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EFFECTIVENESS OF *Trichoderma harzianum* CULTURED IN VARIOUS MEDIA AND APPLIED IN DIFFERENT TIMES TO CONTROL *Colletotrichum* spp. ON CHILI FRUIT

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ABSTRACT
Chili is one of the commodities with high economic value in Indonesia. Chili cultivation is faced with plant diseases, one of which is anthracnose caused by *Colletotrichum* spp. Control effort with *Trichoderma harzianum* is expected to be able to suppress the attack of the pathogen *Colletotrichum* spp. This research was aimed to determine the best propagation media for *T. harzianum* with the right application time to control *Colletotrichum* spp. The research was carried out at the Plant Protection Laboratory of UPN "Veteran" Yogyakarta from January–April 2022. The design used was Completely Randomized Design (CRD). In an in-vitro study, 4 propagation media were tested, namely bran, corn, coconut water, and tempe liquid waste and one control treatment *Colletotrichum* spp. without biological agent. *Trichoderma harzianum* from the best propagation media in in-vitro tests was tested with control treatment (without fungicides and biological agents), Propineb fungicide, *T. harzianum* before pathogen inoculation, and *T. harzianum* after pathogen inoculation. The parameters observed in the in-vitro test were the percentage of inhibition (%) and in-vivo test the incubation period (days), disease incidence (%), disease intensity (%), and AUDPC. The data obtained were analyzed by the F ANOVA test if the results showed significantly different treatments then further tests were carried out using Duncan's Multiple Range Test at 5% level. *T. harzianum* propagated in bran media was effective to suppress the pathogen *Colletotrichum* spp. in in-vitro test, and the application time of *T. harzianum* before pathogen inoculation was as effective as the application of *T. harzianum* after pathogen inoculation.

Keywords: Chili, *Colletotrichum* spp., Anthracnose.

ABSTRAK
Cabai merupakan salah satu komoditas yang bernilai ekonomi tinggi di Indonesia. Budidaya cabai dihadapkan pada penyakit tanaman yang salah satunya yaitu penyakit antraknosa yang disebabkan oleh *Colletotrichum* spp. Upaya pengendalian dengan *Trichoderma harzianum* diharapkan mampu menekan serangan patogen *Colletotrichum* spp. Penelitian ini bertujuan untuk mengetahui media *T. harzianum* yang terbaik dan waktu aplikasi yang tepat dalam mengendalikan *Colletotrichum* spp. Penelitian dilaksanakan di Laboratorium Proteksi Tanaman UPN "Veteran" Yogyakarta pada bulan Januari–April 2022. Rancangan yang digunakan yaitu Rancangan Acak Lengkap (RAL). Pada penelitian *in-vitro* digunakan 4 perlakuan media yaitu dedak, jagung, air kelapa, dan limbah cair tempe serta 1 perlakuan kontrol *Colletotrichum* spp. tanpa agen hayati. *T. harzianum* dari media perbanyakan yang efektif pada uji *in-vitro*, dilakukan uji lanjut *in-vivo* dengan perlakuan kontrol (tanpa fungisida dan agen hayati), Fungisida Propineb, *T. harzianum* sebelum inokulasi patogen, dan *T. harzianum* setelah inokulasi patogen. Parameter yang diamati pada uji *in-vitro* adalah persentase penghambatan (%) dan pada uji *in-vivo* adalah masainkubasi (hari), kejadian penyakit

(%), intensitas penyakit (%), dan AUDPC. Data yang diperoleh dianalisis dengan uji F ANOVA apabila hasil menunjukkan perlakuan berbeda nyata kemudian dilakukan uji lanjut menggunakan Uji Jarak Berganda Duncan taraf 5%. *Trichoderma harzianum* yang diperbanyak pada media dedak efektif menekan patogen *Colletotrichum* spp. pada uji *in-vitro*, serta waktu aplikasi *T. harzianum* sebelum inokulasi patogen sama efektifnya dengan aplikasi *T. harzianum* setelah inokulasi patogen.

Kata kunci : Cabai, *Colletotrichum* spp*.*, Antraknosa.

