Allelopathic Inhibition of Nitrifying Bacteria by Legumes

Uum Umiyati

Department of Agrotechnology, Universitas Padjadjaran
Jatinangor KM21 Bandung West Java, Indonesia, email: umiyati.crb@gmail.com

Received 14 September 2016 / Accepted 24 April 2017

ABSTRACT

The study aimed at understanding the competitive ability of legumes Vigna radiata L. and Mucuna pruriens with weeds and their effects on the activity of nitrifying bacteria in soils and the contents of organic-N in legumes and weeds. The experiment was arranged in a randomized block design with three factors and four replications. The first factor was soil order, i.e. Inceptisol and Vertisol; the second factor was types of legumes, i.e. Vigna radiata L. cultivar Sriti and Mucuna pruriens; and the third factor was weed management, i.e. with weed management and without weed management. The results showed that Vigna radiata L. and Mucuna pruriens indirectly influence the supply of available nitrogen in soils that can be taken up by the coexisted plants or weeds via the inhibition of the growth of Nitrosomonas and Nitrobacter in soils. As a result, the organic-N content in weeds decreases, which is in contrast to the increasing amount of organic-N in Vigna radiata L. and Mucuna pruriens. The results indicated that Vigna radiata L. and Mucuna pruriens are considered as allelophatic legumes, resulting in low organic-N content in weeds.

Keywords: Allelopathic, Mucuna pruriens, Nitrosomonas, Nitrobacter, Vigna radiata

INTRODUCTION

In agricultural point of view, plant consists of crops and weeds that always coexist with different roles, in which the crops are cultivated but the weeds are not. The coexisting plants will have an association because each plant will utilize its genetic potency to control the environment in order to grow optimally. The association between plants can result in positive effects such as mutualism, i.e. two plants of different species exist in a relationship and benefit to each other. On the other hand, plant associations can also have negative effects, which are known as competition and allelopathy.

The allelopathy phenomenon includes all types of chemical interactions between plants, microorganisms, or between plant and...
The study aimed at understanding the competitive ability of legumes *Vigna radiata* L. and *Mucuna pruriens* with weeds and their effects on the activity of nitrifying bacteria in soils and the contents of organic-N in legumes and weeds.

**MATERIALS AND METHODS**

**Experimental Design**

The field experiment was conducted in Cirebon and Brebes, Central Java in February until October 2008. The experiment was arranged in a randomized block design with three factors and four replications. The first factor was soil order, i.e. Vertisol (a₁) and Inceptisol (a₂); the second factor was types of legumes i.e. *Vigna radiata* L. cultivar Sriti (t₁) and *Mucuna pruriens* (t₂); and the third factor was weed management i.e. with weed management (g₁) and without weed management (g₂).

**Field Experiment**

In the experiment, the mung bean seeds (*Vigna radiata* L. cultivar Sriti) and *Mucuna pruriens* were planted. The inorganic fertilizers (urea contains 45% N), SP-36 (36% P₂O₅) and KCl (50% K₂O) were applied at planting time at a dose of 50 kg ha⁻¹, 75 kg ha⁻¹, and 50 kg ha⁻¹, respectively. The fertilizers were placed at holes next to the planting holes and then the fertilizers were covered with soil in order to prevent the loss of fertilizers due to evaporation (Purwono and Hartono 2005).

The plants were nurtured by irrigating and controlling pests and diseases regularly until harvesting time. The soil samples were taken from the field for nitrogen content analysis purpose.

**Bacterial Analysis**

The populations of Nitrosomonas and Nitrobacter were determined using a selective gelatin medium (Ford). The medium used for the determination of Nitrobacter population was made of 1,000 ml aquades, 0.06 g KNO₃, 1 g K₂HPO₄, 0.3 g NaCl, 0.1 g MgSO₄·7H₂O, 0.03 g FeSO₄·7H₂O, 1 g CaCO₃, and 0.3 g CaCl₂. The medium used for the determination of Nitrosomonas population was made of 1,000 ml aquades, 0.5 g (NH₄)₂SO₄, 0.2 g KH₂PO₄, 0.04 g CaCl₂, 0.04 g MgSO₄·7H₂O, and 0.05 g Fe·Citrate (Waluyo 2008).
The populations of nitrosomonas and nitrobacter were determined in the soil samples taken around the rhizosphere of legumes and weeds at 56 days after planting. The populations of the bacteria are expressed in colony forming unit (cfu). The contents of organic-N in mung beans, Mucuna and weeds were determined in the plant and weed samples taken at 42 days after planting for mung beans and at 56 days after planting for Mucuna (Fujii 2012).

RESULTS AND DISCUSSION

Population of Nitrosomonas and Nitrobacter in Inceptisol and Vertisol Soils

Mung beans and Mucuna are able to produce secondary metabolites that are released into the environment through root exudates, and these root exudates can directly affect the growth of other plants and indirectly affect the properties of soil, nutrient status and activity or population of soil microorganisms (Orcutt 2000).

