The Effectiveness of Indigenous Rhizobacteria As Bioherbicide to Control of Weed

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ABSTRACT

Developing method to control weed using rhizobacteria has been increasingly studied recently and environmentally sound friendly strategies. The purpose of this research were to evaluate the effectiveness of indigenous rhizobacteria isolates which are isolated from Buton, Muna and South Konawe Southeast Sulawesi Indonesia on the inhibition of seed germination and growth seedling of several types of weeds. A series of experiment was conducted at the Laboratory of Agrotechnology University of Halu Oleo. The research arranged in completely randomize design (CRD) with five treatments of rhizobacteria isolates: BL03, BL07, MS01, SS01, SS02, and control (non-treatment). Each treatment was repeated three times, totalling 18 experimental units. The results showed that the rhizobacteria treatments had significant effect on weed seed germination parameters, which included percentage of germination and relative growth rate, and weed vegetative growth of seedling height and number of leaves. Rhizobacteria isolates of BL03, BL07, MS01, SS01 and SS02 were consistently able to exhibit inhibitory activity on any weed species tested (Paspalum conjugatum, Ageratum conyzoides, Chromolaena odorata and Amaranthus spinosus) on both seed germination and vegetative growth. The mechanism of inhibition was supposedly enabled by the ability of the four rhizobacteria isolates in producing hydrogen cyanide compounds (HCN). Further study is needed to determine the weed control mechanisms by the isolates and its development as effective bio-herbicide.

INTRODUCTION

A weed is a plant that grows in a situation where it is unwanted. If it is not effectively controlled in a cultivated area, weed may become a biotic factor that can severely affect crops production. As a type of plant, weed can grow in any condition required by other types of plant. Weeds that are not under control, both physically, chemically, and biologically, can cause as much as 69.8% lost (Oerke and Dehne, 2004). Moenandir (1993) propounds that when weed grows in proximity to desirable crops, the two types of plant compete to each other, as a result of their intense interaction. The competition occurs as the plants are trying to get nutrients, water, sun light, CO2, and room to grow (Sembodo, 2010). Weed can reduce crop production by 20-80%, or even by 90% (Fadhly and Tabri, 2009). As one type of organisms considered to be plant pest, weed is a crucial element that must be kept under control in order to minimize any unwanted effects it can have on crops.

A common method to control weed is by using herbicide. This chemical method has been mostly favored due to its practicality and immediate results. However, herbicide can have harmful effects on our environment: it can cause pollution and ecological disturbance, it can leave poisonous herbicide residues, and it can render a weed resistant to particular herbicide. For these reasons, an alternative method is needed to control weed in a more effective yet more environmentally-friendly way. A biological method of controlling weed seems to be much better than others in that it is environmentally safe and it only works during the lifetime of the plant. In addition to biologically combating weed, some agents can also bring out particular hormone-growing compounds, performing another beneficial function to the crop (Silva et al., 2004). One technique to deal with OPT in an earth-friendly way is by implementing a biological control that utilizes microorganism.

Utilizing microorganism as bio control agent is a method of biological control which has recently attracted considerable attention. Microorganisms belonging to the group of rhizosfer bacteria have frequently been used to accelerate plant’s growth rate and are evidently effective to combat various types
of plant disease. It has been reported that rhizobacteria is capable of inhibiting plant growth (*Deleterious Rhizobacteria*), making it potential for controlling weeds. A number of previous studies shows that isolate bacteria (*Pseudomonas kilonensis*) that is isolated from weed host (*Echinocchio crusgalli*) can have a negative effect on weed, in that the growth of weed host is inhibited, if rhizobacteria is inoculated into weed seed prior to planting the plant (Zeller et al., 2007). Some groups of rhizobacteria have also been reported to be capable of producing antimetabolite compounds that have a phytotoxic effect on weed host. The compounds they produce are allelopathic compounds that affect specifically only the weed host, thereby causing no harmful effects on desirable crops or plants (Kramer, 2006 in Indejit and Mukarji, 2006; Cavalho et al., 2007).

Southeast Sulawesi is one of the regions in Indonesia that is rich of specific biological resources with great potentials for development. Characterized by marginal lands which are dominated by ultisol soils, the region can potentially produce specific rhizobacteria isolates needed to develop an environmentally-friendly method of weed control. This research aims to determine the rhizobacteria isolates that are indigenous to Southeast Sulawesi and exhibit the potential to function as agents of weed control, particularly in farmland.

