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Viability formulation with active *Trichoderma asperellum* TR3 in three packaging variation

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Abstract

Trichoderma asperellum TR3 has been applied as a biopesticide and Plant Growth Promotion Fungi (PGPF) but in the form of a suspension and substrate without the addition of carriers and adhesives so for long-term use and short shelf life. This study aims to obtain the right packaging formulation and have good viability, as well as a long storage time using talc and tapioca flour as the basic ingredients. The isolate collection was rejuvenated by growing in PDA medium, after completion of incubation for 48 hours, the growing fungal colonies were counted, isolated and purified, then made in the form of a powder starter. Test of various formulations of *T. asperellum* TR3 fungal powder packaging was carried out by viability test using a Completely Randomized Design (CRD), with packaging treatment on the formulation: P1 = Aluminum foil plastic, P2 = Bottle and P3 = Cetic plastic. Each treatment was repeated 3 times to obtain a combination of 9 treatment units. Furthermore, all packaging combinations were stored at room temperature. In the first month, 1 pack was taken for regrowth which aims to determine the viability of the conidia and then every two weeks for 12 weeks. The results showed that the average number of conidia colonies growing on various packages gave different viability results, but had no significant effect between formulations of various packages and viability. The average number of colonies that grew on aluminum foil plastic packaging was more in each observation of storage time compared to bottle and plastic packaging.

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Introduction

The use of microorganisms as biological control agents has been widely carried out and provides benefits for increasing agricultural production in Indonesia (Hanudin and Marwoto 2012; Nur Amin *et al*., 2015; Ratna *et al*., 2020; Ratnawati *et al*., 2020). However, reports on the use of local or local microorganisms that have potential have not been widely carried out. Local microorganisms work more effectively, because they are supported by various appropriate environmental factors and do not result in ecosystem changes and implementation in the field is easier. According to Cook & Baker (1983), efforts to overcome plant diseases with biological practices provide good opportunities because the microorganisms are already available in the field and their activities can be controlled by the environment and the host.

The group of microorganisms, especially fungal microbes that are able to suppress pathogens, is in the Moniliales family, such as *Verticillum* sp., *Trichoderma* sp and *Gliocladium* sp. The genus *Trichoderma* sp. it is known that several species can parasitize other fungi and have the potential to be used as biological control (Santoso *et al*., 2007; Ratnawati *et al*., 2019; Ratnawati *et al*., 2020).

Microbes that are useful as components of natural habitats have important roles and functions in supporting the implementation of environmentally friendly agriculture through various processes such as decomposition of organic matter, mineralization of organic matter, besides that microbes are positioned as producers of nutrients for plants. Budiarti & Nurhayati (2014), reported that plants and microbes will interact and stimulate each other caused by the presence of root exudates. Fungi are heterotrophs, dominant on acid soils although they are also found in neutral or alkaline soils and some of them are sensitive to pH 9.0 but this sensitivity is important in overcoming plant diseases. The presence of fungi and bacteria as antagonist agents is able to influence the activity of microorganisms so that it is important to take into account the suppression of disease. In addition, many antagonist agents can stimulate plant growth because they are able to produce growth hormones, fix nitrogen, dissolve phosphate, and produce siderophores (Siti Hardiyanti *et al*., 2017).

Antagonistic microbes found in the rhizosphere and rhizoplan areas that can act as controlling agents include the genus *Trichoderma* sp, *Penicillium* sp and *Asperigillus* sp (Agrios., 2005), while Nirwanto & Mujoka (2009) reported that the microbes found in the phyllosphere were the genus *Penicillium* sp., *Fusarium* sp. , *Trichoderma* sp. , *Stemphylium* sp., *Rhizopus* sp., *Curvularia* sp. and *Gliocladium* sp. Furthermore, Sayang (2009) reported that microbial antagonists that are effective as biological controllers include *Bacillus* sp, *Trichoderma* sp, *Penicillium* sp and *Clostridium* sp.

Nur Amin *et al*. (2015) suggested that the application of non-pathogenic fungi to various types of plants would increase the resistance of the host plant to pathogen attack and be able to induce resistance in the upper part of the plant. The use of beneficial microbes in improving soil texture and structure by increasing its aggregation and stability (Rashid *et al*., 2016). The interaction between microbes and plants is an indicator of soil fertility (Hayat *et al*., 2010).

