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The Potential of *Deleterious rhizobacteria* to Control Primary Weed *Echinochloa* sp. on Rice

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Abstract. *Deleterious rhizobacteria* (DRB) is one of the rhizobacteria that can be used as a bioherbicide and can stimulate plant growth. The objectives that can be obtained from this study are to determine the potential of DRB on the growth of weeds *Echinochloa crusgalli* and *Echinochloa colona* on rice. Variables observed in this study were identification of secondary metabolites, measurement of weed sprouts, percentage effectiveness of bacterial isolates against weed seeds, normal sprouts, abnormal sprouts, fresh seeds that did not grow and dead seeds. The aim of this study is to explore biological agents that can be used to control the main weeds of rice crops. This candidate could be a recommendation for environmentally friendly controls. Exploration of the DRB group was obtained by collecting soil samples around the roots of rice crops. Then isolate it, identify as morphologically and physiologically, and test it in vitro studies. The results showed that DRB had the potential to control weed growth and could stimulate the growth of rice. The application of PFA and PFB isolates had a significant effect on inhibiting the length of weed roots and buds, the average percentage of control effectiveness > 80-90%. Novelty in this study could be found as candidate groups of DRB bacteria with specific locations to control the weed *Echinochloa* sp. on rice crops. For society, this research could be an alternative to reduce dependence on synthetic herbicides. DRB has the potential to be formulated into a bioherbicide to control weeds.

Keywords: Bioherbicide; Biological; Cyanide; *Echinochloa*; Phytotoxicity.

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INTRODUCTION

Weeds are a type of plant pest organism that can impact productivity and yield loss of rice (Hossain *et al*., 2020). The presence of weeds becomes a competitor plant for the main crop. Not only that, weeds can become hosts for pests and diseases (Beche *et al*., 2023; Paul *et al*., 2024). Weeds in rice will grow competitively in absorbing nutrients and growing power. Weed growth in rice is difficult to control because the density and height of the plants can compete with cultivated plants. According to Rao *et al*., (2017) cultivating rice without weeding can reduce yield losses. Therefore, the main weeds in rice must be controlled so as not to cause a decrease in production.

The main weeds in rice are dominated by the families Poaceae, Convolvulaceae, Cyperaceae, and Phyllanthaceae (Haris *et al*., 2019; Susanti *et al*., 2021). Jagagoan (*Echinochloa* sp.) is a weed from the Poaceae family that dominates rice (Sitthinoi *et al*., 2017). Current weed control tends to use synthetic herbicides. Synthetic herbicides in the long-term cause risks to the environment, resistance, and residues in agricultural products (Chtourou *et al*., 2024; Piancini *et al*., 2015; Yates *et al*., 2024). Losses caused by weeds can reduce the quality and quantity of production of cultivated plants so that the selling value of the plants becomes low (McCollough *et al*., 2024). Environmentally friendly alternative control can be done mechanically, namely pulling weeds traditionally. However, in large weed populations and areas, this type of control is not effective. The direction of Law Number 22 of 2019 in Indonesia states that the process of cultivating plants must be carried out according to the principles of sustainable agriculture. One implementation of sustainable agriculture is biological control. The advantage of biological control is for specific targets so that it can control weeds without causing damage to cultivated plants. One biological control that can be used is DRB (*Deleterious rhizobacteria*).

Deleterious rhizobacteria (DRB) is one of several types of rhizobacteria that have the potential to be used as bioherbicides and to stimulate plant development and growth (Abbas *et al*., 2018; Phukan *et al*., 2021; Radhakrishnan *et al*., 2016). DRBis an example of biological, environmentally friendly, and sustainable weed control. The mechanism by which DRB affects weeds sick is by producing phytotoxins such as hydrogen cyanide (HCN), which can be absorbed by weeds. DRB does not eradicate weeds but instead suppresses their initial growth and inhibits seed germination (Rakian *et al*., 2015) by releasing these phytotoxins. DRB plays a significant role in controlling weed populations.According to (Zeller *et al*. 2007; Kremer & Souissi, 2001), rhizobacteria can produce secondary anti-metabolite compounds that inhibit the growth of weed seeds. The secondary metabolites released can also control plant diseases (Masnilah *et al*., 2021). Bacteria from the *Pseudomonas* sp group. a type of DRB, can stimulate plant development through the production of phytohormones (Mengstie *et al*., 2024; Rizvi *et al*., 2024). DRB can also be applied to the main plant because it does not cause damage to other parts of the plant.