INTRODUCTION

Chili plant is one of the commodities with high economic value in Indonesia. Efforts to increase the productivity of chili is to protect chilies from pests and plant diseases. The disease that is often found in chili cultivation is anthracnose. This anthracnose disease can be caused by the fungus *Colletotrichum* spp. Anthracnose is a serious disease and can cause losses of as much as 20-90%, especially during the rainy season (Sarwono *et al.* 2013). Controls using synthetic materials should be replaced with controls that do not have a negative impact on the environment and humans. Control using biological agents is an environmentally friendly control. One of the microorganisms that can be used to control plant diseases is *Trichoderma* spp. *Trichoderma* spp. able to suppress the development of anthracnose disease in chili (Nurbailis *et al.* 2017). Application *Trichoderma* spp. is potential as a biological controller of the pathogen *Colletotrichum* spp. which causes anthracnose disease with its cellulolytic activity and parasitic properties against pathogenic fungi (Muliani *et al.* 2019).

The development of the use of biological agents as plant disease control is still slow due to the limited mass production of biological agents and can be used commercially, so technology is needed for mass production of *Trichoderma* spp. with several kinds of propagation media (Wijaya *et al.* 2011). *Trichoderma* spp. can be used as plant protection against certain pathogens. *Trichoderma virens* can be used as a pre-treatment against the pathogen *Rigidoporus microporus* (Amaria & Wardania, 2014; Bruce, 1991; Hightley, 1997).

This study aimed to (i) identify the use of *Trichoderma harzianum* R. propagation media on the percentage of inhibition of *Colletotrichum* spp. in-vitro (ii) To identify the effect of the application time of *T. harzianum* in suppressing anthracnose disease in chili.

RESEARCH METHODS

The research was carried out at the Plant Protection Laboratory, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Yogyakarta from January - April 2022. This study consisted of in-vitro and in-vivo tests. The in-vitro test was carried out in a completely randomized design consisting of 4 treatments of *T. harzianum* propagation media and 1 control treatment of *Colletotrichum* spp. without biological agents. Each treatment was repeated 5 times, and each experimental unit consisted of 3 Petri dishes. The treatment used was *T. harzianum* propagation media, consisted of bran, corn, coconut water, and tempe liquid waste. The materials used in this study were chilli pepper of Nirmala variety, bran, milled corn, coconut water, tempe liquid waste,

T. harzianum isolate, *Colletotrichum* isolate, fungicide, cotton, lactic acid, chloramphenicol, aquades, PDA media, alcohol 70%. The tools were used petri dishes, ose needles, beaker glass, autoclave, laminar air flow, aluminum foil, plastic wrap, micropipette, haemocytometer, Bunsen, matches, erlenmeyer, glass preparations, cover glass, spatula , microwave, 6 mm diameter filter paper, strimin wire. The isolates *T. harzianum* used from collection of Laboratorium Agensia Hayati Yogyakarta, and isolates *Colletotrichum* spp. used came from the results of exploring anthracnose disease in chilies in the field.

In-vitro antagonist test was conducted in Petri dish with PDA media. *Trichoderma harzianum* which had been propagated with bran, corn, coconut water, and tempe liquid waste media for 7 days before being used for the antagonist test were calculated for the density and viability of the spores. The antagonist test was carried out by placing the two fungal colonies as shown in Figure 1. *Colletotrichum* spp. inoculum (diameter 6 mm) was placed in a Petri dish with a distance of 2 cm from the edge of the Petri dish and filter paper with a diameter of 6 mm that had been dipped in suspension of *T. harzianum* from the propagation media according to treatments was placed with a distance of 4 cm on the opposite side. As a control, the isolates of *Colletotrichum* spp. was placed on PDA media in a Petri dish without *T. harzianum*. The growth of colonies for each fungus was measured daily until there was a contact between *T. harzianum* with *Colletotrichum* spp. in dual culture Petri dish. Diameter of *Colletotrichum* spp. in control Petri dish was also measured.

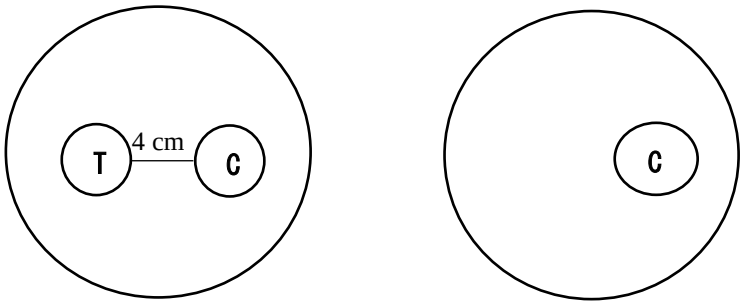


Figure 1. Antagonist Test
1.a. Inoculum placement of *T. harzianum* and *Colletotrichum* spp.
1.b. Control plate (*Colletotrichum* spp.)

