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RESEARCH ARTICLE

THE EFFECT OF INDIGENOUS AZOTOBACTER ISOLATE ON RICE RESULTS OF SRI AND LAND QUALITY METHODS

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Abstract

Nonsymbiotic N-fixing bacteria including rhizobacteria play a role in providing N elements for plants. Some types of rhizobacteria can function as Ryzobacteria Plant Growth Promoting Rhizobacteria (PGPR). Indigenous rhizobacteria are more adaptive and efficient because rhizobacteria colonies develop well in the soil in accordance with their habitat, so N fixation is greater. Pot experiments were carried out using a Completely Randomized Design with a combination of 3 types of indigenous N fixing isolates, namely: I₁: Isolate A₁.bk, I₂: Isolate A₂.bb.p, I₃: Isolate A₃.tt.k, I₄: Isolate (Isolate A₁.bk + A₂.bb.p), I₅: Isolate (Isolate A₁.bk + A₃.tt.k), I₆: Isolate (A₂.bb.p + A₃.tt.k), I₇: Isolate (A₁.bk + A₂.bb.p + A₃.tt.k), repeat 3 times with 21 pots. Analysis of the quality of the initial and final rice field research. Azotobacter single isolate for fixing N is very low so that the protection of vegetative and generative growth is not significantly different, except single isolate I₃ for observation of the number of filled grains and production ha⁻¹ shows significant differences with other single isolates. The response of Azotobacter isolates to the growth and rice production of the SRI method was determined by the type and combination of indigenous Azotobacter isolates. Azotobacter indigenous isolates are stronger giving high yields for vegetative, generative growth and soil quality. The results of rice plants showed that the combination isolate I₄ (A₁.b.k + A₂.bb.p) was the best isolate for all observations of vegetative and generative growth parameters. The stronger the ability of indigenous Azotobacter isolates to live the better N fixation and soil nutrient content. The results of the analysis of soil nutrients at the end of the study there is an increase in soil nutrients in pH, N, P, K, base saturation, which shows the characteristics of moderate soil fertility.

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Introduction:-

Intensification paddy fields are planted with the anaerobic system with low rice yield potential. The new method of saving production facilities, namely SRI (The System of Rice Intensification) is now widely carried out with various

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combinations by 10 million farmers with management reaching 4 million hectares, in more than 50 countries [1]. SRI changes the mix and allocation of inputs, especially water, seeds, fertilizer and labor [2]. SRI rice cultivation water requirements are reduced during the vegetative phase to increase rice production to 15 tons h⁻¹ and make the rice taste better and last longer [3]. Dry conditions allow beneficial microorganisms to live and be active, as well as abundant availability.

Rice intensification so far has been dominated by high artificial fertilizers, especially nitrogen (N) and phosphorus (P). N as an essential macronutrient has an important role in increasing rice production. The availability of N can be a limiting factor in increasing rice production. The problem of element N in wetlands is relatively short availability, easily dissolved in water, carried by percolation, surface runoff and volatile. The availability of soil nitrogen is a major limiting factor [4].

According to [5] the continuous application of N fertilizer at doses of 60 and 80 kg ha⁻¹ year⁻¹ in the dry season and the dry season limits the frequency and diversity of heterotrophic rhizosphere bacteria in the LARR (Long Aromatic Rice-Rice) planting system.

The efficiency of N fertilizer uptake in tropical regions by lowland rice plants is relatively low at around 30-50%. The low efficiency of N fertilizer adds to the number of production costs borne by farmers. Intensive use of chemical fertilizers on agricultural land in the long term will cause a decrease in soil organic content, soil structure is damaged and environmental pollution will occur [6].

An effective and efficient solution is needed, which is a biological approach by utilizing the rhizobacteria group. The existence of indigenous rhizobacteria is very diverse in the soil. This is influenced by biotic and abiotic factors that are in the soil [7].

