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Potential of Various Rice-Washing Water as a Source of Manufacturing Secondary Metabolites of *Trichoderma harzianum* T10 to Control Cucumber Crown and Root Rot

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ABSTRACT
Cucumber is one of the most widely consumed vegetable crops globally. Rice-washing water has not been previously explored as a potential source for producing secondary metabolites from antagonistic fungi. Phytophthora species frequently affect cucumber seedlings, leading to crown and root rot. This research aimed to assess the efficacy of washing water from various rice types on the conidial density of *Trichoderma harzianu*m T10 and its impact on crown and root rot in cucumber seedlings and overall plant growth. We conducted four treatments with rice washing water in vitro under a completely randomized design, comprising six replicates. Five treatments were evaluated in planta under a randomized block design with five replicates. The observed variables included conidial density, incubation period, disease incidence, disease progression expressed as the Area Under the Disease Progress Curve (AUDPC), plant height, fresh weight, and root length. The results indicated that washing water from glutinous rice provided the optimal medium for T. *harzianum* T10, yielding a conidial density of 10.3×10^{-6} conidia mL^{-1}, representing a 66.02% increase compared to washing water from white rice. The crude secondary metabolites produced by *T. harzianum* T10 in glutinous rice washing water significantly extended the incubation period and reduced disease incidence and AUDPC values by 40.34, 62.07, and 69.41%, respectively, compared to the control. Furthermore, the secondary metabolites from *T. harzianum* T10 in glutinous rice washing water enhanced plant height, fresh weight, and root length by 91.81, 92.42, and 95.21%, respectively, compared to the control.

Keywords: Antagonistic fungi; Biocontrol; *Cucumis sativus;* Cucumber seedlings; Soil-borne pathogen

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INTRODUCTION
Cucumber (*Cucumis sativu*s L.) is a horticultural commodity highly valued by the public due to its numerous benefits for daily life. This vegetable is rich in various nutrients and bioactive compounds that are beneficial for consumption and medicinal purposes (Uthpala et al. 2020). In Indonesia, cucumber production reached 4,440,567 tons in 2022; however, in 2023, production declined to 4,167,281 tons, representing a decrease of 6.15% (BPS-Statistics Indonesia 2023). Multiple factors can contribute to the decline in cucumber production, including the quality of superior seeds, soil fertility conditions, climatic influences, as well as pest infestations and plant diseases (Daunde et al. 2020; Parkash et al. 2021; Aparna et al. 2023).

One of the major diseases affecting cucumbers is seedling crown and root rot, which is caused by the pathogenic fungus *Pythium* sp. (Sigillo et al. 2020). Cucumber plants infected by this pathogen can experience significant losses. *Pythium* sp. begins to invade the seeds during germination in the soil and continues to affect the sprouts as they break through the soil's surface, resulting in symptoms such as lodging and plant death (Lamichhane et al. 2017). Several strategies can be employed to prevent and manage seedling crown and root rot; however, disease management predominantly relies on the use of synthetic chemical fungicides (Wu et al. 2023). The continuous and indiscriminate application of synthetic fungicides can have detrimental effects, not only on human health but also leading to environmental pollution (Okagu et al. 2023). Furthermore, chemical interventions have proven inadequate in fully addressing seedling crown and root rot. Alternative approaches, including the use of botanical fungicides and the development of resistant

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cucumber varieties, have yet to manage this disease successfully, and resistant varieties are not widely available in the market. Additionally, the efficacy of resistant varieties can be short-lived, as they may lose their resistance by the subsequent growing season (Alam et al. 2024).