The in-vivo test consisted of 4 treatments and each treatment was repeated 6 times. Each treatment replication contained 10 samples of chili, so the total sample of chili was 240 pieces. *Trichoderma harzianum* from the best propagation media obtained from in-vitro test was tested in-vivo with the different application time. The treatments used in the in-vivo test were Control (without biological agents and fungicides), Propineb Fungicide, *T. harzianum* application before pathogen inoculation, and *T. harzianum* application after pathogen inoculation.

Trichoderma harzianum propagated in the best propagation media for with a culture age of 7 days was taken as much as 1 gram and diluted into 100 mL aquadest to obtain a spore density of 10^7 conidia/mL. *Colletotrichum* spp. suspension was prepared to have a conidia density of 10^6 conidia/mL. Negative

control treatment was conducted by dipping chilies in *Colletotrichum* spp. suspension. Applications were made by dipping the chilies in suspension for 1 minute according to each treatment. Chili fruit that has been inoculated with *Colletotrichum* spp. was air-dried for 1 hour. The application of *T. harzianum* was carried out according to the treatment time i.e. before and after inoculation of the pathogen. The parameters observed in the in-vivo test were incubation period, disease incidence, disease intensity, and AUDPC. The placement of chilies in the in-vivo test was presented in Figure 2.

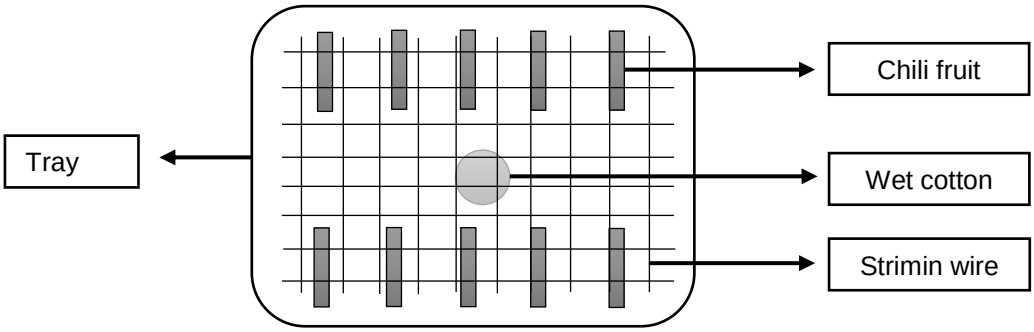


Figure 2. Illustration of in vivo test

Statistical analysis was performed by F test (ANOVA). If the analysis results show an effect, then it was continued with Duncan's Multiple Range Test (DMRT), with a significance level of 5%. The percentage data which were not normally distributed were transformed to transformation arcsin$\sqrt{x}$.

RESULT AND DISCUSSION

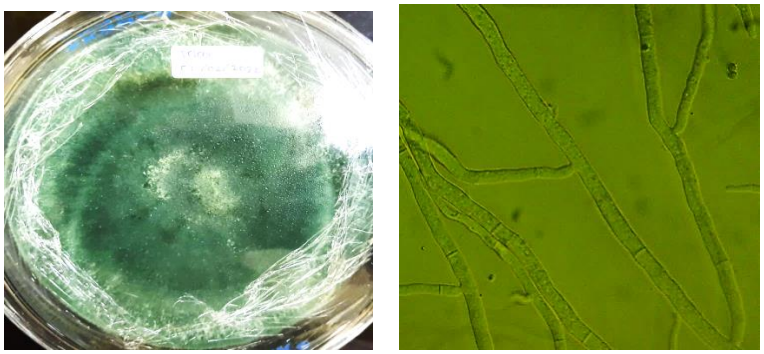
a. Identification of *Colletotrichum* spp. and *T. harzianum*

Macroscopic observation showed that *Colletotrichum* spp. has a reddish-white conidia color (Figure 3a). Microscopic observations showed that *Colletotrichum* spp. has a insulated and branched hyphae. The conidia were hyaline, uninsulated, transparent, and oblong (Figure 3b). It is documented that *Colletotrichum* spp. has characteristics, namely hyaline conidia, single-celled, non-insulated conidia between conidiophores, dark brown hairs (setas), reddish-brown conidia, insulated and branched hyphae and produce transparent and elongated conidia with rounded or tapered ends (Sudirga, 2016).