Rhizobacteria interact with plant root systems, both directly and indirectly will affect plant growth [8]. The type of rhizobacteria is expected to be able to increase the availability of special nutrients N is the type of native N fastening. Rhizobacteria are capable of tethering N from the air, both symbiotically (root-nodulating bacteria) and non-symbiotic (free-living nitrogen-fixing rhizobacteria) [9]. Some types of rhizobacteria can function as Rhizobacteria Plant Growth Promoting Rhizobacteria (PGPR) [10]. The type of non-symbiotic N-fasting rhizobacteria and also capable as PGPR which is commonly found in Gramineae plants such as rice are *Azotobacter*, *Azospirillum* and *Beijerinckia* spp [11].

According to [12] *Azotobacter* found in the SRI method in rice cultivation. The characteristics and characteristics of this indigenous *Azotobacter* are more effective, adaptive and efficient in their development and growth because they are empowered in their natural ecosystems.

Efforts to utilize indigenous *Azotobacter* can be done by breeding it and giving it back to the root zone of the bacteria's origin in optimal amounts and conditions (reinoculation) to rice plants. The reinoculation of this indigenous *Azotobacter* is expected to be a reliable alternative to enhance the level of efficiency of N fertilization, to increase rice production [13].

According to [14] increased vegetative plant growth due to the synthesis of several growth hormones inoculated with indigenous *Azotobacter*. The provision of microbial inoculants in rice plants using the SRI method was higher nutrient availability and nutrient uptake in the presence of nitrogen fixation from *Rhizobium* sp compared to conventional systems.

The availability of P in the soil and translocation of P through root to leaf increases significantly as a result of cyanobacterial inoculation. Besides the N and P content of rice seeds is higher due to the administration of microbes in the SRI method [15].

The biological N cycle by microbes gives the effect of exploiting more N fixation. Besides that, it can inhibit nitrification and reduce denitrification thereby minimizing the application of inorganic fertilizers and reducing losses due to the impact of inorganic fertilizers [16].

Based on the description above, the utilization of *Azotobacter* indigenous non-symbiotic from the intensification of paddy soils planted with SRI method is believed to increase the ability to tether free nitrogen from the air so that it can reduce the use of inorganic N fertilizer and maintain environmental sustainability and farm costs are low. The purpose of this study was to determine the types of indigenous *Azotobacter* isolates that could increase the SRI method of rice production and the quality of paddy soils

Material And Method:-

Experimental design:-

The pot experiment was carried out in a greenhouse using a completely randomized design with a combination of 3 types of *Azotobacter* indigenous PGPR. The treatments are I₁: Isolate A₁.bk, I₂: Isolate A₂.bb.p, I₃: Isolate A₃.tt.k, I₄: Isolate (A₁.bk + A₂.bb.p), I₅: Isolate (Isolate A₁.bk + A₃.tt.k), I₆: Isolate (A₂.bb.p + A₃.tt.k), I₇: Isolate (A₁.bk + A₂.bb.p + A₃.tt.k), repeat 3 times so there are 21 pots. To test the effect of the treatment of the observed responses, analysis of variance was performed using the Statistical Analysis System (SAS) program. The Duncan New Multiple Range Test (DNMRT) was then tested to see differences in treatment at the 5% level.

Azotobacter isolate collection and implementation:-

Azotobacter indigenous isolates derived from rice plants rhizosphere with the SRI method in the farmers' rice fields in Harau District, Limapuluh Kota Regency. The results of the previous trial selection, obtained three types of indigenous *Azotobacter* isolates with good potential (number of cells > 10⁵ × 10⁴ cells / mL) are A₁.bk, A₂.bb.p, and A₃.tt.k, conducted with potential *Azotobacter* indigenous species test source of inoculum in rice plants SRI method (Fig. 1).