An environmentally friendly approach to controlling seedling crown and root rot lies in the use of biological control agents. Among these, the antagonistic fungi genus Trichoderma has been widely utilized for its potential to suppress disease and promote plant growth (Usman Ghazanfar et al. 2018). In addition to controlling pathogens, Trichoderma species (TS) can stimulate plant growth and increase biomass production (Kumar et al. 2021; Contreras-Cornejo et al. 2024). This is attributed to the production of plant growth-promoting substances, including enzymes such as β-1,3-glucanase and chitinase (Kumar et al. 2023). Moreover, TS produces growth hormones, earning it the designation of a plant growth-promoting fungus (PGPF) (Kumar et al. 2021; Contreras-Cornejo et al. 2024). Notably, Trichoderma-based approaches are typically applied as preventative measures, whereas their effectiveness in mitigating established plant infections remains unclear (Zin and Badaluddin 2020). Furthermore, the application of Trichoderma can cause unintended damage to mushroom cultivation (Hatvani et al. 2017; Aydoğdu et al. 2020). Field applications of Trichoderma are further hindered by abiotic (Fadiji et al. 2023) and biotic constraints (Negi et al. 2023).

In light of these challenges, there is a pressing need for innovative approaches, including the utilization of secondary metabolites (SMs) produced by *Trichoderma* sp. Secondary metabolites are bioactive compounds that have negligible effects on organism or microorganism growth and development (Khan et al. 2020; Lv et al. 2024). However, the production of Trichoderma SMs is currently hindered by high costs and impracticality associated with laboratory culture conditions (Khan et al. 2020; Zhang et al. 2021). Rice-washing water has been used as a medium for Trichoderma cultivation, but its utility is limited by instability and poor yield (Hewavitharana et al. 2018; Asiandu et al. 2021; Ramadhana et al. 2022). Thus, there is a need to explore alternative sources of secondary metabolite production media. Interestingly, various types of rice, including white rice, brown rice, black rice, and glutinous rice, are commercially available but have not been utilized as sources of *Trichoderma* cultivation medium or as substrates to produce its secondary metabolites. This study aimed to investigate the efficacy of washing water from various rice types on *T. harzianum* T10 production and its impact on cucumber seedling crown and root rot, as well as its effects on seedling growth.

MATERIALS AND METHODS

The research was conducted at the Crop Protection Laboratory and greenhouse of the Faculty of Agriculture, Universitas Jenderal Soedirman, over a period of four months, from November 2023 to February 2024.

Preparation of *T. harzianum* T10

The *Trichoderma harzianum* isolates T10, sourced from the rhizosphere of ginger, were rejuvenated on Potato Dextrose Agar (PDA). One plug (1 cm diameter) of fungal colonies was extracted using a sterile cork borer and incubated for five days at room temperature.

Preparation of rice washing water

The rice varieties used in this study included white rice (IR 64), brown rice (Inpari 24 Gabusan), black rice (Super), and glutinous rice (Cempo Ireng). Fifty grams of each variety were weighed and placed in separate containers. Each rice sample was washed with 250 mL of water and stirred manually for two minutes (Nabayi et al. 2021). The washing water for each rice type was collected separately in 500 mL Erlenmeyer flasks and subsequently sterilized by autoclaving at 121 °C with a pressure of 15 psi for 20 minutes.

Preparation of *Pythium* sp.

Pythium sp. were isolated from cucumber seedlings exhibiting crown and root rot symptoms in the field. The seedlings were surface-sterilized for two minutes using 70% ethanol and then rinsed three times with sterile water. The roots were then isolated on PDA (Parnell et al., 2024) and incubated at room temperature (approximately 28 °C). The growing isolates were morphologically identified using a microscope, with reference to existing literature (Schroeder et al. 2013; Chenari Bouket et al. 2015; Toporek and Keinath 2021).

Production of secondary metabolites

Sterile rice washing water media derived from white, glutinous, black, and red rice was inoculated with two plugs (1 cm diameter) of the *T. harzianum* T10 isolate in 250 mL Erlenmeyer flasks (Soesanto et al. 2019). The flasks were shaken on an orbital shaker (Daiki) at 135 rpm for seven days at room temperature (Khan et al. 2020). Following the shaking period, the conidial density was assessed using a hemocytometer. After determining the density of *T. harzianum* T10 conidia, the solution was centrifuged at 9,000 rpm for two minutes to obtain crude secondary metabolites for further testing (Shehata et al. 2019).

