The effectiveness of yam (*Pachyrhizus erosus*) juice in maintaining the viability of *Trichoderma viride* spores

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Abstract. *Trichoderma viride* is a potential biological agent to control main estate crop diseases. One of the disadvantages of this fungus is susceptible to ultraviolet (UV) from sunlight. This study aimed to evaluate the effectiveness of yam juice (YJ) in protecting *T. viride* spores against UV exposure. The research was conducted from March to July 2019 at the Plant Protection Laboratory of IIBCRI, Sukabumi. The suitability as a growth medium was performed by incorporating 0%, 1%, 5%, 10%, and 20% concentration of YJ into potato dextrose agar (PDA) medium. The spore viability test used a 20% concentration of YJ in the talc carrier medium compared to control (without YJ) and exposing treatment for 0, 60, 120, and 180 minutes. The results showed that YJ was suitable for *T. viride*. The original viability remaining (OVR) were 77.9%, 32.6%, and 13.67% after exposing 60, 120, and 180 minutes under sunlight, and 40.01%, 35.78%, and 32.62% respectively under UV lamp. The relative efficiency (RE) value of YJ treatment after exposure 60, 120, and 180 minutes under sunlight were 4.72, 3.52, and 4.42 respectively, and under UV lamp were 1.69, 1.65, and 1.73 respectively. YJ effectively protects *T. viride* spores from UV exposure.

Keywords: Biological agents, estate crop, UV protectant.

1. Introduction

The main technology for controlling estate crops diseases developed is the use of biological agents. So far, farmers have only relied on the use of chemical pesticides that pollute the environment, and are ineffective to control estate crop's diseases. Biological agents can naturally grow, spread, and actively infect pathogens so that they are expected to be more effective and inexpensive than synthetic pesticides. *Trichoderma* is one of the biological agents that has been widely developed to control the main diseases of estate crops. *Trichoderma* species that has been known as one of the potential biological agents of several crops diseases that can be developed as a biofungicide is *T. viride*. The fungus *T. viride* was proved as an effective biocontrol agent against *Fusarium oxysporum* [1][2][3][4], *Fusarium moniliforme* [5], *Phytophthora palmivora* [6][7][8], *Pythium aphanidermatum* [9], *Meloidogyne javanica* [10], and *Meloidogyne incognita* [11]. *T. viride* produces gliotoxin [12], viridin [8][13], and glioeverin [8] which are an antifungal substances that are able to control plant pathogenic fungi.

One of the disadvantages of biological agents for controlling plant pests and diseases is the low level of persistence in the field due to ultraviolet (UV) exposure. This is because UV-C (100-280 nm) from sunlight is germicidal with a broad spectrum that can inactivate bacteria and fungi [14][15]. *Clonostachys rosea* is effective for controlling *Botrytis cinerea* at the laboratory but is very susceptible...
to ultraviolet radiation, so its pathogenicity decreases in the field when exposed to sunlight [16][17]. Some entomopathogenic fungi also decrease their effectiveness in controlling pests, due to the influence of ultraviolet exposure [18].

Yam (Pachyrizus erosus) tuber contains saponin compounds, vitamin C and starch that are opaque can be used to protect the skin from sun exposure [19][20]. Yam tuber also contains flavonoid compounds [21] which one of its functions is UV absorption [22]. Samsudin et al. [23] reported that the yam juice can protect Spodoptera exigua nucleopolyhedrovirus (SeNPV) particles 86.18% after being exposed to ultraviolet for 30 minutes. To improve T. viride in planted fields, it is necessary to add natural ingredients that can protect spores from exposure to UV rays (UV protectant). This study aimed to evaluate the effectiveness of yam juice (YJ) in protecting T. viride spores against ultraviolet exposure.

2. Materials and methods

The research was conducted from March to July 2019 at the Plant Protection Laboratory of Indonesian Industrial and Beverage Crops Research Institute (IIBCRI), Sukabumi, West Java. The T. viride isolate used was TNAU-1 isolate which was propagated on potato dextrose agar (PDA) medium in the laboratory.

2.1. Test of the suitability of YJ as an in-vitro medium for growth of T. viride.

A total of 0.5 kg of yam tubers were washed, peeled, and grated. The grated result is then immediately squeezed without adding water because the water content of the yam tubers reaches 86-90% [20]. The results of the juice were used for this research.

The suitability test for YJ as a growing medium for T. viride was carried out in-vitro on PDA. The YJ treatments were performed by incorporating 0% (control), 1% (P1), 5% (P2), 10% (P3), and 20% (P4) concentration of YJ into PDA. The test was carried out by mixing the material with the media in a sterile petri dish in a laminar flow. T. viride on PDA media was cut with a diameter of 0.5 cm using a corbom placed in a position in the middle of the petri dish. Then incubated for 3 days at room temperature. The study used a completely randomized design (CRD) with each treatment repeated 4 times. Parameters observed were colony diameter for 2 and 3 days after treatmens (DAT), and observations were made every day.