The element phosphate is needed by plants, but it is not always available in the soil. The presence of phosphate-solubilizing bacteria in the soil helps in its availability to plants. Rhizobacteria such as *Pseudomonas* sp. and *Bacillus* sp. It is a root bacterium that can dissolve phosphate. These bacteria can release organic ions so that they are absorbed by plants (Blanco-Vargas *et al*., 2020; Fitri *et al*., 2020). According to Nikfarjam & Aminpanah (2015), bacteria *P. fluorescence* has an impact on the growth of rice, increases the availability of P elements (Phosphorus) in the soil, and stimulates cell and tissue division in rice. *Pseudomonas fluorescence* which synergizes with plant roots can produce and stimulate phytohormones for plant needs such as auxin, gibberellins, and cytokinins (Nepali *et al*., 2018). The research aims to examine the potential of *Deleterious rhizobacteria* (DRB) as a candidate for biological control of champion weeds in rice. The advantage of DRB bacteria is that they have the potential as biological agents to control weed populations by infecting crops. Apart from that, it produces several growth hormones for plant growth.

METHODS

Research sites

The study was conducted at the Disease Laboratory, Department of Plant Protection, Faculty of Agriculture, University of Jember. The research period starts in January 2022 – August 2023. The sampling location is in Sumber tengah Village, Mumbusari District, Jember Regency. Soil samples were taken in the rhizosphere area of rice where there were no weeds around which showed signs of disease. Soil samples were taken in the rhizosphere area of the rice where there were no weeds infected with the disease. Soil samples were taken from the root soil of rice which were healthy and had no weeds around them. The soil samples obtained were immediately taken to the laboratory for isolation and identification.

Isolation and Identification Bacteria

The isolation of candidate root bacteria was carried out as follows: First,10 g of rice roots with soil granules were taken and placedin a 100 mL Erlenmeyer flask containing sterile distilled water.

The Erlenmeyer flask was then shaken using a shaker at 150 rpm for 30 minutes to homogenize the solution. The resulting suspension was then diluted in a serious from 10^{-1} to 10^{-10}. The dilutedsolution was poured onto King's B agar medium which is characteristic for Pseudomonas spp and the resulting isolate were observe for fluorescence. A total of 0.1 mL of the suspension was taken, spread on King's B medium, incubated for 2 days. A single colony was then picked with a loop needle, streaked onto King's B medium, incubated for 48 hours, and stored as a pure culture (Schaad *et al*., 2001; Yusran, 2023).

Rejuvenation and Multiplication of Bacteria

The isolated results were then rejuvenated and grown on NA (Sodium Agar) media. One cycle of the isolate was obtained and added with 5 mL of distilled water and vortexed for 3 minutes until homogeneous. After homogenizing one cycle, the suspension was grown and incubated at room temperature for 2 days (Figure 1).