Preparation of planting media

Polybags containing pasteurized planting media, consisting of soil and manure in a 1:1 weight ratio, were prepared. Planting holes were created 1 cm deep and inoculated with one plug (1 cm diameter) of *Pythium* sp. culture. Subsequently, one cucumber seed was placed in each hole and covered with a thin layer of the planting media.

Experimental design

The in vitro experiment employed a completely randomized design consisting of four treatments: white rice washing water, brown rice washing water, black rice washing water, and glutinous rice washing water, with six replicates for each treatment. Sterile filter paper disks measuring 0.5 cm in diameter were immersed in each type of rice washing water, drained, and placed on PDA in Petri's dishes at a distance of 3 cm from the edge of the dish. One plug (0.5 cm diameter) of Pythium sp. culture was positioned opposite the filter paper at a distance of 3 cm. The in-planta experiment utilized a randomized block design with five treatments: control, white rice washing water, brown rice washing water, black rice washing water, and glutinous rice washing water, each with five replicates. Each treatment received 10 mL of secondary metabolites of *T. harzianum* T10, derived from the washing water of the respective rice varieties, which was applied to the soil surface every three days for a total of five applications.

Maintenance of seedlings

Cucumber seedlings were maintained through regular watering and weeding. Watering was performed based on the moisture level of the soil media, while weeding was carried out manually.

Observation and data collection

Data were collected through various observations. The density of conidia was calculated using the following formula [1]:

$$S = \frac{X}{Lxtxd} x10^3 \text{..................................}[1]$$

Where *S* represents conidia density, *L* is the area of the counting box (0.2 mm²), *t* is the depth of the counting field (0.1 mm), ddd is the dilution factor, and *X* is the average number of conidia in five samples boxes (a, b, c, d, e) (Akagi et al. 2015). The incubation period was monitored from Pythium inoculation until the first symptoms of seedling damping-off appeared, recorded in days after inoculation (dai).

Disease incidence was assessed subsequent to treatment applications, with observations made every two days, calculated using the formula [2] used by Nathawat et al. (2020) as follows.

$$DI = \frac{n}{N} x100\% \text{ ....}[2]$$

Where DI denotes disease incidence (%), nnn is the number of affected plants, and NNN is the total number of observed plants.

The Area Under the Disease Progress Curve (AUDPC) was calculated using the formula [3] used by Paraschivu et al. (2013) as follows.

$$AUDPC = \sum_{i=1}^{n} \left(\left(\frac{(Y_i\text{-}Y_{i+1})}{2} \right) x(t_{i+1}\text{-}t_1) \right) \text{...........}[3]$$

Where AUDPC represents the disease progression curve (% per day), n is the number of observations, Y_i is the disease incidence at the initial (previous) observation, Y_{i+1} is the disease incidence in the subsequent observation, t_i is the time of the initial observation, and t_{i+1} is the time of the next observation. The height of the plants was measured every five days from the base to the apex using a ruler (cm). The wet weight of the plants was measured 21 days after planting (dap) using a digital scale (g). The root length was measured from the root collar to the root tip using a ruler (cm).

Data analysis

The data were analyzed using Analysis of Variance (ANOVA) at a 5% significance level, followed by Tukey's Honestly Significant Difference (HSD) test at the same significance level.