**MATERIALS AND METHOD**

**Evaluating the effectiveness of rhizobacteria on weed seed germination:**

The inhibitory capacity of seed germination was examined in a laboratory by running a viability test and seed vigor *Paspalum conjugatum*, *Ageratum conyzoides*, *Amaranthus spinosus*, and *Chromolaena odorata*. The rhizobacteria used in this study was the bacteria explored from weed rhizoster at four districts in Southeast Sulawesi, comprising two isolate originated in Buton (BL03 and BL07), one isolate in Muna (MS01), and two isolates in South Konawe (SS01 and SS02), all were the results of selection done in 2012.

**Multiplying rhizobacteria isolates:**

The rhizobacteria isolates were multiplied through TSA (*Tryphonic Soy Agar*) media, and were incubated for 48 hours. The colony of growing bacteria was suspended in 50 ml of aquades sterile, until the colony reached a population density of 10^7 cfu mL^-1 (Bai et al., 2002).

**Treatments of Weed Seeds:**

Prior to receiving treatment with rhizobacteria, the weed seeds *P. conjugatum*, *A. conyzoides*, *A. spinosus*, and *C. odorata* were disinfected on their outer layer with 2% of natrium hypochlorite for 1 minute, 70% of alcohol for 1 minute, before they were washed five times with aquadest sterile and were air-dried on filter papers (Gearly et al., 1996). Weed seeds (0.25 gr) from each type of weed were soaked in separate suspensions of isolate bacteria (50 ml), and then shook by rotary shaker for 48 hours at a temperature of 28°C. Following this, the weed seeds were planted in some plastic petri dishes (Ø 9 cm) with damp scratch paper covering the bottom of the dishes. Thirty (30) weed seeds were planted on each petri dish, which were then stored in a germinator. This experiment involving completely randomized design was repeated three times.

The variables being observed were germination capacity (DB) and relative growth rate (*K*\_CT\(^R\)), which were calculated as follows:

1. Germination capacity (DB) indicates the seed’s potential viability (Sadjad et al., 1999), and is calculated based on the percentage of normal germination observed on the last day, according to the following formula:

\[
DB = \frac{\sum \text{Normal Seed}}{\text{Planted Seed}} \times 100\%
\]

2. Relative growth rate (*K*\_CT\(^R\)) concerns with the seed vigor and compares the value of *K*\_CT to the maximum *K*\_CT\(^R\) The value of maximum *K*\_CT\(^R\) itself was obtained based on the assumption that the normal seed has grown 100% at the first count. *K*\_CT\(^R\) was calculated by taking account the accumulated daily growth rate (Sadjad et al., 1999), using the following formula:

\[
K_{CT} = \frac{\sum N}{t_{n}} \times 100%
\]

Legend:

\( t \) = observation time
\( N \) = % of normal seed at each observation
\( t_n \) = last observation time

Example: To calculate the *K*\_CT\(^R\) of *Ageratum conyzoides* seed, the following formula is used:

\[
K_{CT} = \frac{\sum N}{t_{n}} \times 100%
\]

**Testing rhizobacteria isolates capacity to inhibit growth of weed seeds:**

Individual weed seed obtained from germination test on seeds *P. conjugatum*, *A. conyzoides*, *C. odorata* and *A. spinosus* was moved and planted in plastic pots measuring a diameter of 10 cm and 6 cm, at top and down part respectively, and 7.5 cm height, and containing a mixture of soil and husk charcoal at 2:1 ratio (v/v). The plants were watered every mornings and afternoons. The growth of the weed seed was observed by employing plant height variables at the age of 14 and 28 days after the seed was moved and planted, whereas the number of leaves was observed at the age of 28 days after the seed was moved and planted.

**Rhizobacteria isolates capacity to produce Hydrogen cyanides (HCN):**

The compound of HCN was qualitatively determined by employing a method developed by Lorck (1948), later modified by Alstrom and Burns (1989). Rhizobacteria isolates were grown in petri
dishes with TSA media that was complemented with glycine (4.4 gL⁻¹). At the center of the lid of the petri dish was attached a piece of filter paper that has previously been soaked with liquid to detect the compound of HCN (2 g of picric acid, 8 g of natrium carbonate in 200 ml of water). The bacterial culture was incubated for 4 days at a temperature of 24°C. Changes in the colors of the filter paper indicated the formation of HCN compound. The paper that remained yellow was an indication that no HCN compound has been produced by the rhizobacteria, whereas a change of color from light brown to orange, brown, or brick red indicates increased production of HCN compound.

**Data Analysis:**

A variety analysis was used to analyse the data. If the results of this analysis indicate significant effect, then the analysis may proceed to Duncan Multiple Range Test at the significance level of 95%.