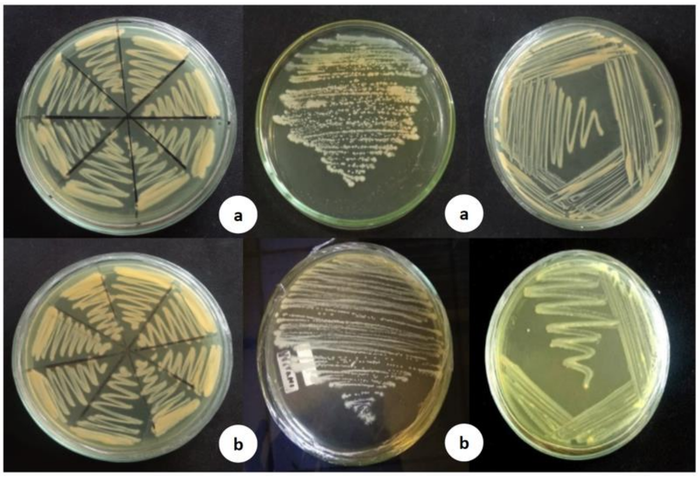

Figure 1. *Pseudomonas* sp. isolates from the collection. Description: a) PFA isolate (b) PFB isolate.

Physiological Characterization Test

The physiological characterization was carried out through several stages, including the hypersensitivity test, gram stain test, catalase test, fluorescent pigment test on Kings-B medium, starch hydrolysis test, gelatinase test, phosphate solubilization test, and hyrdrogen cyanide (HCN) production test.

Experimental Design and Data Analysis

Sterilized seeds were germinated in petri dishes that had been filled with cotton and moistened with 20 mL water. Seeds that had pre-germinated were selected uniformly based on the criterion of seed coat breaking. A total of 10 *Echinochloa sp.* seeds were place into eachsterile petridish. A bacterial suspension, 30 µl in volume was applied to each seed using a micropipette. The dishes were then incubated for 14 days. The experiment was repeated three times for each test isolate with six treatments, resultingin 18 experimental units (see Table 1).

Table 1. Experimental design

Treatment	Information
P0G1	: Control of *E. crusgalli*
P0G2	: Control of *E. colona*
P1G1	: PFA + *E. crusgalli*
P2G2	: PFA + *E. colona*
P2G1	: PFB +*E. crusgalli*
P2G2	: PFB +*E. colona*

Data obtained from the relationship between isolate application and *Echinochloa sp*. weed types will be analyzed using linear regression, with the day of observation as the independent variable and symbolized by (X) and the length of the weed roots as dependent variable (Y). The significance of the results will be assessed using the F-test.

RESULTS AND DISCUSSION

Characterization and Identification of Bacteria

The results of the exploration produced candidate *Deleterious rhizobacteria* which will be identified morphologically and physiologically. Each group of rhizobacteria has different colony shapes and characteristics. Morphologically, the bacteria obtained were observed under a microscope with direct observation according to the characteristics of the target bacterial colony, with several observation parameters, namely: colony shape, colony color, and colony edges. Morphological identification of two types of bacterial groups was obtained. The PFA and PFB isolate codes have the same characteristics, namely colonies are round, flat edges, fluid, white colonies. Based on the results of research, the bacteria obtained are bacteria belonging to the Pseudomonas sp. group, and it is suspected that the type of bacteria that has been obtained is Pseudomonas fluorescens, this has been adjusted to the morphological and physiological characteristics according to Schaad *et al*. (2001) namely, it has the morphological characteristics of round colonies, flat edges, fluidity, and white colonies.

Deleterious rhizobacteria are obtained in the rhizosphere area of rice where there are no pests or competing plants (weeds) around them and have an important role in plant health and soil fertility. According to Rabari *et al*. (2023) states that the main requirement for bacteria that can be used as *Deleterious rhizobacteria* is that they do not cause poisoning in the main plant (phytotoxicity). Rhizobacteria were obtained from the root area of healthy rice with the suspicion that this area had many rhizobacteria colonies that had the potential to become *Deleterious rhizobacteria* and there was no visible weed growth around the roots. Plant root bacteria obtain energy availability from metabolite compounds released by plants through the roots. These metabolite compounds can be sugar compounds, amino acids, organic acids, glycosides, nucleotide compounds, vitamins, enzymes and indole compounds (Asghari *et al*., 2020; Yilmaz & Karik, 2022). Metabolic activity and metabolite compounds released by plants into the soil through the roots are factors that greatly determine the microbiological condition of the soil in the root area. *Deletious rhizobacteria* (DRB) which have been formulated with several types of carrier materials have the ability to interact with soil properties (Zdor and Alexander, 2005).