RESULTS AND DISCUSSIONS

Conidial density

The conidial density of *T. harzianum* T10 varied significantly across different rice-washing water media (Table 1). Notably, glutinous rice washing water exhibited the highest conidial density at 66.02%, which was significantly greater than that observed in white rice washing water and the other rice washing media. This phenomenon is likely attributed to the higher nutrient content, particularly carbohydrates, present in glutinous rice washing water, which is essential for the growth of *T. harzianum* T10. This finding is consistent with the observations of Amrinola et al. (2021) and Ali and Hashim (2024), who noted that white glutinous rice contains a carbohydrate content of 68%, which is higher than that found in other rice varieties. Furthermore, Harman et al. (1991) suggested that *T. harzianum* produces a higher number of conidia when the media provide adequate nutrients. According to Muñiz-Paredes et al. (2017), the diversity in fungal conidial production is closely related to the type of media used and its nutrient content.

Table 1. Conidial density of *T. harzianum* T10 in various rice washing water media

Treatments	Conidial density (× 10^6 conidia mL^{-1} solution)
White rice washing water	3.5±0.001 b
Brown rice washing water	5.9±0.001 b
Black rice washing water	4.0±0.001 b
Glutinous rice washing water	10.3±0.001 a
ANOVA Sig.	0.041

Remarks: Numbers followed by the same letter indicate no significant difference according to the HSD test with a 5% error rate.

The production of conidia in *Trichoderma harzianum* can be stimulated by the presence of substantial amounts of carbohydrates (Rai and Tewari 2018). According to Ali and Hashim (2024), the growth of Trichoderma species relies on carbohydrates as their primary energy source. Furthermore, Legodi et al. (2023) noted that fungi grown in media with high cellulose content can be utilized to produce cellulase enzymes; however, both fungal growth and cellulase production may be inhibited if the sucrose and glucose concentrations in the media are too low.

Incubation period analysis

The results of the analysis (Table 2) indicated that the application of various rice-washing glasses of water significantly altered the incubation period compared to the control. Specifically, the application of all types of rice washing water resulted in delays of 3.66%, 35.58%, 31.37%, and 40.34% in the incubation period of seedling damping-off, with glutinous rice washing water leading to the greatest delay. This enhanced delay is likely due to the high production of bioactive compounds in the crude secondary metabolites of *T. harzianum* T10, as suggested by the elevated conidia density (Table 1) observed in this treatment compared to other rice washing waters. These bioactive compounds may inhibit the development of Pythium species and slow the penetration of fungal pathogens into plant tissues and seeds, thereby extending the incubation period.

The production of secondary metabolites is closely correlated with the density of fungal conidia (Singh and Kumar 2023). As the density of fungal conidia in the solution increases, so does the production of secondary metabolites. This finding aligns with research by Soesanto et al. (2020), which indicates that higher densities of *T. harzianum* conidia in the media correlate with prolonged incubation periods. As a beneficial fungus, T. harzianum is frequently employed as a biological control agent to mitigate plant diseases and can indeed extend the incubation period of specific plant pathogens. Notably, Soesanto et al. (2019) confirmed that the administration of secondary metabolites derived from *T. harzianum* T10 can prolong the incubation period by up to 15 days compared to untreated controls.

Trichoderma harzianum produces secondary metabolites that contain various bioactive compounds. These include the enzymes β-1,3-glucanase and chitinase, which have the ability to lyse the cell walls of pathogenic fungi, as well as other compounds such as glyoxin, viridin, and trichomidin, which inhibit the development of pathogenic fungi (Lakhdari et al. 2023). Furthermore, secondary metabolites can penetrate the tissues of cucumber seedlings due to their polar nature (Soesanto et al. 2019). Once these metabolites are absorbed into plant tissues, they can systemically promote plant resistance mechanisms, enhancing the plants' ability to withstand fungal pathogen infections (Zhou et al. 2023).

