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Allelopathic Inhibition of Nitrifying Bacteria by Legumes

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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 results, 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*

ABSTRAK

Penelitian ini bertujuan untuk mendapatkan kemampuan bersaing genotipe *Vigna radiata* dan *Mucuna pruriens* dengan gulma dalam menghambat aktivitas mikroorganisme Nitrifikasi hingga mempengaruhi kandungan N organik pada tanaman dan gulma. Percobaan dilakukan dengan menggunakan rancangan acak kelompok dengan tiga faktor dan empat ulangan. Faktor pertama adalah dua ordo tanah yaitu Inceptisol dan Vertisol (a). Faktor kedua adalah genotipe tanaman legum (t) dan faktor ketiga adalah dua jenis pengelolaan gulma (g). Percobaan lapangan menunjukkan bahwa *Vigna radiata* L. kultivar Sriti dan *Mucuna pruriens* mempengaruhi secara tidak langsung ketersediaan nitrogen dalam tanah, yang menyebabkan N tersedia menjadi N tidak tersedia bagi tanaman lain atau gulma, dengan mempengaruhi populasi bakteri Nitrosomonas dan Nitrobacter pada tanah, sehingga terjadi penurunan kandungan N-organik dalam gulma tetapi kandungan N-organik pada *Vigna radiata* L. kultivar Sriti dan *Mucuna pruriens* meningkat. Hal ini menunjukkan bahwa *Vigna radiata* L. kultivar Sriti dan *Mucuna pruriens* merupakan dua tanaman legum yang mengandung alelopati. Selanjutnya hasil eksperimen menunjukkan bahwa *Vigna radiata* L. kultivar Sriti dan *Mucuna pruriens* secara tidak langsung mempengaruhi populasi bakteri *Nitrosomonas* dan *Nitrobacter,* sehingga gulma mengandung N organik yang rendah.

Kata kunci: Alelopati, *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 microorganism (Einhellig 1996). According to Rice (1983) the interactions include inhibition and stimulation of the growth and the development of other organisms directly or indirectly via compounds excreted by a certain organism (plant, animal or microbe). The chemical compounds excreted by organisms play a role in the mechanism called allelochemistry. The effect of allelochemistry on other organisms is selective.

Legumes that produce allelochemicals include *Vigna radiata, Vigna unguiculata, Phaseolus vulgaris* and *Mucuna pruriens*. Cereals that produce allelochemicals are *Oryza sativa, Triticum sativum, Zea mays,* and *Sorghum bicolor* (Chou 1986). The chemical compounds produced by mung beans are C-glycosyl flavonoids, which include in flavonoid group. Certain compounds are able to inhibit the activity of nitrogen in soil, which further decreases the population and activity of nitrifying bacteria (*i.e.* nitrosomonas bacteria), resulting in soil nitrogen deficiency.

Other flavonoids can cause inhibition on the microorganism associated with nitrogen-producing plants by inhibiting the formation of NH_4^+ in plants that will be used for germination and growth. A study conducted by Alderich (1984) showed that the phenolic compounds in soil can inhibit the oxidation of NH_4^+ into NO_3^-. In addition, the study of Bremner and McCarty (1993) indicated that phenolic compounds in soil are able to enhance NH_4^+ immobilization due to inhibition of Nitrosomonas bacterial activity.

The study of Nilsson *et al.* (1993) showed that the extract of *Empetrum hermaphroditum* decreased mycorrihza infection on roots of pinus seedlings. Moreover, seed germination of *Pinus sylvestris* was inhibited due to the application of *E. hermaphroditum* extract (Zackrisson and Nilsson 1993).

Allelochemical compounds produced by Mucuna are flavonoids, galic acid and L-Dopa. L-Dopa compound contained in Mucuna seeds has been used as a cure for Parkinson's disease, while flavonoids and isoflavonoids produced by green beans can suppress the growth of bacteria in soil (Eilitta *et al.* 2002). The addition of organic materials to soil can increase microbial activity, which would take up nutrients in soil. The toxic effect of allelopathy can not be separated from the effects of soil microbial activity (Inderjit and Foy, 1999). Therefore, it is necessary to conduct a study about the ability of legumes to compete with weeds by producing allelochemicals that can affect the activity of nitrifying bacteria in various limited abiotic environmental conditions.

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 (a_1) and Inceptisol (a_2); the second factor was types of legumes *i.e. Vigna radiata* L. cultivar Sriti (t_1) and *Mucuna pruriens* (t_2); and the third factor was weed management *i.e.* with weed management (g_1) and without weed management (g_2).

Field Experiment

In the experiment, the mung bean seeds (*Vigna radiata* L. cultivar Sriti) and *Mucuna pruriens* were planted. The inorganic fertilizers of urea (contains 45% N), SP-36 (36% P_2O_5) and KCl (50% K_2O) 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 KNO_2, 1 g K_2HPO_4, 0.3 g NaCl, 0.1 g $MgSO_4.7H_2O$, 0.03 g $FeSO_4.7H_2O$, 1 g $CaCO_3$, and 0.3 g $CaCl_2$. The medium used for the determination of Nitrosomonas population was made of 1,000 ml aquades, 0.5 g $(NH_4)_2SO_4$, 0.2 g KH_2PO_4, 0.04 g $CaCl_2$, 0.04 g $MgSO_4.7H_2O$, and 0.05 g Fe-Citrate (Waluyo 2008).

The populations of nitrosomonas and nitrobacter were determined in the soil samples taken around the rhyzosphere 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 (Robertson 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	80.70×10^6	61.70×10^6	52.10×10^6	37.20×10^6
2	t_1g_2	54.60×10^6	52.40×10^6	46.00×10^6	36.10×10^6
3	t_2g_1	154.70×10^6	138.70×10^6	50.00×10^6	46.30×10^6
4	t_2g_2	159.00×10^6	49.50×10^6	153.30×10^6	101.10×10^6

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 availalibility 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 2. The contents of organic-N in legumes and weeds grown on Vertisol and Inceptisol.

No	Treatment	Vertisol		Inceptisol	
		Plant/Weed Group	Organic-N Content (g)	Plant/Weed Group	Organic-N Content (g)
1	t_1g_1	Mung beans	3.85	Mung beans	5.56
		Sedgegrass	0.88	Sedgegrass	0.94
		Grass	0.98	Grass	2.31
		Broadleaf weed	0.11	Broadleaf weed	0.32
2	t_1g_2	Mung beans	6.96	Mung beans	7.48
		Sedgegrass	3.59	Sedgegrass	1.74
		Grass	1.62	Grass	3.28
		Broadleaf weed	0.76	Broadleaf weed	0.82
3	t_2g_1	*Mucuna*	5.50	*Mucuna*	14.37
		Sedgegrass	2.25	Sedgegrass	0.54
		Grass	1.82	Grass	7.92
		Broadleaf weed	0.17	Broadleaf weed	1.54
4	t_2g_2	*Mucuna*	5.23	*Mucuna*	9.48
		Sedgegrass	0.67	Sedgegrass	0.72
		Grass	2.09	Grass	8.61
		Broadleaf weed	1.29	Broadleaf weed	1.17

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 NH_4^+ into NO_3^-. The NH_4^+ 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.* NO_2^- 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 that 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 nitrobacter 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.

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