multiplication and enhanced population of *T. harzianum* (CPTD 28) with longer shelf life (24 months).

Thus, the areca leaf sheath substrate could be used for regular multiplication and the long-term preservation of the nucleus culture of *T. harzianum*. For further multiplication of *T. harzianum* (CPTD28) at the farmer's level, nucleus culture maintained in the areca leaf sheath can be directly used as an inoculum without using mycelial disc from the *Trichoderma* culture grown on synthetic media. In addition, by maintaining *T. harzianum* in the areca leaf sheath, we could retain its virulence because by regular subculturing in the synthetic media, there might be chances of loss of virulence. *Trichoderma* multiplication using the areca leaf sheath is a very simple, cost-effective and eco-friendly technology for mass production of *Trichoderma,* especially for farmers and startup groups.

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