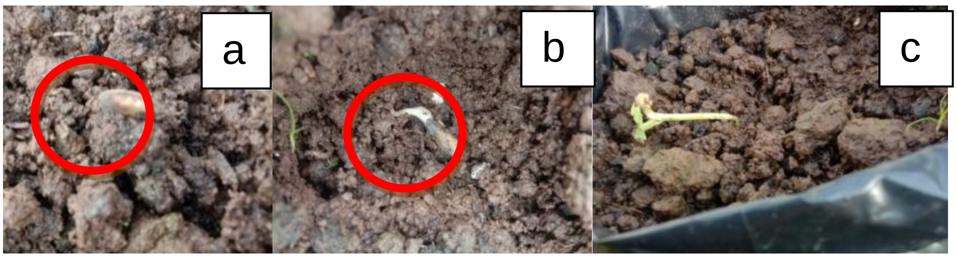
Cucumber seeds infected by *Pythium* species exhibit altered symptoms, including changes in seed color and texture. The seed coat typically becomes blackish brown and adopts a soft texture (Toporek and Keinath 2021). This phenomenon is consistent with the findings of Roberts et al. (2021), which indicate that cucumber seeds affected by *Pythium* species initially display a blackish-brown discoloration on their surface, eventually leading to seed softening or rot (Figure 1a). In seedlings, symptoms manifest as brown discoloration and a soft texture in the radicle (Figure 1b), along with watery or oily lesions on the root (hypocotyl) (Figure 1c).

In the absence of protection provided by the application of *T. harzianum* secondary metabolites, *Pythium* species infected cucumber seeds, resulting in symptoms of seed rot, particularly in seeds that had not yet emerged to the soil surface. The development of *Pythium* species is influenced by environmental factors such as soil moisture and temperature. According to Liu et al. (2020), high soil moisture significantly promotes the development and growth of *Pythium* species. Additionally, Bickel and Koehler (2021) noted that the optimum development of *Pythium* species is highly dependent on temperatures ranging from 25-36°C.

Table 2. Metabolites effect of Trichoderma grown in rice washing water media on pathosystem components of Cucumber dumping off.

Treatments	Incubation period (dai)	Disease incidence (%)	AUDPC (%. days)
Control	10.5±0.001 a	87±0.001 a	533±0.001 a
White rice washing water	10.9±0.001 b	80±0.001 ab	397±0.001 ab
Brown rice washing water	16.3±0.001 b	47±0.001 abc	210±0.001 bc
Black rice washing water	15.3±0.001 b	60±0.001 bc	263±0.001 bc
Glutinous rice washing water	17.6±0.001 b	33±0.001 c	163±0.001 c

Remarks: Numbers followed by the same letter in the same column show no significant difference in the HSD test at the 5% error level. Disease incidence data were transformed in arcsin √x + 0.5. dai= days after inoculation



Note: Picture Documentation before the seed emerges on the soil surface. a. seed coat, b. radicle, and c. after emerging to the soil surface.

Figure 1. Symptoms of cucumber damping off

Disease incidence

Statistical analysis revealed significant differences in disease incidence among the various rice washing water treatments (Table 2). The application of glutinous rice washing water, black rice washing water, brown rice washing water, and white rice washing water suppressed disease incidence by 62.07%, 31.03%, 46%, and 8.04%, respectively, compared to the control. Notably, glutinous rice washing water exhibited the highest suppression of seedling damping-off incidence, which corresponded with the longest delay in the incubation period. This can be attributed to the highest conidial density observed in glutinous rice washing water (Table 1), as high conidial densities are associated with increased production of bioactive compounds in secondary metabolites, thereby enhancing disease suppression (Soesanto et al. 2020).

The control crops displayed the highest incidence of disease compared to other treatments, primarily due to the lack of protection provided by *T. harzianum* T10 secondary metabolites. As a result, seeds were more rapidly infected by pathogens, and the crops lacked an effective resistance system to prevent pathogen infection (Saldaña-Mendoza et al. 2023). In contrast, the application of secondary metabolites of *T. harzianum* T10 from diverse rice-washing water substrates resulted in lower disease incidence, as these compounds inhibited pathogen development within the host plants. The secondary metabolites of *T. harzianum* contain enzymes such as chitinase, cellobiase, 1,3-β-glucanase, and cellulase, which can lyse the cell walls of pathogens, leading to the breakdown of pathogen cell walls and thereby delaying or inhibiting pathogen development (Markovich and Kononova 2003). Furthermore, the production of secondary metabolites utilizes water or polar materials, enabling these compounds to enter seeds through imbibition and subsequently increasing seed resistance while inhibiting pathogenic fungal infections (Zhou et al. 2023).