**RESULTS AND DISCUSSION**

**Testing the effect of rhizobacteria on the viability and vigor of weed seeds:**

The result of applying isolates of indigenous rhizobacteria to weed seeds at a laboratory scale showed that the germination of the seeds (P. conjugatum, A. conyzoides, C. odorata and A. spinosus) could be inhibited. Five isolates of rhizobacteria used, which included BL03, BL07, MS01, SS01 and SS02, had different effects on the germination capacity and the relative growth of the seeds under examination. Overall, however, all of the five isolates were able to inhibit the germination capacity and relative growth of the seeds, a fact not evident in the controlled treatment (which did not involve rhizobacteria) (Table 1).

**Testing the effectiveness of rhizobacteria on the weed growth:**

Compared to the non-treated rhizobacteria (control), all of the rhizobacteria isolates of BL03, BL07, MS01, SS01, and SS02 were able to inhibit the vegetative growth of the weed, as was indicated by the growth of the seedling height and the number of leaves of P. conjugatum, A. conyzoides, C. odorata and A. spinosus (Figure 1 and Figure 2). Each rhizobacteria isolate exhibits different level of capability to inhibit the growth of the weed seeds. In comparison to other treatments, the rhizobacteria isolate of BL07 (Buton isolate), SS01 (South Konawe isolate), and MS01 (Muna isolate) could significantly inhibit the height growth of the seedlings P. conjugatum and A. spinosus, whereas isolate of BL03 (Buton isolate) was able to severely inhibit the height growth of A. conyzoides.

<table>
<thead>
<tr>
<th>Viability and Vigor of Weed Seeds</th>
<th>Isolat</th>
<th>Type of Weed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination Capacity (%)</td>
<td>P. conjugatum</td>
<td>A. conyzoides</td>
</tr>
<tr>
<td>Control</td>
<td>89,33ᵃ</td>
<td>41,33ᵃ</td>
</tr>
<tr>
<td>BL03</td>
<td>60,00ᵃ</td>
<td>2,67ᵇ</td>
</tr>
<tr>
<td>BL07</td>
<td>48,00ᵇ</td>
<td>4,00ᵇ</td>
</tr>
<tr>
<td>SS01</td>
<td>58,67ᶜ</td>
<td>6,67ᶜ</td>
</tr>
<tr>
<td>SS02</td>
<td>48,00ᵇ</td>
<td>6,67ᶜ</td>
</tr>
<tr>
<td>MS01</td>
<td>44,00ᶜ</td>
<td>5,33ᶜ</td>
</tr>
</tbody>
</table>

| Relative Growth (%/etmal)        |        |              |            |            |
| Control                          | 92,00ᵃ | 44,44ᵇ      | 49,50ᵇ     | 71,52ᵇ     |
| BL03                             | 35,88ᵇ | 1,39ᵇ       | 26,01ᵇ     | 9,82ᵇ      |
| BL07                             | 26,05ᵇ | 1,94ᵇ       | 8,11ᵇ      | 1,48ᵇ      |
| SS01                             | 33,43ᶜ | 5,83ᶜ       | 16,68ᵇ     | 10,81ᶜ     |
| SS02                             | 25,49ᵇ | 4,05ᵇ       | 24,70ᵇ     | 4,58ᵇ      |
| MS01                             | 31,59ᵇ | 2,78ᵇ       | 21,31ᵇ     | 5,26ᵇ      |

Note: The figures in column having the same letter indicate insignificant difference according to results of Duncan Multiple Range Test at a significance level of 95%

**Testing the effectiveness of rhizobacteria on the weed growth:**

Compared to the non-treated rhizobacteria (control), all of the rhizobacteria isolates of BL03, BL07, MS01, SS01, and SS02 were able to inhibit the vegetative growth of the weed, as was indicated by the growth of the seedling height and the number of leaves of P. conjugatum, A. conyzoides, C. odorata and A. spinosus (Figure 1). Each rhizobacteria isolate exhibits different level of capability to inhibit the growth of the weed seeds. In comparison to other treatments, the rhizobacteria isolate of BL07 (Buton isolate), SS01 (South Konawe isolate), and MS01 (Muna isolate) could significantly inhibit the height growth of the seedlings P. conjugatum and A. spinosus, whereas isolate of BL03 (Buton isolate) was able to severely inhibit the height growth of A. conyzoides.