Fig. 1:- A₁) *Azotobacter* A₁.bk isolates focused cells, intact colony edges, yellowish creamy pigment colors, the total population of 10⁵ CFU / gram of soil. A₂) *Azotobacter* isolates A₂.bb.p, Yarn-shaped cells, wavy edge colonies, white pigment colors, total population 4 × 10⁴ CFU / gram of soil, A₃) *Azotobacter* isolates A₃.tt.k., irregularly shaped cells, the edge of toothed colonies, yellowish beige pigment color, total population 5 × 10⁴ CFU / gram soil.

Media and initial soil analysis:-

Growing media comes from the intensification of paddy fields of farmers planted with SRI method rice. The soil is air-dried and sieved with a 10 mesh sieve, then mixed with cow manure with a ratio of 3: 1 (v / v). The media was sterilized in the autoclave with a temperature of 140 °C with a pressure of 1 Atm for 1 hour in order to kill all organisms contained in the soil so that only indigenous *Azotobacter* isolates would grow. Sterile soil is put in a 20 kg/pot. Initial soil analysis is taken from 100 gram sterilized soil for initial soil fertility analysis. The results of soil analysis with the criteria of soil chemical properties based on [17] are presented in Table 1.

Table 1:- Results of initial soil analysis.

Parameter	Analysis Method	Results	Criteria
pH 1:1	H2O	5,25	Somewhat sour
C-Organic (%)	Walky % Black	0,75	Very low
N-Total (%)	Kjeldahl	0,09	Very low

P – available (ppm)	Bray 2	9,00	Low
K (me.100 g⁻¹ soil)	(me.100 g ⁻¹ soil)	0,11	Low
CEC (me.100 g⁻¹ soil)	N NH4OAc pH 7.0	13,74	Low
Base Saturation %		4,22	Very low

Inoculation and Planting:-

Inoculation of indigenous Azotobacter isolates was 20 mL (cell density 104-105) / pot according to the isolated treatment and incubated for one week. Seed was carried out by biopriming seeds with three isolates and Azotobacter indigenous isolates. Biopriming was carried out on Sijunjung variety rice seeds soaked for 24 hours in a suspension of indigenous Azotobacter isolates (48 hours old) with a concentration of 108-109 CFU mL⁻¹ sterile equates. Seeds are planted in a plastic tub measuring 30cmx20cmx10cm which contains sterile husk media. The 12-day-old seedlings are moved 1 stem/pot.

Irrigation:-

Watering is done once in 3 days with criteria up to field capacity. This condition is maintained until entering the initial flowering period. Flowering period, the water level is maintained up to 3 cm above the surface of the soil until the reproductive period. The physiological maturity of the water level is maintained at 5 cm. Ten days before harvest the soil is left dry.

Maintenance:-

Care for plants is carried out mainly weeding because weeds are easier to grow on the SRI method due to aerobic systems. Weed is carried out every week and buried by hand so that erase is better.

Fertilization:-

Fertilization using half the recommended dosage of Urea (Urea 150 kg ha⁻¹ or 0.75g / pot), SP-36 (100 kg ha⁻¹ or 0.50g / pot), KCL (50kg ha⁻¹ or 0.25 g / pot) procedure according by [18]. Observations were made: (1) Plant Height (cm), (2) Number of panicles per clump (panicle), (3) Number of grain per panicle (grains), (4) Weight of 1000 seeds (g), (5) Production of grain dry per hectare (ton). Soil nutrient analysis end of the research.

Harvest and Post Harvest:-

Harvesting is done after the plants meet the harvest criteria of 110 days. Post-harvest activities include threshing and drying in the sun so that the grain moisture content reaches 14%. Harvesting is done with a sickle

Results And Discussion:-

The effect of a combination of Azotobacter indigenous PGPR isolates on the growth and yield of rice using the SRI method. The results of the vegetative growth savings of the indigenous PGPR Azotobacter isolate combination test after statistical analysis are presented in Table 2.

Table 2:-Vegetative observation from the combination test of indigenous Azotobacter isolates in the SRI method of rice cultivation.