Macroscopic observations showed that the growth of *T. harzianum* was fast as it only takes 3-5 days to fill the Petri dish. Colonies of *T. harzianum* at the beginning of growth were white, then turned dark green (Figure 4a). The microscopic observations of *T. harzianum* showed that the hyphae were upright, insulated, branched, and round conidia (Figure 4b). The characteristics of *T. harzianum* are well documented as follows: it has a fast growth rate as it only takes 3-4 days to fill the petri dish, colonies of *T. harzianum* are white at the beginning of growth and will turn dark green, have upright and branched hyphae, and have conidia that are round (Sudantha, 1997).

3a. 3b.

Figure 3. Observations of Colletotrichum spp. Description: 3a. Macroscopic Observation of *Colletotrichum* spp.; 3b. Microscopic Observation of *Colletotrichum* spp.



4a. 4b.

Figure 4. Observations of *T. harzianum*.
Description: 4a. Macroscopic Observation of *T. harzianum*; 4b. Microscopic Observation of *T. harzianum*.

b. Spore Density and Spore Viability of *T. harzianum* on Propagation Media

Trichoderma harzianum propagated using bran media had the highest total spore density (4,54 x 10^7/mL) and spore viability (100%) (Table 1). The bran media contains high carbohydrates as much as 84,36%. Carbohydrates contain sugars that fungi use for metabolic processes. Sugar will be transformed into fungal cells and also carry protein. Carbon in addition to coming from carbohydrates (sugars) is utilized by fungi for biosynthetic purposes, this shows the occurrence of gluconeogenesis in the effect of reversing the glycolytic pathway in fungi. Bran also contains approximately 11,35% protein so that it can help the growth and development of *T. harzianum* (Uruilal *et al.* 2012).

Table 1. Spore Density and Spore Viability of *T. harzianum* on Propagation Media

Propagation Media	Spore Density	Spore Viability
Bran	4,54 x 10^7	100%
Corn	1,28 x 10^7	93,94%
Coconut water	0,79 x 10^7	84,40%
Tempe Liquid Waste	0,40 x 10^7	84,07%

c. Percentage of Inhibition in-vitro Test

Trichoderma harzianum propagated on bran media resulted in the highest percentage of inhibition rate on the growth of *Colletotricum* spp. than those propagated on other media on day 5 (Table 2). The percentage of inhibition of *T. harzianum* propagated on bran media was the highest because it had a higher spore density and spore viability than *T. harzianum* propagated on other media. According to Surtikanti & Yasin (2009), the success of a microorganism in inhibiting pathogens is not only influenced by environmental factors and the number of spores, it is also influenced by germination or spore viability.

Table 2. Percentage of Inhibition of Pathogen *Colletotrichum* spp. by *T. harzianum* by Propagation Media (%)

Media	Percentage of Inhibition Day to (%)				
	1	2	3	4	5
Bran	11,85a	44,89a	64,04a	69,04a	70,61a
Corn	8,11a	41,25a	59,71a	60,42b	61,99b
Coconut water	4,05a	28,28b	50,61b	55,93c	60,16b
Tempe Liquid Waste	4,05a	24,72b	47,18b	55,07c	59,11b

Note: Values followed by the same letter in one column show no significant difference based on Duncan's 5% multiple comparison test.

The microscopic observation of the antagonist mechanism showed that parasitism was occurring, because the hyphae of the antagonist agent grew on the hyphae of the pathogenic fungus, and the hyphae of the antagonist fungus were found wrapped around the hyphae of the pathogen in the contact area (Figure 5).