**The ability of Rhizobacteria isolate to produce Hydrogen Cyanide (HCN):**

The results of testing the ability of indigenous rhizobacteria isolate shown that all of the five isolates tested were capable of producing HCN compound at different degrees. Isolate of SS01 was able to produce the highest level of HCN (score +++), compared to other rhizobacteria isolates (Table 2). A scoring example of the production of hydrogen cyanide (HCN) compound by weed-originated rhizobacteria can be seen in Figure 2.
Fig. 1: The effect of applying rhizobacteria isolate on the height of weed seed *P. conjugatum*, *A. conyzoides*, *Chromolaena odorata* and *A. spinosus* at the age of 14 and 28 days after transplanting.

Table 2: The ability of various rhizobacteria isolates to produce Hydrogen cyanide (HCN) compound.

<table>
<thead>
<tr>
<th>No.</th>
<th>Isolat Code</th>
<th>HCN produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>BL03</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>BL07</td>
<td>++</td>
</tr>
<tr>
<td>3.</td>
<td>MS01</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>SS-01</td>
<td>++</td>
</tr>
<tr>
<td>5.</td>
<td>SS-02</td>
<td>+++</td>
</tr>
</tbody>
</table>

Note: HCN was detected by the color of filter paper +++(brick red), ++ (dark brown), + = light brown, - = yellow.

Fig. 2: A scoring sample of the production of hydrogen cyanide (HCN) compound by rhizobacteria. The color of filter paper changed into (a) brick red (score +++), (b) dark brown (score ++), (c) light brown (score+), and (d) yellow (score -).

**Discussion:**

Utilizing microorganism as agents of bio control is a strategy for biological control that has recently been receiving considerable attention. Rhizobacteria has been reported to exhibit a potential to inhibit plant growth, a particular rhizobacteria exhibiting this potential is called *Deleterious Rhizobacteria* (DRB). The result of this study showed that different treatments by various isolates have resulted in different levels of inhibition on each type of weed being tested, although overall five rhizobacteria isolates tested (BL03, BL07, MS01, SS01, SS02) were able to inhibit more than 50% of the germination of seed *P. conjugatum*, *A. conyzoides*, *C. odorata* dan *A. spinosus*, compared to the controlled treatment (without rhizobacteria isolate) (Table 1). The result of testing on four types of weed also showed that treatments of rhizobacteria isolate could inhibit vegetative growth, as was indicated by plant height, a result that was better than what was showed by controlled treatments (Figure 1).

Rhizobacteria isolates were able to inhibit the germination and growth of weed due to the roles of rhizobacteria applied to the seeds of the weed. The mechanism demonstrated by rhizobacteria to inhibit weed growth has been made possible by the production of phytotoxic compounds, substances that regulate plant growth, or interaction with other microorganism (Kremer *et al.*, 2006). Omer and Balah (2011) state that phytotoxic-producing rhizobacteria isolates generate substance capable of inhibiting growth of weed seeds, a substance that is believed to be potentially DRB bacteria, which is known for its ability to reduce germinated seeds and to significantly inhibit weed growth. In line with this, the results of testing on five rhizobacteria isolates
(BL03, BL07, MS01, SS01, SS02) indicated that they were all capable of producing HCN compound (Table 2).

HCN has been considered as the main factor in the inhibition of weed growth since the compound of HCN exhibits enormous potential to inhibit growth of weed, in that it takes part in the metabolism process, which include inhibiting respiratory path, CO2, and it is capable of binding plastocyanin protein in order to block photosynthetic electron transport and to inhibit oxygen release during electron transport, the whole process of which can eventually cause the plant to die from hypoxia cellular (cells suffering from lack of oxygen) (Kremer and Souissi, 2001). The ability of a variety of indigenous rhizobacteria isolates found in Southeast Sulawesi (BL03, BL07, MS01, SS01, SS02) to inhibit germination and growth of weed seeds of *P. conjugatum*, *A. conyzoides*, *C. odorata* dan *A. spinosus*, indicates that those rhizobacteria isolates have the potential to become deleterious rhizobacteria and can therefore be utilized as bio herbicide in order that the uses of synthetic herbicide can be reduced and agro-ecosystem can be sustainably maintained through biological control that utilizes local rhizobacteria.

**Conclusion:**

Based on the results of this study concluded that rhizobacteria indigenous Southeast Sulawesi, namely isolate BL03, BL07, MS01, SS01, and SS02 have the same ability to inhibit weed germination and seedling growth *P. conjugatum*, *A. conyzoides*, *C. odorata* and *A. spinosus*. The fifth isolate potentially as deleterious rhizobacteria through its ability to produce hydrogen cyanide compound.

**REFERENCES**