2.2. T. viride spore propagation

Propagation of T. viride spores was carried out using a liquid medium containing 5% molasses (5 ml molasses dissolved in 95 ml distilled water). A total of 50 ml of liquid media that has been sterilized is put into a gallon. Colony stock of T. viride in PDA media with a diameter of 7.7 mm was put into each bottle, then covered with aluminum foil. Then incubated in a room with a temperature of 25˚C for at least four days so that the spores grow to meet the surface of the media. The spore culture layers were harvested, blended, added with 10 ml of distilled water, and stirred. The spore suspension was taken 1 ml and put into a test tube containing 9 ml of distilled water (10⁻²). 1 ml was taken back and put into a test tube containing 9 ml of distilled water (10⁻³). The number of spores in the suspension with a dilution of 10⁻³ was calculated with a hemacytometer of 0.0025 mm² under a microscope.

2.3. Viability test of T. viride spores after exposure under ultraviolet from the sunlight and UV lamp

A total of 100 ml of T. viride spore suspension with a density of 108/ml was mixed with 200 grams of talc in a sterilized container. The dough was divided into two parts, one part was added with 25 ml of YJ, and the other part was only added with 25 ml of distilled water without YJ (control). Then 75 ml of distilled water was added to each of the mixtures while stirring evenly so that the spore density in the formula was about 2 x 10⁷ spores/gram. The formula dough was dried at room temperature, then sieved using a flour sieve.

Formulas containing T. viride spores with 20% YJ and without YJ each 10 grams were taken and sprinkled evenly into a small baking dish. The exposure treatment under the sun was made for 60 (T1), 120 (T2), and 180 (T3) minutes. Each treatment used 3 small baking dishes as replicates. Exposure to
ultraviolet was carried out from 10.15 to 13.15 noon. As a negative control using a formula containing *T. viride* spores without YJ and not exposed to ultraviolet. After treatment, 1 gram of formula was taken from each small baking dish and dissolved in sterile distilled water to a volume of 10 ml. Each sample was made in 4 dilutions and the fourth dilution was taken to be grown on PDA media. The exposure treatment under UV lamps was made the same as the exposure treatment under sunlight. All small baking dishes were put into the laminar flow and irradiated with a 15 watt UV lamp for 60, 120, and 180 minutes. Observations were made with the parameter of the number of colonies (spores growing) of *T. viride*.

To assess the effect of YJ treatment on the viability of *T. viride* spores, the percentage original viability remaining (OVR) was used with the formula:

$$OVR(\%) = \frac{\%\ viability\ after\ exposure\ UV}{\%\ viability\ without\ exposure\ UV} \times 100$$

The effectiveness of YJ in protecting spores from UV exposure was calculated using the relative efficiency (RE) by the formula:

$$OVR(\%) = \frac{\%\ OVR\ on\ YJ\ treatment}{\%\ OVR\ on\ control\ (without\ YJ)}$$

### 3. Results and discussion

#### 3.1. Test of the suitability of YJ as an in-vitro medium for growth of *T. viride*.

The results showed that the YJ was suitable as a growth medium for *T. viride*, there was even an indication that it was able to increase the growth of *T. viride* on PDA media. All colonies in the YJ treatment had covered the surface of a 90 mm petri dish at 3 DAT, and beat the growth in the control which had not covered the surface (Figure 1).

On the 3rd day after treatment (DAT), the mean colony diameters of *T. viride* at concentrations of YJ 1%, 5%, 10%, and 20% were 83.43, 89.30, 88.89, and 88.89 mm respectively which are all significantly different from the control (80.04 mm). The increase in the diameter of the *T. viride* colonies on PDA media after 2 DAT and 3 DAT showed a significant difference, where after 2 DAT, the control was seen to be wider in diameter than all of the YJ treatments, but after 3 DAT, on the contrary, all treatments were more area compared to the control (Figure 2.).

![Figure 1. *T. viride* colony diameter on PDA after YJ treatment 0%, 1%, 5%, 10%, and 20% (left to right).](image-url)
3.2. Test of the viability of spores after treatment of exposure to ultraviolet light from sunlight and lamps. The average number of colonies in the formula that was not exposed to sunlight, either on 20% YJ treatment or without YJ was not significantly different, namely 31.67 and 32.33, respectively. Meanwhile, in the 60, 120, and 180 minutes of sun exposure, the average number of colonies in the YJ treatment was 24.67, 10.33, and 4.33, which was significantly different from the control (without YJ) of 5.33, 3.00, and 1.00, respectively (Figure 3).

Figure 2. Growth of *T. viride* on PDA that has been treated with YJ.

Figure 3. Effect of YJ treatment on the viability of *T. viride* spores after exposure under sunlight.
The test results on exposure under UV lamps in the laboratory for 60, 120, and 180 minutes, the average number of colonies in YJ treatments were 12.67, 11.33, and 11.33 which were significantly different from control (without YJ) 7.67, 7.00, and 6.67 respectively (Figure 4).

The results showed that the addition of 20% YJ to the _T. viride_ formula was able to maintain spore viability after exposure until 3 hours under the sunlight or UV lamps. The UV protectant performance of YJ decreased after 2 to 3 hours of exposure, although it was still able to protect some of the spores and its viability was still better than the control.