Soil organism is classified into microflora (bacteria, fungi, actinomycetes and algae) and soil fauna. Bacteria is the most predominant organism in soil with the population of $10^8$-$10^{10}$ per gram soil. Good soil aeration and soil drainage are required by soil microorganisms to grow optimally (Inderjit and Moral 1997; Inderjit and Dakshini 1994).

The larger the surface area of a soil particle, the greater the role of this particle in managing the chemical and biological properties of the soil, such as the ability to bind water and nutrients and the population of soil organisms. Therefore, the population of soil microorganisms in clay particles is higher than that in other soil particles. Table 1 shows that the population of Nitrobacter and Nitrosomonas in Vertisol is higher than that in Inceptisol.

The population of Nitrosomonas and Nitrobacter in the soils grown with mung beans ($t_1$) is lower than that in the soils grown with Mucuna ($t_2$). The result showed that the inhibition of soil nitrogen availability for weeds and other plants by mung beans is stronger than that of Mucuna.

The lack of nutrient availability in soil has stimulated mung beans to produce secondary metabolites, such as C-Glycosyl Flavonoids, which are released as root exudates. This mechanism is called allelochemistry. Then, the secondary metabolites that are released into root zone are decomposed by microorganisms into HCOO$^-$ and CN$^-$ and further used as an energy source for soil microorganisms, resulting in the increase of microorganism activity and CO$_2$, and the decrease of O$_2$ in the soil. This soil condition further affects the growth of Nitrosomonas that require O$_2$ to perform ammonia oxidation activity (Dixon 1983).

Mucuna produces allelochemical, i.e. L-DOPA (L-3,4-Dihydroxyphenyl Alanine), which can hamper the growth of Nitrosomonas population and further inhibit the oxidation of NH$_4^+$ into NO$_3^-$. Allelochemistry activity generated by mung beans and Mucuna to other crops is affected by soil texture, pH, CEC and organic ions in the soil (Weston 1996). A clay soil with slightly acidic up to nearly neutral pH and high CEC stimulates the allelochemistry activity, which further inhibits the growth of microorganism that play a role in the nitrification process in Vertisol or Inceptisol, resulting in the decrease of N availability in the soil for other plants (Roberton and Vitousek 1981).

The Effect of Allelopathy on the Organic-N Contents in Legumes and Weeds Grown on Different Soil Order

Among other nutrients, nitrogen is the nutrient that in most cases limits plant growth. Besides

| Table 1. Population of Nitrosomonas and Nitrobacter in Inceptisol and Vertisol soil samples. |
|---|---|---|---|---|
| No | Treatment | Vertisol | Inceptisol |
| | | Nitrobacter (cfu ml$^{-1}$) | Nitrosomonas (cfu ml$^{-1}$) | Nitrobacter (cfu ml$^{-1}$) | Nitrosomonas (cfu ml$^{-1}$) |
| 1 | $t_1g_1$ | $80.70 \times 10^6$ | $61.70 \times 10^6$ | $52.10 \times 10^6$ | $37.20 \times 10^6$ |
| 2 | $t_1g_2$ | $54.60 \times 10^6$ | $52.40 \times 10^6$ | $46.00 \times 10^6$ | $36.10 \times 10^6$ |
| 3 | $t_2g_1$ | $154.70 \times 10^6$ | $138.70 \times 10^6$ | $50.00 \times 10^6$ | $46.30 \times 10^6$ |
| 4 | $t_2g_2$ | $159.00 \times 10^6$ | $49.50 \times 10^6$ | $153.30 \times 10^6$ | $101.10 \times 10^6$ |

$t_1$ = Mung beans; $t_2$ = Mucuna; $g_1$ = with weed management; $g_2$ = without weed management
required in large quantities, nitrogen plays important roles in enhancing the growth of plants. This is because nitrogen is an essential element involved in the process of plant biochemistry, i.e. the formation of cells, proteins, cytoplasm, nucleic acids, chlorophyll and other cell components.

Different soil types will have different nutrient content, pH, and microorganisms activity, so that the response of plants on the nutrient availability in soil will be different, which further affect the nutrient content in plants.

According to Dixon and Whiller (1983), environmental contribution on the differences in plant populations is affected by variation in soil nitrogen source and uptake by plants as well as variation in soil pH. pH in general affects the activity of soil microorganisms. Soils with a neutral pH are favorable for the growth of soil microorganisms. However, at pH < 5.50, the growth of microorganisms is hampered. The range of soil pH measured in our study was 5.6 for Inceptisol and 6.8 for Vertisol, so that the organic-N contents in mung beans and Mucuna grown on both soils were different (Table 2).

The differences in the use of nitrogen by different plants caused different nitrogen content in the tissues of Mucuna and green beans. The study of Dixon (1983) showed that the available nitrogen in soil could not be utilized by cucumber plants due to the chemistry competition between Mung beans or Mucuna with cucumber, so the mung beans or Mucuna released allelochemicals through root exudates in the form of C-glycosyl flavonoid or L-Dopa into the soil. The release of allelochemicals into the soil decreased the pH of rhizosphere of green beans or Mucuna, which further inhibited the growth of roots of other plants and nitrifying bacteria, so that the supply of nitrogen in the non-rhizosphere that could be taken up by other plants was low, resulting in the low nitrogen content in other plant’s tissues.

