THE EFFECT OF BIOFERTILIZER FUNGI ON CIHERANG RICE GROWTH AT SOME LEVEL OF SOIL SALINITY

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ABSTRACT

A research about the effect of fungus contained biofertilizer on Ciherang rice that was growth on different level of soil salinity was conducted. One of the effect of global climate changes is the increase of sea water level. It leads to the expansion of sea water submerged land for agriculture. Salt intrution to the agriculture area considerably decrease soil fertility because of the high salinity. Some of microbes especially soil fungi such as Aspergillus sp and Penicillium sp. are able to grow at high salinity environment. Those fungi were also able to degrade lignocellulose, sollubilize in organic phosphate and provide organic phosphat and produce plant growth hormon especially IAA. Such activities benefit to improve soil fertility in high salinity land as a bio-fertilizer.The objective of this research was to know the growth of rice plant that treated with fungus contained bio-fertilizer on land with different level of salinity. The rice were planted in Green house of Cibinong Science Centre, Cibinong.The research was set up as complete random design with five replication. The rice were watered by 5 conditions: 50% of sea water, 100% of sea water, 100% sea water + 2 % NaCl , fresh water + 5 % NaCl and 100% fresh water as the control. Fertilizer was added to the medium twice. Ten grams of fertilizer were used per polybag (10g/7 Kg), 2 weeks after planting and before flowering subsequently. The observed parameters were plant height, number of tiller, leaves colour, biomass dry weight, soil organic carbon content, cellulosic and lignin degrading activities of the fungus, fungus phosphate-solubilizing potency and fungus production of IAA.The watering treatment lead to 5 level of salinity i.e. : 5,93 dS/m (50% sea water), 9,15 dS/m (100% sea water), 10,42 dS/m (sea water + 2% NaCl), 12,43 dS/m (fresh water + 5% NaCl) and 0,74 dS/m (fresh water). The result showed that among those 5 watering condition, the rice grew best on 5,93 dS/m (watering 50% of sea water). This result was considered as the best since the plant height and number of tiller were not significantly different with those of the control. Plants height and number of tiller were 74,4 cm and 11 respectively. On the higher salinity level the plant growth was inhibited. The ability of rice to withstand the high salinity possibly was supported by the fungus activities of providing organic phosphate and IAA growth substance.

Keywords*:bio-fertilizer, fungi, phosphate solubilizing, rice growth, salinity*

INTRODUCTION

The climate global changes has lead to some changes in agriculture. Wet season which comes late and last longer resulted in crop failure of some commodities. The rising of sea level triggers the tidal sea water flood in the coastal area. The flooded area which used to be fertile turn out to be poor-nutrient soil since it contains salt thus has a high salinity level. In the saline soil, water absorption is inhibited and the plant growth would be hampered in water stress condition. The high salinity level on the soil will also cause unbalanced nutrient vailability, accumulation of toxic compound in the plant, and the decreasing of water infiltration.

As we know some of soil fungus can survive living on dry or high salinity condition, one of them is *Aspergillus niger*. According to Mert and Dizbay (1977) *A. niger* could produce conidia on 1% NaCl containing medium and it grow well on 3% NaCl containing medium.This fungus can also withstand high temperature. *Penicillium* sp is the other soil fungus that could live in high salinity condition. Radwan *et al.* (1985) succeed to isolate *Penicillium* sp. from saline soil. The fungus could grow on 10% NaCl medium. According to Urja & Meenu (2010b), *Fusarium, Gliocladium, Penicillium* and *Trichoderma* are fungus with high tolerance of stress condition.

Aspergillus niger is able to degrade solulose substance. Subowo (2011) succed to isolate *Aspergillus niger* PS 1.4 from coastal soil. The fungus has selulosic activity of 0,127 unit/ml. It also could dissolve phosphate rock into organic phosphate which could be absorbed by plants. Urja & Meenu (2010) stated that *Aspergillus, Penicillium* and yeast are able to dissolve inorganic phosphate.

Penicillium sp. is able to degrade lignin substances. Subowo (2009) succeed to isolate *Penicillium* sp. R7.5 from peat soil at Kalimantan. The fungus could decompose Poly R-478 as much as 18,05% of the material in 90 minutes. *Penicillium* sp. SB42, *Trichoderma* sp. CB21 and *Aspergillus* sp. CB23 are PGPF (Plant Growth Promoter Fungi) and able to boost plant growth. These fungi can increase productivity of cucumber plant (Worosuryani *et. al*., 2006).