Area Under Disease Progress Curve (AUDPC) value

The disease progression curve can be utilized to calculate the Area Under the Disease Progress Curve (AUDPC) value, derived from the disease incidence data of plants over a specified time period (Simko and Piepho 2012). The AUDPC values resulting from the application of secondary metabolites derived from various substrates exhibit variability, as illustrated in Figure 2. A higher incidence of disease in the plants correlates with an elevated AUDPC value (Bock et al. 2022).

In accordance with Simko and Piepho (2012), the Area Under the Disease Progress Curve (AUDPC) is a parameter used to evaluate disease progression over time. Figure 2 illustrates the AUDPC results, which demonstrate significant differences among the treatments. Notably, the application of glutinous rice washing water yielded the smallest AUDPC value at 69.41%, relative to the control (Table 2). This observation is consistent with conidial density (Table 1) as well as disease incidence and incubation period (Table 2). It is postulated that the application of *T. harzianum* T10 has an effect on disease development or AUDPC value. This assertion is corroborated by Bock et al. (2022), who noted that ineffective treatments will lead to an increase in the AUDPC value, whereas treatments with high efficacy will result in a low AUDPC value.

The curve generated by the application of *T. harzianum* T10 secondary metabolites exhibits a slower disease development rate compared to the control, which demonstrates a faster disease development curve. Research by Saldaña-Mendoza et al. (2023) suggests that disease development in control plants is higher due to the absence of plant protection applications. Conversely, the application of secondary metabolite *T. harzianum* T10 in glutinous rice washing water substrate effectively protects seeds from *Pythium* sp. pathogen infection and results in a low AUDPC value. The application of secondary metabolites leads to a decrease in the AUDPC value because plants possess the ability to inhibit disease development through a systemic plant resistance mechanism (Zhou et al. 2023). A lower AUDPC value is indicative of reduced plant disease development and greater plant health (Bock et al. 2022).

Crop height

The data presented in Table 3 reveal a significant difference in crop height among the various rice washing water treatments relative to the control. The application of various rice washing water increases crop height by 91.81% for glutinous rice washing water, 86.31% for brown rice washing water, 83.50% for black rice washing water, and 79.37% for white rice washing water, respectively, compared to the control. This increase in crop height is attributed to the inhibition of seedling crown and root rot development, as well as the presence of bioactive compounds, including hormones, in the secondary metabolites of *T. harzianum* T10, which stimulate crop growth.

Role of *Trichoderma harzianum* in plant growth enhancement

According to Contreras-Cornejo et al. (2024), *Trichoderma harzianum* is classified as a Plant Growth-Promoting Fungus (PGPF) due to its ability to absorb nutrients and active minerals from the soil, thereby enhancing plant growth. Additionally, *T. harzianum* facilitates the breakdown of organic matter in the soil, rendering it more soluble and accessible for plant uptake (Zin and Badaluddin 2020). Vitti et al. (2022) reported that crown length can be significantly increased through the application of *T. harzianum*. Furthermore, (Li et al. 2015) indicated that *T. harzianum* enhances the availability of key nutrients, including manganese (Mn), phosphorus (P), potassium (K), nitrogen (N), aluminum (Al), and iron (Fe), which contribute to improved plant growth. Therefore, when nutrients in the soil become more available due to the application of *T. harzianum*, plant growth can be expected to increase.