Based on the results of morphological observations of bacteria, shows that these bacteria belong to the Pseudomonas sp group. Gamez *et al*. (2019), stated that the morphological characteristics of Pseudomonas sp., are round colonies and white colonies. The results of the bacterial isolates from exploration had the morphological characteristics of round colonies, even edges, and white colonies in both isolates obtained from exploration results. Physiological characterization was carried out by carrying out several tests on the bacterial isolates that had been obtained. The results of the physiological characterization of bacterial isolates resulting from the exploration that has been carried out can be seen in Table 2.

Based on observations of physiological characterization which have been adjusted to the determination book according to Schaad *et al*. (2001), the two bacterial isolates resulting from exploration were *Pseudomonas* fluorescens. This is also supported by the results of morphological identification, where the colonies of the two isolates have the same characteristics *P. fluorescens*. According to Korany *et al*. (2021), *P. fluorescens* has round bacterial colonies, flat edges, fluid, and is capable of producing greenish-yellow pigment on Kings'B medium. King's B medium is a medium that contains little Fe ions (Kumar *et al*., 2019). The group of bacteria that it is part of *P. fluorescent* will form siderophores in chelating Fe (iron) ions. Siderophores were detected as greenish yellow pigments that diffused into King's B medium (Figure 2). Pigments that diffuse into the medium become more clearly visible when observed under an ultraviolet lamp with a wavelength of 365 ns. The following is a picture of the physiological characterization of bacterial isolates.

Table 2. Physiological Characterization of *Deleterious Rhizobacteria* Isolates

Test	Characteristics*Pseudomonas fluorescens* menurut Schaad (2001)	Isolate PFA	Isolate PFB
Hypersensitive	Negative (-)	Negative (-)	Negative (-)
Gram	Negative (-)	Negative (-)	Negative (-)
Catalase	Positive (+)	Positive (+)	Positive (+)
Fluorescent Pigments	Positive (+)	Positive (+)	Positive (+)
Starch Hydrolysis	Positive (+)	Positive (+)	Positive (+)
Melting Gelatin	Positive (+)	Positive (+)	Positive (+)

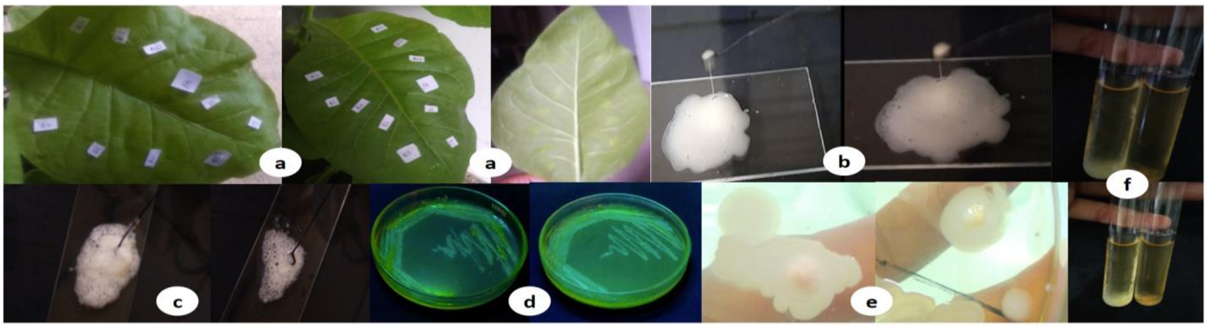

Figure 2. (a) hypersensitive test (b) gram test (c) catalase test (d) fluorescent pigment test (e) starch hydrolysis test (f) gelatin melting test.