Many studies using the fungus *Trichoderma* sp. as an effective biological agent to control various pathogens, however, it is still experiencing obstacles related to storage time (Suhera *et al*., 2018). *Trichoderma* sp. is a group of fungi that are always associated with plants and soil. If this fungus is in plant tissue, it is called an endophytic fungus (Nur Amin *et al*., 2017). Trichoderma sp. is one of the endophytic fungi that can live in all parts of plant tissue both below ground and above ground, namely roots, stems and leaves (Kusari *et al*., 2012; Rosmana *et al*., 2018).

Ratnawati's previous research (2020) was in vitro starting from the microbial isolation stage, identification of microbes both macroscopically and microscopically, microbial screening, molecular identification, compatibility tests, antagonist tests, until superior and potential microbes were found as biopesticides and as plant growth microbes. Promotion Fungi (PGPF) obtained as many as 9 microbes that could potentially be used as biological agents. One of them is *Trichoderma asperellum* TR3.

Trichoderma asperellum TR3 is one of the rhizosphere fungi which is known as an antagonist fungus in both in vitro and in vivo tests which has the ability to suppress various types of pathogens in various plants. *T.asperellum* TR3 is a fungus with biopesticide and PGPF properties (Suhera 2018; Ratnawati *et al*., 2020; Ismail *et al*., 2020). *T. asperellum* (TR3) has been applied as a biopesticide and PGPF but in the form of a suspension and substrate without the addition of carriers and adhesives so that it is for long term use and has a short shelf life. Therefore, it is necessary to develop a formulation in powder form with various forms of packaging which will then be monitored for viability during storage.

Based on the description above, it is necessary to conduct research on the development of powder formulations of the fungus *T.asperellum* strain TR3 isolate in various forms of packaging formulations to determine the viability of the fungal formulation. This study aims to obtain the right packaging formulation and have good viability, with a duration of storage.

Materials and methods

This research was carried out at the Microbiology Laboratory of the Faculty of Agriculture, Alkhairaat University, Palu from January to June 2022.

The materials and tools used in this research include 70% alcohol, *Trichoderma asperellum* TR3 isolate, Rice, Talk, Tapioca Flour, Garlic Powder, Brown Sugar, Aluminum Foil Plastic, Bottles, Cetic Plastic, PDA medium, KOH, sterile water, aluminum foil, filter paper, transparent cling pack, cotton, label, polybag. While the tools are petri dishes, electric microscope, stirrer, test tube, erlenmeyer, dropper, ose needle, spatula, bunsen burner, beaker, measuring cup, syringe, scales, Laminar Air Flow, digital camera, isolate shooting box, box storage isolates and stationery.

Isolation of the fungus Trichoderma asperellum TR3

The isolate collection was rejuvenated by growing in PDA medium, after completion of incubation for 48 hours, the growing fungal colonies were counted, isolated and purified, then made in the form of a powder starter.

Trichoderma asperellum TR3 Fungus Powder Formulations Test and Their Viability

Viability test using *T. asperellum* TR3 isolate using Completely Randomized Design (CRD), with various packaging treatments consisting of:

P1 = Alumunium foil

P2 = Bottle

P3 = Cetic Plastic

Each treatment was repeated 3 times to obtain a combination of 9 treatment units. Furthermore, various packages are stored at room temperature until the observation process is complete. Observations were made by taking 1 g of mushroom formulation from each package (Fig. 1) for regrowth in the first month every two weeks. Subsequent observations were made every two weeks until the third month (12 MSI), modified method of Suhera *et al*. (2018).

The viability of fungal conidia was measured by suspending 1 g of the fungal formulation in distilled water to dilute it with a density of 10^6 CFU mL^{-1}. After 24 hours, the number of growing conidia was observed.

Conidia population level on the storage time of each package was calculated by the formula:

P = ∑N x TP x 10

P = Number of conidia CFU mL^{-1}

∑N = Total number of conidia

TP = Dilution rate

10 = Constanta

The formulation was considered feasible if the conidia population was still above 10^5 CFU mL^{-1}.

Data analysis

The data obtained were quantitatively analyzed using RAL (Completely Randomized Design). If it shows a significant effect, then it is continued with the BNT test (Least Significant Difference) with a confidence level of = 0.05. Qualitative data were observed visually and analyzed descriptively.