Rice is stapple food for Asian habitant, various variety of rice are planted in many areas of Asia. One of them is Ciherang rice which is planted by most of Balinese farmer because it is tastier than any other variety of rice. Rice Ciherang generally was grown in irrigated land and rainfed land. In rainfed land could produce 7.1 tonnes/ha (Polakitan and Taulu, 2009) but few grown in soil containing high salinity or coastal areas. This research was conducted because there had never been a research about the effect of fungus contained biofertilizer on Ciherang rice growth on saline soil. The objective of this research was to know the growth rate of rice that treated with fungus contained bio-fertilizer on land with different level of salinity.

MATERIALS AND METHODS

Rice seedling

Rice seed was put in a bucket filledwith saline water then the sink rice seed from the floated rice seed. Then the sink rice seed were washed with water until clean. Rice seed was soaked in fresh water for 24-48 hours. The rice seed was drained until germination. The rice seed which already germinated sown in the plastic tray and covered with soil. Rice seedling was ready to move to pot after 10 days (the seedling has 2 leaves).

Biofertilizer

Fungal cells *Aspergillus niger* PS1.4 and *Penicillium* sp. R7.5 were multiplied in liquid media, as much as 10 ml of fungal cells were grown in 100 ml of medium. Fungi mycelium was harvested after one week incubation on the shaker. The mycelium was mixed with compost Bioposka from Bogor Botanical Garden. Into mycelium and compost mix were added 10% NPK and 10% guano fertilizer, then incubated at room temperature for 3-4 weeks. Fertilization was done 2 times, first two week after planting and the second when the rice plant was ready for flowering. Each polybag was given biofertilizer as much as 100 g/7 kg soil.

Planting

Polybag that was used for planting 10 kg sized. The media for plants were a mix of 5 kg soil and 2 kg compost. Rice seedlings were planted in the polybag with 2 plants per polybag. After rice plants grew, watering is started. The watering was done every 2 days with composition as follows:

I. Rice plants was watered with fresh water
II. Rice plants was watered with 50% sea water
III. Rice plants was watered with 100% sea water
IV. Rice plants was watered with 100% sea water + 2% NaCl
V. Rice plants was watered with fresh water + 5% NaCl

In order to maintain the activity of the fungus, into each polybag was added straw as much as 100 g. Straw addition was done when rice plants aged a month. Into each pot was embedded test block to determine the activity of soil fungi.

The research was conducted in the green house Cibinong Science Centre, Cibinong. Complete Randomized Trial arranged with 5 replications. The parameters observed were plant height, number of tillers, leaf colour, dry weight biomass, soil salinity, soil pH, soil organic C content, fungal cellulase and ligninase activity (Subowo, 2010), the dry weight of test block. The ability of fungi dissolving phosphate compound and produced IAA were measured followed Ames(1966) and Bilkay*et.al*.(2010) methods.

RESULTS

The soil salinity level was vary from one treatment to another. The highest soil salinity happened in treatment with freshwater+5% NaCl (12.43 dS/m), then sea water +2% NaCl (10.42 dS/m), 100% seawater (9.15 dS/m), 50% sea water (5.93 dS/m) and finally fresh water treatment (0.74 dS/m).Similarly, pH of the soil was affected by salts contained in sea water, the highest value was in treatment with seawater+2%NaCl and the lowest in freshwater watering (Table 1).

Table 1.The soil salinity level and pH of rice experiments

No	Treatment	Salinity (dS/m)	pH
1	I	0,74	6,64
2	II	5,93	7,45
3	III	9,15	7,30
4	IV	10,42	7,50
5	V	12,43	7,40

Fertilization with fungal biofertilizer was to support rice plants growth in soil with high salinity. The salinity affected on soil microbial activity but fertilizing with soil fungi that resistant on salinity was expected to support plants growth. In treatment with 50% seawater (salinity of 5.93dS/m) the height of rice plants was not significantly different to fresh water treatment, which was74.4cm and 83.2 cm respectively. The best rice plants growth was in 50% sea water treatment or soil salinity5.93dS/m. In addition, the number of tiller was not significant different between salinity and fresh water treatments.

In morphological observation, the rice plants with 50% seawater treatments was not different to fresh water treatment. Colour leaves were same i.e. green, which indicate healthy plant. The time of flowering, plant height and number of tiller from both salinity (50%) and fresh water treatment were not different as well. However, the others of treatment were different, the plants were not yet to produce flower (Table 3).