Potential of Rhizobacteria Isolates as *Deleterious rhizobacteria* (DRB)

Rhizobacterial isolates resulting from exploration that have the potential to be DRB were tested for effectiveness on weed seeds that are dominant in rice. The weeds used were *Echinochloa crusgalli* and *Echinochloa colona*. The application of bacteria in all treatments can inhibit root growth compared to the control treatment (no bacteria applied). The effectiveness of bacteria in inhibiting the growth of weed seeds *E. crusgalli* and *E. colona* seen in Table 3.

Table 3. Average Root Length and Buds

Treatment	Average Length (cm)	
	Root	**Bud**
P0G1	0.4	1.29
P0G2	0.37	1.23
P1G1	0.032	0.18
P2G2	0.027	0.22
P2G1	0.033	0.26
P2G2	0.03	0.24

Based on the data in the table above, it can be concluded that the two isolates of the DRB type were effective in suppressing the growth rate of roots and buds, this was because the results of observing the length of the roots and buds were smaller than the control treatment. The most effective type of isolate in suppressing root growth in weeds was PFA because it produced root lengths that were much smaller than the control, namely 0.032 cm in the weed Echinochloa crusgalli and 0.027 cm in the weed *Echinochloa colona* (Figure 3). The isolate that was most effective in inhibiting bud growth in weeds was PFA because it produced much smaller root lengths than the control, namely 0.18 cm in the weed *Echinochloa crusgalli* and 0.22 cm in the weed Echinochloa colona. The allelopathic compounds can also impact root formation, inhibition and growth of target plants (Głąb *et al.*, 2017; Hussain *et al*., 2022; Rodríguez-Mejías *et al.*, 2023). Environmental factors such as temperature, pH, and light intensity (Han *et al.*, 2021; Shan *et al.*, 2023). The application of *Deleterious rhizobacteria* has a real effect on weed growth, this can be seen from the length of weed roots and buds that are inhibited after PFA and PFB bacteria are applied, which can be seen in the figure 3. DRB inoculation with plant roots in soil media can have a phytotoxic effect that plants become infected (Brinkman *et al*., 1999).

Based on Figure 5, it is known that the correlation value between the observation day variable (X) and the root length variable (Y) is 0.9632 which is included in the very strong category based on the correlation category table. The coefficient of determination or R Square value is 0.9278 or equal to 92.78%. This figure means that the observation day variable (X) affects on the root length variable (Y) by 92.78% after applying PFA to weeds *E. crusgalli*, while the remaining 7.22% is influenced by other variables outside this regression equation or variables that are not studied (Figure 4). It can be concluded that the application of *Deleterious rhizobacteria* affects on weed growth, this can be seen from the calculated F value which is 154.25, while the resulting significance value is 0.00 which is smaller than 0.05, thus it can be It was concluded that the observation day variable (X) had a significant effect on the weed root length variable (Y) after PFA was applied *E. crusgalli*.

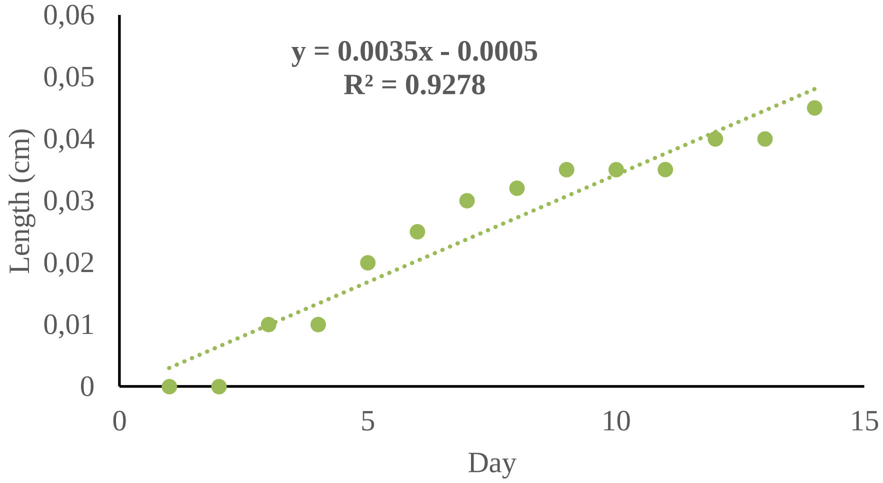

Figure 3. PFA isolates in the weed *Echinochloa crusgalli* (root length)