Table 3. Metabolites effect of Trichoderma grown in rice washing water media on cucumber seedlings growth components

Treatments	Crop height (cm)	Crop fresh weight (g)	Root length (cm)
Control	1.13±0.001 b	0.84±0.001 c	1.19±0.001 c
White rice washing water	5.48±0.001 ab	3.54±0.001 bc	9.71±0.001 b
Brown rice washing water	8.28±0.001 ab	6.82±0.001 ab	14.23±0.001 b
Black rice washing water	6.85±0.001 ab	5.30±0.001 bc	10.63±0.001 b
Glutinous rice washing water	13.80±0.001 a	11.09±0.001 a	24.89±0.001 a

Note: Numbers followed by the same letter in the same column show no significant difference in the HSD test with a 5% error rate

Crop fresh weight

Statistical analyses demonstrate that crop fresh weight exhibited significant differences among treatments involving various rice washing waters enriched with secondary metabolites from *T. harzianum* T10 (Table 3). The application of glutinous rice washing water resulted in a 92.42% increase in crop fresh weight, followed by black rice washing water at 84.15%, brown rice washing water at 87.68%, and white rice washing water at 76.27% compared to the control. These findings are consistent with crop height observations, suggesting that the application of secondary metabolites from *T. harzianum* T10 contributes to enhanced crop growth. Guo et al. (2022) supported this notion, indicating that the secondary metabolites of *T. harzianum* facilitate rapid photosynthesis and increased nutrient absorption by promoting root hair development, leading to greater fresh weights in treated plants compared to controls (Li et al. 2015).

The highest crop fresh weight was recorded for the glutinous rice washing water treatment, showing an increase of 92.42% relative to the control, which aligns with the observed crop height (Table 3). The elevated fresh weight in this treatment likely results from the secondary metabolites produced by *T. harzianum* T10, which effectively break down soil nutrients, making them available for crop uptake and stimulating growth (Vitti et al. 2022). Healthy crop growth inherently contributes to increased fresh weight.

Root length

As shown in Table 3, the root length of cucumber crops revealed highly significant differences due to the application of various rice washing waters. The application of white rice washing water resulted in an increase in root length by 87.74%, while brown rice washing water enhanced root length by 91.63%, black rice washing water by 88.80%, and glutinous rice washing water by 95.21% compared to the control. The longest root length was observed with the application of glutinous rice washing water, correlating with increased crop height and fresh weight. This enhancement in root length is attributed to the presence of bioactive compounds in the secondary metabolites of *T. harzianum* T10, including growth regulators that promote root elongation and overall plant growth.

Tyśkiewicz et al. (2022) noted that the potential of *Trichoderma* species can stimulate root growth, facilitating greater nutrient absorption, reducing pathogen populations, and providing protection against pathogen attacks. Guo et al. (2024) also highlighted that the application of *T. harzianum* can enhance root length in kidney beans. Moreover, Xiao et al. (2023) emphasized that plants can produce growth-stimulating hormones, such as gibberellins (GA3), indoleacetic acid (IAA), and benzylaminopurine (BAP), as the chemical compounds generated by *T. harzianum* can trigger substantial hormone production. According to Edelmann (2022), the presence of IAA can significantly enhance the growth of plant roots, including lateral, primary, and adventitious roots.

CONCLUSIONS AND SUGGESTIONS

Based on the findings from the conducted research, it can be concluded that glutinous rice washing water is the most effective growth medium for *Trichoderma harzianum* T10, exhibiting a conidial density of 10.3 × 10^6 conidia.mL^{-1}. The secondary metabolites from *T. harzianum* T10 present in glutinous rice washing water were the most effective in managing seedling crown and root rot. This treatment successfully delayed the incubation period, reduced disease incidence, and lowered the AUDPC value by 40.34, 62.07, and 69.41%, respectively, compared to the control. Moreover, glutinous rice washing water significantly enhanced crop growth, leading to increases in crop height, fresh weight, and root length by 91.81, 92.42, and 95.21%, respectively, when compared to the control.

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