Treatment type	Plant height (cm)	No. of Tillers (tillers)
I ₁	114.67 ^d	20.00 ^b
I ₂	124.00 ^b	21.00 ^b
I ₃	125.00 ^{ab}	23.67 ^b
I ₄	127.00 ^a	26.00 ^a
I ₅	124.67 ^{ab}	22.67 ^b
I ₆	119.33 ^{bc}	20.33 ^b
I ₇	117.33 ^c	21.33 ^b

The numbers in the columns followed by the same upper case letter are not significantly different at a 5% significance level as determined by DNMRT.

In Table 1, it can be seen from seven indigenous Azotobacter isolates that observed the highest plant height in treatment I₄, not significantly different from I₃ and I₅, but significantly different from other treatments. The observation for the highest number of tillers in I₄ treatment was significantly different from the six other indigenous Azotobacter isolates.

These results indicate that the indigenous Azotobacter I₄ isolate is a combination of A₁.bk isolate population density (1 x 10⁵ CFU / g soil) and A₃.tt.k isolate population density (5 x 10⁴ CFU / g soil) is the total population density of Azotobacter bacteria effective indigenous to increase vegetative growth of rice plants with the SRI method.

I₄ combination isolates in addition to the total population and combination of indigenous Azotobacter isolates are an appropriate combination so that they are mutually reinforcing in N fixation and soil nutrient providers that affect the vegetative growth of rice plants.

According to [19] in general, plants that have a suitable population density of Azotobacter sp have effective ability to provide nutrients N. Azotobacter sp is one of the most important and beneficial soil bacteria where the amount and distribution are influenced by the physicochemical and biological nature of the soil and land use [20].

Bacteria as a biological component have many roles positioned as producers of nutrient supply in the soil. Soil as a biosynthetic medium and the work of bacteria is seen as a major provider of nutrient requirements for plants [21]. Soil bacteria is one of the effective indicators in determining the soil quality index. Soil inoculation by rhizosphere bacteria influences inter-phase arsenic transition in rhizosphere soils significantly influences the process of immobilization and mobilization of arsenic in plant-soil systems and plant bioavailability [22].

Observations on the generative growth of the SRI method of rice from the influence of indigenous Azotobacter isolates observed were the number of productive tillers, number of grains/panicles, number of filled grains/panicles, the weight of 1000 seeds and production ha⁻¹. The results of the analysis of variance are presented in Table 3.

Table 3:- Generative Observation From The Combination Test Of Indigenous Azotobacter Isolates In SRI Method Of Rice Cultivation.

Treatment type	No. of Productive tillers (tillers)	No. of grains/panicle	No. of filled grains/panicle	1000 seeds weight (g)	Yield ha ⁻¹ (ton)
I ₁	18.67 ^b	164.00 ^b	151.11 ^b	19.01 ^b	5.95 ^c
I ₂	19.00 ^b	174.44 ^b	158.78 ^b	19.25 ^b	6.55 ^c
I ₃	20.67 ^b	181.22 ^b	182.22 ^a	19.41 ^b	8.11 ^b
I ₄	24.33 ^a	193.44 ^a	185.33 ^a	20.96 ^a	10.67 ^a
I ₅	20.00 ^b	178.56 ^b	280.67 ^a	20.45 ^a	8.21 ^b
I ₆	19.33 ^b	173.67 ^b	150.44 ^b	19.89 ^b	7.26 ^b
I ₇	19.67 ^b	174.33 ^b	158.00 ^b	19.35 ^b	6.65 ^b

The numbers in the columns followed by the same upper case letter are not significantly different at a 5% significance level as determined by DNMRT.