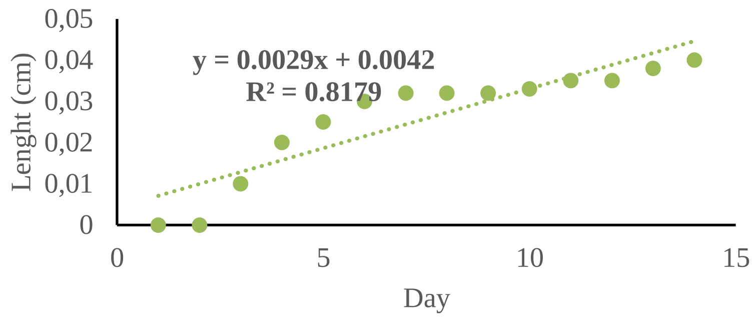

Figure 4. PFA isolates in weeds *Echinochloa colona* (Root Length).

Weeds that grow around rice can reduce the productivity of rice so they must be controlled. The main weeds that need to be controlled in rice are: *E. crusgalli* and *E. colona* which can reduce rice production by 100%. Based on the results of research that has been carried out, growth *E. crusgalli* and *E. colona* those that had been treated experienced abnormal growth, in the germination of weeds that were treated with PFA and PFB producing HCN, a growth process did occur, but the growth became abnormal. The mechanism by which DRB infects weeds is by producing phytotoxins such as cyanide acid (HCN) which are absorbed by weed seeds. DRB carries out activities by inhibiting the initial growth of weeds and seed germination (Phukan *et al*., 2021; Verdugo-Navarrete *et al*., 2021). The higher the HCN content in the bacteria, the more effective the level of bacterial control against dominant weeds in rice. The following are the results of the HCN test on bacteria.

According to Ahmad *et al*. (2023); Fatima *et al*. (2024); Reddy *et al*. (2023); Simarmata *et al*. (2020), stated that *P. fluorescens* can induce plant resistance, produce antibiotic compounds and can act as Plant Growth Promoting Rhizobacteria. Plant Growth Promoting Rhizobacteria (PGPR) is a biological agent that has been widely used and tested to control pest and diseases (Safriani *et al*. (2020). Another advantage of using antagonistic rhizobacteria as biological control agents is that they can be combined with one bacterium with another. The advantage of the combination of rhizobacteria is that can to increase adaptability to the environment, and is able to control weeds simultaneously and in synergy so that it is superior. Research result Kremer & Souissi (2001) strains of Pseudomonas that can produce secondary metabolites in the form of cyanide acid (HCN) are *P. chlorophis*, *P. aureofaciens*, *P. aeruginosa*, *P. flourecens*.

Based on the results of the HCN test on PFA and PFB isolates, it showed a color change in the first and second repetitions. In the first repeat on PFA and PFB, the color of the filter paper changed to brick red, which indicated that the test isolate was producing HCN (+++). In the second repeat on PFA and PFB, the color of the filter paper changed to brown, which indicated that the test isolate was producing HCN (+++). Figure 5 presents the development of weeds introduced with PFA and PFB isolates which are experiencing problems because not only are the root size and bud length shorter than the control but the growth is abnormal or deformed, this is a reflection of disruption of plant physiological processes.