In addition, the allelochemical that is released by mung beans through root exudates in the form of HCN can inhibit the synthesis of protein in other plants, which is indicated by the decrease of root length, so that the absorption of nutrients, especially nitrogen is hampered, causing the organic-N content in weeds becomes low (Einhelling 2004).

Table 2 shows that the contents of organic-N in mung beans and Mucuna grown on both Vertisol and Inceptisol are higher than that in weeds. The result shows that both legumes have good ability to compete with weeds (competitive ability) in term of utilization of nitrogen.

The result of our study is supported by the study of Orcutt (2000), showing that allelochemicals released by plant species can affect the nutrient cycle in an ecosystem that ultimately affects both

<table>
<thead>
<tr>
<th>No</th>
<th>Treatment</th>
<th>Vertisol Plant/Weed Group</th>
<th>Organic-N Content (g)</th>
<th>Inceptisol Plant/Weed Group</th>
<th>Organic-N Content (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>t_{1g1}</td>
<td>Mung beans</td>
<td>3.85</td>
<td>Mung beans</td>
<td>5.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sedgegrass</td>
<td>0.88</td>
<td>Sedgegrass</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grass</td>
<td>0.98</td>
<td>Grass</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Broadleaf weed</td>
<td>0.11</td>
<td>Broadleaf weed</td>
<td>0.32</td>
</tr>
<tr>
<td>2</td>
<td>t_{1g2}</td>
<td>Mung beans</td>
<td>6.96</td>
<td>Mung beans</td>
<td>7.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sedgegrass</td>
<td>3.59</td>
<td>Sedgegrass</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grass</td>
<td>1.62</td>
<td>Grass</td>
<td>3.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Broadleaf weed</td>
<td>0.76</td>
<td>Broadleaf weed</td>
<td>0.82</td>
</tr>
<tr>
<td>3</td>
<td>t_{2g1}</td>
<td>Mucuna</td>
<td>5.50</td>
<td>Mucuna</td>
<td>14.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sedgegrass</td>
<td>2.25</td>
<td>Sedgegrass</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grass</td>
<td>1.82</td>
<td>Grass</td>
<td>7.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Broadleaf weed</td>
<td>0.17</td>
<td>Broadleaf weed</td>
<td>1.54</td>
</tr>
<tr>
<td>4</td>
<td>t_{2g2}</td>
<td>Mucuna</td>
<td>5.23</td>
<td>Mucuna</td>
<td>9.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sedgegrass</td>
<td>0.67</td>
<td>Sedgegrass</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grass</td>
<td>2.09</td>
<td>Grass</td>
<td>8.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Broadleaf weed</td>
<td>1.29</td>
<td>Broadleaf weed</td>
<td>1.17</td>
</tr>
</tbody>
</table>

Notes: t_{1} = Mung beans; t_{2} = Mucuna; g_{1} = with weed management; g_{2} = without weed management.
below ground and above ground population diversity in the ecosystem.  

The release of allelochemicals through root exudates of mung beans or Mucuna into the rhizosphere results in the disruption of the oxidation of NH₄⁺ into NO₃⁻. The NH₄⁺ is bound by carboxylic acid released by mung beans and Mucuna, so it will not move out from the rhizosphere of mung beans and Mucuna. On the other hand, the nitrogen outside of rhizosphere, i.e. NO₃⁻ is easy to evaporate, therefore it can not be utilized by the roots of weeds.  

The ability of legumes to produce allelochemicals can affect soil microorganisms, especially nitrosomonas nitrobacter that are involved in the nitrification process (Blum and Shafer 1988). The inhibition of nitrification process by allelochemicals released by both mung beans and Mucuna caused the low amount of nitrogen that could be absorbed by weeds and stored in their tissues. On the other hand, the amount of nitrogen that was taken up and stored in the tissue of legumes was higher than that in weeds because the soil nitrogen was not available (immobile) for weeds due to the allelopathy effect from both legumes.  

According to Orcutt and Nilsen (2000), in the diverse ecosystem (mung beans, Mucuna and weeds), the effect of allelochemistry can be identified easily, i.e. when certain species of plants predominantly affect the soil chemistry (such as the results in Table 2) and the ecosystem. Table 2 shows that the content of organic-N in mung beans or Mucuna is higher than that in weeds, indicating that both legumes have allelochemical activity.

CONCLUSIONS

The presence of allelopathic plants can affect the activity of soil microorganisms, especially nitroabacter and nitrosomonas that play important role in the nitrification process. This phenomenon will further influence the nutrient cycle in an ecosystem that ultimately affects the diversity of both below ground and above ground populations. The allelopathic effects are mainly found in the soils grown with climax vegetation.

REFERENCES