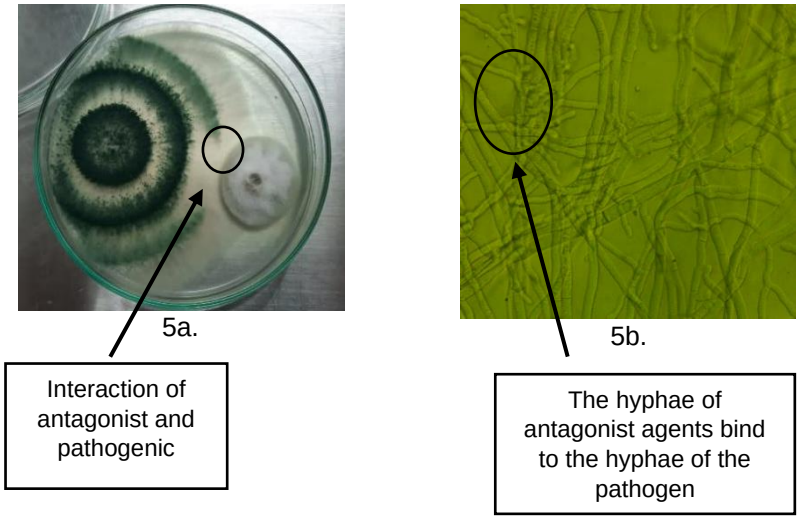


Figure 5. Observations of the Antagonist Mechanism.
Description: 5a. Macroscopic observations;
5b. Microscopic observation

Pathogen growth inhibited by *T. harzianum* occurs through microparasitic mechanisms that can occur through colonization, competition, antibiosis, and parasitism. Colonization of biological agents

occurs by narrowing the growth space for pathogenic infections, because biological agents have the ability to grow and develop faster in covering surfaces or organs in plants. Competition between biological agents and pathogenic fungi occurs when they are present in the same place and compete for sources of nutrients such as carbon (C), nitrogen (N), iron (F), oxygen (O_2), light, and water (H_2O). This competition causes changes in conditions that are less favorable for pathogens so that they cannot develop fully. Antibiosis is the inhibition of growth and development and the destruction of organisms from the metabolism of other organisms. The products of metabolism are toxic and are called antibiotics. Parasitism occurs when antagonistic fungi parasitize a pathogen and take up the nutrients contained in that pathogen. Antagonistic fungi parasitize pathogenic fungi through hyphae by forming haustoria and causing hyphal lysis in pathogenic fungi (Muslim, 2019).

d. Incubation Period of *Colletotrichum* spp.

The average incubation period *Colletotrichum* spp. treated with fungicide was not significantly different from the incubation period of *Colletotrichum* spp. on chili peppers treated with *T. harzianum* before inoculation of the pathogen *Colletotrichum* spp. and after inoculation of the pathogen *Colletotrichum* spp (Table 3).

Table 3. Incubation period of *Colletotrichum* spp. on Chili Fruit (day)

Treatment	Incubation Period (Days)
P1 (Control)	3,13 b
P2 (Propineb Fungicide)	4,03 a
P3 (*T. harzianum* before pathogen inoculation)	3,92 a
P4 (*T. harzianum* after pathogen inoculation)	3,60 a

Note: The *T. harzianum* used is propagated on bran media. Values followed by the same letter in one column indicate no significant difference based on Duncan's Multiple Range Test of 5%.

The incubation period of the three treatments was significantly longer than the incubation period of the control treatment (*Colletotrichum* spp. that was not treated with fungicide Propinep or *T. harzianum*). The control treatment showed that the pathogen would infect more quickly so that the fruit would show symptoms of disease attack more quickly. This is because there are no antagonistic agents or chemicals that can inhibit the growth of the pathogen *Colletotrichum* spp. to infect chili peppers. *Trichoderma harzianum* applied before inoculation of the pathogen *Colletotrichum* spp. had a mean incubation period that was not significantly different from the application of *T. harzianum* after inoculation of the pathogen *Colletotrichum* spp. Diseases can arise and develop if there is an interaction between susceptible plants and virulent pathogens in an environment that supports the growth of the pathogen or an environment that is not suitable for plants (Sopialena, 2017).

e. Disease Incidence

The results showed that the disease incidence of anthracnose on chili peppers on day 4, 8, and 12 on control treatment (without treatment with

Fungicide Propinep and *T. harzianum*) was not significantly different from those treated with Propineb fungicide treatment, *T. harzianum* treatment before pathogen inoculation, and *T. harzianum* treatment after inoculation (Table 4).