Table 2.Plant height, number of tiller and colour leaves of Ciherang rice at different levels of salinity

No	Treatment	Salinity (dS/m)	Plant height (cm)	Number of tiller	Color leaves
1	I	0,74	83,2 ^{a}	15,2 ^{a}	green
2	II	5,93	74,4 ^{a}	11,0 ^{a}	green
3	III	9,15	54,3 ^{b}	4,8 ^{b}	green
4	IV	10,42	52,4 ^{b}	4,0 ^{b}	green
5	V	12,43	53,6 ^{b}	4,0 ^{b}	green

Note : the same notation in one column meant was not significantly

Dry weight of biomass per polybag was different among all treatment, the highest dry weight was in the 50% sea water treatment, i.e.39,06 g, then fresh water+5% NaCl, sea water, and the last sea 100% water+2% NaCl watering (Table 4).

Apparently, in this research, fungi activity in has positive effect on rice growth. The biofertilizer was composed of mixture *Penicillium* sp. R7.5, *Aspergillus niger* PS1.4, compost, 10% NPK, and 10% guano fertilizer. *Penicillium* sp. R7.5 has ligninase enzymes that able to degrade lignin compound to be C organic. *Penicillium* sp. R7.5 could decrease the Poly R-478 concentration as much as 18,05% in 90 minutes.

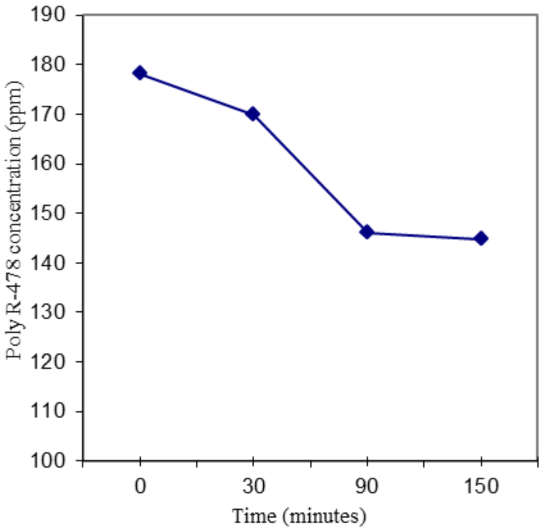

Figure 1. The Poly R-478 decreasing concentration by *Penicillium* sp. R7.5

Table 3.The growth of rice plants on some soil salinity levels

No	Treatment	Salinity (dS/m)	Plant morphology
1	I	0,74	All rice plants were flowering, colour leaves were green, BWD 5
2	II	5,93	All rice plants were flowering, The height plant were same with treatment I, color leaves were green, BWD 5
3	III	9,15	All plants were not flowering yet, plant height were lower than treatmen I, The number of tiller was lower than treatment I, plants growth was disturbed
4	IV	10,42	Some rice plants were death, All plants were not flowering yet, The number of tiller was lower than treatment I, plants growth was disturbed
5	V	12,43	All plants were not yet flowering, The number of tiller was lower than treatment I, plants growth was disturbed

Table 4. Dry weight of rice straw

No	Treatment	Salinity (dS/m)	Dry weight of rice straw (g)
1	I	0,74	58,05 [a]
2	II	5,93	39,06 [b]
3	III	9,15	9,00 [c]
4	IV	10,42	4,48 [c]
5	V	12,43	10,60 [c]

Note : the same notation in one column meant was not signify-cantly

Table 5. C organic content in the soil on some level of salinity

No	Treatment	Salinity (dS/m)	C organic (ppm)	
			I	II
1	I	0,74	374,92	1003,25
2	II	5,93	325,92	983,33
3	III	9,15	340,17	1001,75
4	IV	10,42	335,58	997,75
5	V	12,43	294,33	1013,75

Table 6. The decreasing of dry weight test block by fungal enzymes activity

No	Treatmen	Decreasing of test block weight (%)			
		I	II	III	Average
1	I	17,77	15,98	19,89	17,88 [a]
2	II	9,7	10,43	8,8	9,64 [b]
3	III	10,22	12,07	9,63	10,64 [b]
4	IV	7,33	5,53	2,61	5,16 [b]
5	V	19,96	18,26	15,27	17,83 [a]

Note : the same notation in one column meant was not signify-cantly

Table7.The ability of fungal isolates dissolved phosphate

No	Fungi	P available (ppm)	
		0 day	6 day
1	*Aspergillus niger* PS1.4	1,2995	4,5637
2	*Penicillium* sp. R7.5	1,1242	3,1200

Table 8.The ability of fungal isolates produced IAA

No	Fungi	IAA content on media (ppm)	
		0 day	3 day
1	*Aspergillus niger* PS1.4	0,6273	2,4907
2	*Penicillium* sp. R7.5	0,6522	1,2547