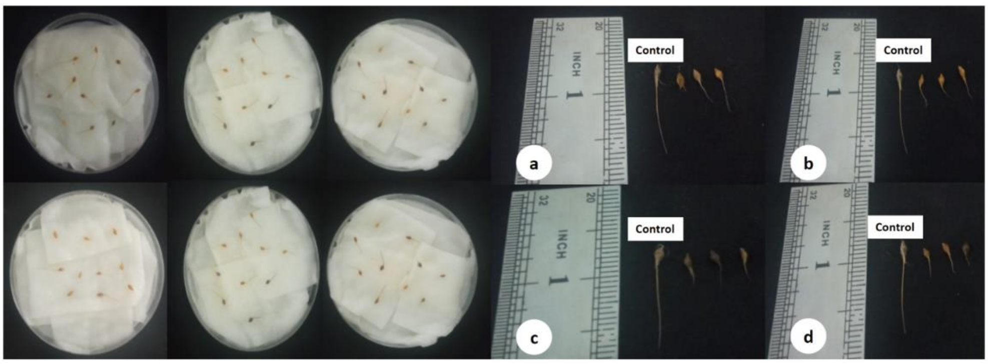

Figure 5. Germination of weeds treated with isolate *Deleterious Rhizobacteria.* Note: (K: Control, a: P1G1 (PFA Treatment on *E. crusgalli*), b: P1G2 (PFA Treatment on *E. colona*), c: P2G1 (PFB Treatment on *E. crusgalli*), d: P2G2 (PFB Treatment on *E. colona*).

Table 4. Effectiveness of Bacterial Control on Root and Bud

Treatment	Effectiveness Value (%)	Category	Effectiveness Value (%)	Category
	Roots		Bud	
P1G1	92	Very capable	86	Very capable
P2G2	92	Very capable	84	Very capable
P2G1	91	Very capable	83	Very capable
P2G2	91	Very capable	83	Very capable

Control effectiveness is the level of a biological agent's ability to inhibit the growth of weed roots and buds. The control effectiveness value is calculated based on the length of the weed roots and buds. The effectiveness values obtained are then classified according to the category of whether or not the biological agent can control weeds in rice. Table 5 presents the effectiveness of the test isolates in controlling *E. crusgalli* and *E. colona*. The results show that the presence of bacteria exerts a demonstrated inhibitory effect on *E. crusgalli* and *E. colona*. The percentage of control effectiveness shows the level of efficiency of the test isolates in controlling dominant weeds. The higher the percentage value of control effectiveness, the higher the level of effectiveness of each isolate in inhibiting growth and controlling dominant weeds. Based on this table, it can be explained that the percentage of control effectiveness *E. crusgalli* and *E. colona* the average is >80-90% (Table 4) so it can be concluded that the application of PFA and PFB isolates is very capable of controlling dominant weeds in rice.

PFA and PFB isolates were able to suppress weed growth in the roots and buds. PFA and PFB isolate candidates have high effectiveness. The bacterial isolate that has the highest effectiveness value is the PFA isolate, however the PFB isolate also has an effectiveness value that is not much different from PFA, only slightly related. Based on the level of effectiveness value for controlling biological agents can be concluded that the application of PFA and PFB isolates was declared very capable with a control effectiveness value of >80% for controlling the main weeds in rice.

CONCLUSION

The application of *Deleterious rhizobacteria* (DRB) has a potential and significant effect on the growth of weeds and rice. The application of bacteria has a real effect in inhibiting the growth of weed roots and buds. The most effective isolate in inhibiting root and bud growth in weeds is PFA. The application of PFA and PFB isolates had a significant effect in inhibiting the length of weed roots and buds, having an average percentage of control effectiveness >80-90%. The application of PFA and PFB isolates is stated to be very capable of controlling the main weeds in rice with a control effectiveness value of >80% so that they can be used as an alternative weed control. The advance on this study is needed for formulation of bacteria and application on a field scale for its effectiveness in suppressing weeds and its effect on main plant growth.

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