Table 4. Percentage of Disease Incidence of Anthracnose on Chili Fruit

Treatment	Percentage of Disease Incidence (%)		
	4 day	8 day	12 day
P1 (Control)	76,36a	90,00a	90,00a
P2 (Propineb Fungicide)	62,57a	78,07a	82,50a
P3 (*T. harzianum* before pathogen inoculation)	67,00a	86,93a	86,93a
P4 (*T. harzianum* after pathogen inoculation)	70,57a	86,93a	86,93a

Note: The *T. harzianum* used is propagated on bran media. The results of the ANOVA analysis showed that there was no effect between treatments.

Pathogen infection occurs in a temperature range of 27ºC with 80% high humidity which is optimum for the development of anthracnose disease. The stage of fungal infection generally starts from the germination of spores on the surface of plant tissue, producing a germination tube, after penetration, intra- and intercellular hyphae are formed which spread through plant tissues (Anggraeni *et al.* 2019).

f. Disease Intensity

The percentage of disease intensity of the three treatments was significantly lower than the percentage of disease intensity of the control treatment (without the Fungicides Propineb and *T. harzianum*) (Table 5).

Table 5. Percentage of Anthracnose Disease Intensity on Chili Fruit (%)

Treatment	Percentage of Disease Intensity (%)		
	4 day	8 day	12 day
P1 (Control)	30,79a	51,06a	71,97a
P2 (Propineb Fungicide)	23,55b	39,75b	60,82b
P3 (*T. harzianum* before pathogen inoculation)	24,26b	40,00b	60,79b
P4 (*T. harzianum* after pathogen inoculation)	25,34b	41,34b	62,01b

Note: The *T. harzianum* used is propagated on bran media. Values followed by the same letter in one column indicate no significant difference based on Duncan's Multiple Range Test.

Trichoderma harzianum has been able to suppress the growth of the pathogen *Colletotrichum* spp. the cause of anthracnose disease in chili, this is due to the fast growth and antagonistic properties of *T. harzianum.* Previous study showed that *Trichoderma* spp. will release toxin compounds that can inhibit growth and kill plant pathogens*. Trichoderma* spp. produce volatile and non-volatile antibiotics*. Trichoderma* spp. able to create a degree of acidity (pH) that is not optimal for pathogens, so that the rate of pathogen infection does not increase (Muslim, 2019).

g. AUDPC

The results of the calculation of the AUDPC value of disease incidence and disease intensity showed that control (without the fungicide Propineb and

T. harzianum) had the highest value than the treatment with the Propineb fungicide, the treatment with *T. harzianum* before inoculation of the pathogen *Colletotrichum* spp., and *T. harzianum* after inoculation of the pathogen *Colletotrichum* spp. The higher the incidence of disease and the intensity of the disease, the greater the AUDPC value (Table 6).

Tabel 6. AUDPC Value of Disease Insidence and Disease Intensity

Treatment	AUDPC Value	
	Disease Insidence	Disease Intensity
P1 (Control)	845,44	471,34
P2 (Propineb Fungicide)	726,66	374,84
P3 (*T. harzianum* before pathogen inoculation)	789,58	378,62
P4 (*T. harzianum* after pathogen inoculation)	803,86	390,66

Note: The *T. harzianum* used is propagated on bran media.

The use of *Trichoderma* in plant disease control can reduce the impact of using chemical fungicides which can have a negative impact on the environment. The application of pesticides can pollute the environment, especially if they are applied uncontrollably (Nuryanto, 2017). Excessive and continuous use of pesticides can leave residues. *Trichoderma harzianum* is effective in controlling anthracnose disease caused by *Colletotrichum* spp. on chilies. The effectiveness of *T. harzianum* in controlling anthracnose was not significantly different from the use of the fungicide Propineb. *T. harzianum* is a fungus that has a mutualistic symbiosis with plants. Plants benefited in terms of growth and disease control, while *T. harzianum* benefited because it could absorb nutrients produced by plants. The use of *Trichoderma* spp. is expected to increase the production and control of diseases in chili plants.

CONCLUSION

Based on the analysis of the results of the antagonist test of *T. harzianum* against *Colletotrichum* spp. it can be concluded that *T. harzianum* propagated on bran media was effective in inhibiting the pathogen *Colletotrichum* spp. in in-vitro tests and the application time of *T. harzianum* before pathogen inoculation, application of *T. harzianum* after pathogen inoculation, and the application of the fungicide Propineb has same effective.

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