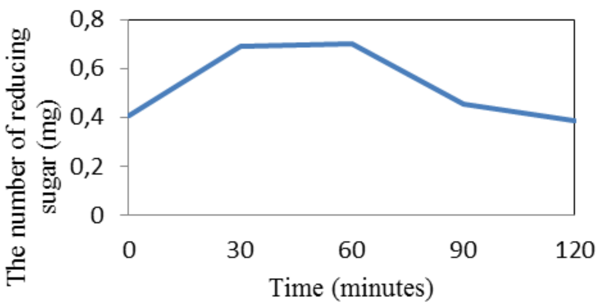

Figure 2.The number of reducing sugar formed by *Aspergillus niger* PS1.4

In among consortium of these fungi,*Aspergillus niger* PS1.4 has significant cellulase activity. The cell-ulase enzymes decomposed cellulose compounds into polysaccharide. *Aspergillus niger* PS1.4 has cellulase acti-vity as much as 0,127 unit/ml.

The content of C organic was different before and after addition of biofertilizer. C organic in the soil was small before bio-fertilization addition but it increased af-ter fertilization. The increasing of C organic in the soil was result of decomposition process by fungal lingocellu-loses.

Fungal enzyme activity in the soil could be observed by decreasing of test block dry weight. The highest decreasing occurred on fresh water treatment (17,88%)

then fresh water + 5% NaCl treatment, sea water, 50% sea water and last sea water+2% NaCl treatment (Table 6).

Fungal containing in biofertilizer was able to dissolve inorganic phosphate into organic phosphate. This compound was readily absorbed by plants. Between two fungi, *Aspergillus niger* PS1.4 has greater ability to provide organic phosphate than *Penicillium* sp. R7.5 (Table 7).

It is known that some soil fungi are able to produce IAA, as well as fungal isolates used to develop biofertilizers. Between the two fungi, *Aspergillus niger* PS1.4 has higher capacity to provide IAA than *Penicillium* sp. R7.5 (Table 8).

DISCUSSION

Soil salinity can affect the soil fertility. The soil that contain high salt concentration, cause the plant absorb water and nutrient poorly. The plants could grow well by using bio-fertilizer containing resistant microbes. Fungi which is resistant on salinity will be active under high salinity condition. They decompose lignocelluloses compound, dissolved inorganic phosphate and produced hormones to support plant growth.

The watering with 50% sea water resulted in high soil salinity, which is 5.93 dS/m. It was too high for the growth of rice plants. Rice plant could grow normally at 0 dS/m to 8 dS/m (Mass, 1986). According Kotuby *et al.* (2000), some plants can be grown in salinity above 6 dS/m, for example, barley, sugar beet, grain etc. While some plants can be grown at salinity below 6 dS/m as bean, canola, corn etc. Soil salinity can be reduced by multiplying the drainage so that salt levels reduced. The rain water will also reduce the levels of soil salinity.

Watering with 50% sea water made the soil salinity5.93dS/m, this salinity level was quite high because it was above the standard salinity for rice(2.5dS/m). At this salinity level, rice var. Ciherang could grow almost equally well with fresh water treatment especially in plant height, number of tillers and leaf colour. This means that Ciherang rice could still grow normally at this level of salinity. It was certainly supported by the absorption of nutrients that still running well, enough nutrients, hormones required were available. The addition of *Aspergillus niger* PS1.4 and *Penicillium* sp R7.5 may actually enhance plant growth. This can be seen on plant height and number of tillers in control treatment, plants were shorter and less number of tillers. Similarly with Urja & Meenu research (2010a). They inoculated chick pea plants in pots with *Penicillium* sp. S11 and *Aspergillus* sp. S12B. Inoculation of the fungus enhanced plant growth in soil salinity levels of 2%. Microbial activity were disturbed in higher levels of soil salinity. The absorption of nutrients was disturbed in high salinity conditions.

The morphology of rice plants with50% seawater treatment was not different to rice plants fresh water treatment. Flowering time was also similar between two treatments. This suggests that physiological processes and microbial activity were almost the same between the two treatments.

Dry weight of rice straw results associated with rice plant growth. When rice crops grew well, it produced more straw. But when plant growth was disturbed straw will be reduced. Similarly the best plant growth occurred in 50% sea water treatment. The highest biomass produced by this treatment. When rice plants growth was disrupted the absorption of nutrients from the soil was disturbed. According Worosuryani *et al*. (2006) the addition of inoculants *Penicillium* sp., *Trichoderma* sp. and *Aspergillus* sp. could increase the dry weight of cucumber plants.