![Figure 4](image)

**Figure 4.** Effect of YJ treatment on the viability of _T. viride_ spores after exposure under UV lamp.

The ability of YJ to maintain the viability of _T. viride_ spores can be determined from the percentage of original viability remaining (OVR), namely the percentage of spore viability after exposure to UV light divided by the percentage of spore viability in the formula without UV exposure (control). The OVR percentage after exposure 60, 120, and 180 minutes under sunlight were 77.9%, 32.62%, and 13.67% respectively, and under UV lamp were 40.01%, 35.78%, and 32.62% respectively.

The effectiveness of YJ in maintaining the viability of _T. viride_ spores can be seen from the relative efficiency value (RE), namely the percentage of OVR from YJ treatment divided by the percentage of OVR formula without YJ (control). The RE value of YJ treatment after exposure 60, 120, and 180 minutes under sunlight were 4.72, 3.52, and 4.42 respectively, and under UV lamp were 1.69, 1.65, and 1.73 respectively. According to the standards of Mehrvar et al. (2008) [24], YJ is a good material to use as a natural UV protectant because it has an ER value more than 1.5 (Table 1).

These results showed that YJ is good to be used as a UV protectant for _T. viride_ spores. YJ contains alkaloids and saponins [19] which can protect spores from ultraviolet exposure as a reflectance [23]. Saponins have a characteristic that is to become foam when mixed with water so that it can function as a surface tension-reducing agent. The workings of these YJ materials are thought to be similar to detergents and optical brighteners which have been studied intensively as UV protectors. In addition, it is suspected that the YJ also contains flavonoids which are a type of secondary metabolite that is dominant in protecting fungal spores from exposure to UV rays [25]. This is based on the function of flavonoid complexes contained in plant materials which are known as plant resistance agents against ultraviolet B (UV-B) (280 – 315 nm) and can absorb UV light (UV absorber) [21]. Previous research has noted that some materials are effectively used as a UV protectant to protect the fungal spores from UV exposure from the sunlight. Hemalatha _et al_ [26] reported that skim milk, yeast extract, starch, molasses, and tinopal were able to protect the _Beauveria bassiana_ conidia after being exposed to
ultraviolet for 60 minutes with a germination rate of more than 70%. Emulsified mineral and vegetable oil were able to protect *Trichoderma asperellum* TV190 spores which were exposed to ultraviolet for 5 minutes under UVLMS-38 model lamp with wavelengths of 254 nm (UV-C) with maintaining viability of 37-43% and 56-63%, respectively [10].

**Table 1.** The effectiveness of yam juice in protecting *T. viride* spores from UV exposure.

| Treatment<sup>a</sup> | Number of colonies (cfu) | OVR<sup>b</sup> (%) | RE<sup>c</sup> |
|-----------------------|--------------------------|---------------------|----------------|
| T<sub>0</sub>YJ       | 31.67 ± 2.51             | 100                 | 1.00           |
| T<sub>0</sub>WYJ      | 32.33 ± 1.53             | 100                 |                |
| **Sunlight**          |                          |                     |                |
| T<sub>1</sub>YJ       | 24.67 ± 1.53             | 77.89               | 4.72           |
| T<sub>1</sub>WYJ      | 5.33 ± 2.89              | 16.49               |                |
| T<sub>2</sub>YJ       | 10.33 ± 1.53             | 32.62               | 3.52           |
| T<sub>2</sub>WYJ      | 3.00 ± 2.65              | 9.28                |                |
| T<sub>3</sub>YJ       | 4.33 ± 1.53              | 13.67               | 4.42           |
| T<sub>3</sub>WYJ      | 1.00 ± 1.00              | 3.09                |                |
| **UV Lamps**          |                          |                     |                |
| T<sub>1</sub>YJ       | 12.67 ± 1.53             | 40.01               | 1.69           |
| T<sub>1</sub>WYJ      | 7.67 ± 2.89              | 23.72               |                |
| T<sub>2</sub>YJ       | 11.33 ± 2.52             | 35.78               | 1.65           |
| T<sub>2</sub>WYJ      | 7.00 ± 1.00              | 21.65               |                |
| T<sub>3</sub>YJ       | 11.33 ± 2.08             | 35.78               | 1.73           |
| T<sub>3</sub>WYJ      | 6.67 ± 1.53              | 20.63               |                |

<sup>a</sup> YJ (yam juice); WYJ (without yam juice); T<sub>0</sub> (no exposure); T<sub>1</sub> (60 minute exposure); T<sub>2</sub> (120 minute exposure); T<sub>3</sub> (180 minute exposure)

<sup>b</sup> OVR (original viability remaining)

<sup>c</sup> RE (relative efficiency)

4. **Conclusions**

Yam juice added to PDA media was suitable for the growth of *T. viride*. The addition of 20% YJ in the *T. viride* formula resulted in a better percentage of remaining original viability (OVR) after exposure for 60, 120, and 240 minutes under sunlight and UV lamps compared to without YJ. The average relative efficiency (RE) value of YJ is greater than 1.5 which indicates a good UV protectant. YJ is one of the protectors of *T. viride* spores from sun UV exposure which has the potential to be developed as a natural UV protectant.

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