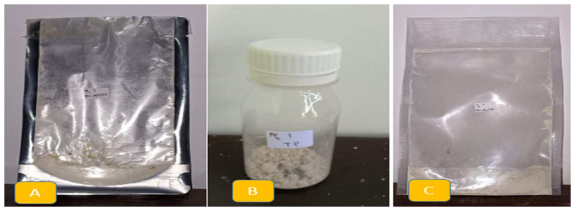

Fig. 1. Treatment of various packaging (A) Aluminum foil plastic (B) Bottles (C) Cetic Plastic.

Results and discussion

The results of observations of the average viability of conidia (CFU mL^{-1}) on various packages of the fungus *Trichoderma asperellum* TR3 formulation with observation time intervals of 2, 4, 6, 8, 10 and 12 MSI (Week After Inoculation). The variance test showed that the treatment of various packaging formulations had no significant effect on the conidia viability test of *Trichoderma asperellum* TR3, but the tendency for the average number of colonies to grow on aluminum foil plastic packaging was more in each observation period of storage compared to bottles and plastic packaging (Fig. 2).

The use of clique plastic gave the average number of colonies that grew more at the time of observation of 10 MSI and 12 MSI compared to bottled storage, although the average number of colonies that grew was less than the previous observation (Fig. 2).

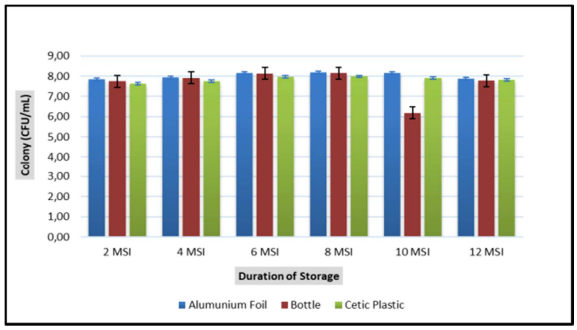

Fig. 2. Average viability of conidia in various packaging formulations. CFU = Colony Forming Unit Per Mililiter.

Aluminum foil plastic as a container or storage place for *Trichoderma asperellum* TR3 gave good results or the average number of fungal colonies that grew more, although the effect was not significant with plastic containers and bottles. This is because aluminum foil packaging is stronger and more resistant, not easily torn and good enough to protect the product inside. The existing adhesive system also allows for better opening and closing, so it is not easy for oxygen to enter which can affect the viability of the formulation. This is different from a bottle with a wider opening and closing system, there may be air gaps that can enter the bottle and affect the product inside. The same thing applies to the use of plastic wrap, which is usually thin and easily torn, so that it often affects the moisture of the product inside, which in turn can affect the viability of the formulation.

According to Novita *et al.* (2021) aluminum foil plastic has a higher density value than ordinary plastic. The greater the density value, the smaller the permeability of the material to gas and water vapor. The rate of transmission of oxygen gas and the rate of transmission of water vapor in the packaging of aluminum foil plastic has a small permeability value, meaning that aluminum foil plastic is good enough to protect the product with its properties and is breakable and not easily torn.

The same thing was also found in the research of Suhera *et al.* (2018) that the combination packaging of aluminum foil + plastic gave the highest average viability of *P.ostreatus* PO2 conidia and was significantly different from the treatment of ordinary bottles and plastics. The average viability of the formulation can last up to 24 weeks of storage, provided that a formulation is considered feasible if the viability of the conidia is above 10^5 CFU mL^{-1}. There was a decrease in the number of colonies that grew, especially at the 10 MSI and 12 MSI week observations in all packaged treatments; this was presumably due to the availability of nutrients in the formulation which also continued to decrease with the length of storage time. In addition, it can also be caused by contamination during the fungal breeding process in the PDA media used. Lopes-Arevalo *et al.* (1996) mentioned that contaminant fungi were often found in propagation media such as sawn media and rice. Contamination is largely determined by environmental factors, sources of inoculum and wind gusts.

Conclusion

The number of conidia colonies growing on various packages gave different viability results, but the effect was not significant between various packaging formulations (aluminum foil plastic, bottles and cetic plastic). The average viability of the number of colonies growing on aluminum foil packaging was higher at different observations of storage time than bottles and plastic packaging.

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