Soil fungi activity could be observed from the number of carbon organic were formed. Measurements was done twice before and after addition of fungi biofertilizer. The increasing Carbon organic indicated that fungi containing biofertilizer degraded lignin and cellulose compounds and produce Carbon organic. *Penicillium* sp.- R7.5 has high ability in lignin degradation (Subowo, 2009) and *Aspergillus niger* PS1.4 has high cellulose activity.

The greatest dry weight decreasing test block was occurred in fresh water treatment. Fungal activity was not inhibited by salinity. *Penicillium* sp. R7.5 was able to decompose lignin. And *Aspergillus niger* PS1.4 has ability to decompose cellulose. The decreasing of dry weight of test block proves that fungi can do enzymatic activity in the soil experiments.

Aspergillus niger PS1.4 and *Penicillium* sp. R7.5 were able to dissolve inorganic phosphate into organic phosphate compounds that readily absorbed by crops. This suggests that both *Aspergillus niger* PS1.4 and *Penicillium* sp. R7.5 could be used in the preparation of biofertilizers. *Aspergillus niger* PS1.4 has greater in phosphate dissolving than *Penicillium* sp. R7.5. Pradhan & Sukla (2005) reported that *Aspergillus* sp. and *Penicillium* sp capable of dissolving tricalcium phosphate (TCP). *Aspergillus* sp. dissolved 480ug/ml and *Penicillium* sp. dissolved 275 ug/ml P of 0.5% TCP after 3 – 4 days of the growth.

Both *Aspergillus niger* PS1.4 and *Penicillium* sp. R7.5 that was used to develop biofertilizer were also able to produce IAA. *Aspergillus niger* PS1.4 has greater ability in IAA producing than *Penicillium* sp. R7.5. So both fungi can be used in the preparation of fungi biofertilizer for plants growing in soil salinity. Hasan (2002) reported that *Aspergillus flavus, A. niger, F. oxysporum, Penicillium corylophilum, P.cyclopilum, P.funiculosum* and *Rhizopus stolonifer* were able to produce GA and IAA.

REFERENCES

Ames, B. N, 1966. Assay of inorganic phosphate and phosphatases. *Meth Enzymol*. 8: 115-116.

Bilkay, I. S., Karakoc, S and Aksoz, N, 2010. Indole-3-acetic acid and gibberellic acid production in *Aspergillus niger*. *Turk J Biol*. 34: 313-318.

Economic Empowerment and Culture-Based Environment and Initiation Formation Environment Association of Indonesia, Universitas Sebelas Maret Surakarta, 15-16 August 2009. ISBN: 978-979-185-162-6.

Hasan, H. A. H, 2002. Gibberellin and auxin production by plant root-fungi and their biosynthesis under salinity calcium interaction. *RostlinnaVyroba*, 48 (3): 101-106.

Kotuby, J. A., Koenig, R and Kitchen, B, 2000. Salinity and Plant Tolerance. *Utah state University*. 8pp

Mass, E. V, 1986. Salt tolerance of plants. *Applied Agricultural Research* 1:12-26.

Mert, H.H and Disbay, M, 1977. The effect of osmotic pressure and Salinity of the medium on the growth and sporulation of *Aspergillus niger* and *Paecilomyces lilacinum*. *Mycopathologia* 61 (2): 125-127.

Polakitan, A and Taulu, L, 2009. Study of some VUB lowland rice productivity with PTT approach on rainfed rice in the district of Minahasa. Regional Seminar on Agricultural Technology Innovation, North Sulawesi Agriculture Program, 189-194.

Pradhan, N and Sukla, L.B, 2005. Solubilization of inorganic phosphates by fungi isolated from agriculture soil. *African Journal of Biotechnology* 5(10): 850-854.

Radwan, S.S, El-Essawy, A.A and Helal, G.A, 1985. Salinity-loving fungi in Egyptian Soil. II Preliminary notes on antibiotics from halophilic *Penicillium spp*. *Zentralbl Mikrobiol*, 140 (2): 149-154.

Subowo, Y.B, 2009. Isolation and selection of lignin degrading fungi of Ascomycetes from some extreem environment in West Kalimantan. Proceeding National Seminar Sector

Subowo, Y.B, 2010. Enzyme activity assays cellulose and ligninase support fungal growth of Egg plant. *Berita Biologi* 10(1): 681 – 690.

Subowo, Y.B, 2011.Selection of soil fungi degrading cellulose and Deltamethrin of several Environment in West Kalimantan.*In press*