In Table 3 the combination of indigenous Azotobacter isolates on the number of productive tillers and the number of rice grains/panicles of the SRI method gave the highest significant effect on the combination of indigenous Azotobacter isolates I₄ (A₁.b.k + A₂.bb.p). The combination of indigenous Azotobacter isolates I₄ (A₁.b.k + A₂.bb.p) can also play a role in producing growth regulators that function as Rhizobacteria Plant Growth Promoting Rhizobacteria (PGPR) capable of increasing generative growth. This condition provides a positive response to the growth in the number of productive tillers and the amount of grain/panicle.

According to [23] that root microorganisms related to rhizosphere contribute to the protection of arsenic toxicity in the rhizosphere in stimulating plant growth and their effects can be mediated by direct or indirect mechanisms. The direct effect is often associated with the supply of biological nitrogen, production of plant hormones such as auxin, gibberellin, and cytokinin. Indirect mechanisms include suppressing pathogens and producing antibiotics.

The amount of filled grain showed high results in treatments I₃, I₄, and I₅ that were significantly different from other treatments. This result is in line with plant height observations (Table 2). The three types of isolates I₃, I₄ and I₅ can live in the same environmental and host conditions to fix nitrogen from the atmosphere so that the photosynthesis process takes place perfectly, photosynthate yield increases the amount of filled grain. This is in line with the ability of nutrient absorption after soil analysis during harvest. The N and P elements indicate the number of nutrients absorbed a lot in treatments I₃, I₄, and I₅ (Table 4).

In contrast to I₁, I₂, I₆, and I₇ the absorption capacity is small and leaves a little nutrient above the initial soil nutrient (Table 1), but all treatments still have an increase in nutrient content compared to the initial soil nutrient analysis (Table 1).

At a weight of 1000 seeds, only I₄ and I₅ isolates were able to give the best results. Isolates I₄ and I₅ can survive on their host, which is still sufficient to survive so that it still gives a positive response to the weight parameters of 1000 seeds, but the two isolates showed results that were not significantly different. In line with opinion [24], the different effects of indigenous Azotobacter isolates were tested because isolates can live in certain environmental and host conditions to fix nitrogen from the atmosphere, so photosynthetic results are also different.

Observations on the production per pot of all yield components show that only I₄ isolates were able to give the highest yield. This phenomenon shows that isolate I₄ is a combination of indigenous Azotobacter isolates which acts as a nutrient supply producer capable of synthesizing N fixation perfectly so that it is superior to single isolates and other combination isolates as shown by vegetative and generative observation parameters (Tables 2 and 3).

The difference in influence shows that the seven isolates of a single indigenous Azotobacter and combination can live in certain environmental and host conditions and the ability to fix N from the atmosphere that is not the same. Therefore the type and number of populations of indigenous Azotobacter isolates gave different responses to vegetative and generative observations of rice plants.

According to [25] Azotobacter sp inoculation in peanut plants increased Azotobacter sp population and peanut growth was not significant. According to [26] the application of biological fertilizers containing various N-fixing bacteria can increase the morphological parameters of *Catharanthus roseus* L as well as secondary nutrient and metabolic absorption. The use of Arbuscular Mycorrhizal Fungi microbes in SRI method rice cultivation can increase SRI method rice production to 13.86 tons ha⁻¹ [27]. Application of Bioorganic Plus containing *Trichoderma harzianum* and *P. fluorescens* microbes can increase the SRI method of rice production by 229.05% [28].

Analysis of the quality of the final rice field research:

Table 4:- Results Of The Final Analysis Of The Study After Being Treated.

No	Soil Chemical Characteristics	Treatment (I)						
		I ₁	I ₂	I ₃	I ₄	I ₅	I ₆	I ₇
1	pH H ₂ O (1:1)	5,72	5,75	6,05	6,08	6,02	5,92	5,88
2	C-organic (Walkley and Black) %	1,45	1,75	1,63	2,74	2,62	2,26	1,65
3	N-total (kjeldahl) %	0,17	0,22	0,19	0,26	0,19	0,21	0,19
4	P-available (Bray-2) ppm	16,01	15,14	10,0	17,12	14,0	14,58	14,97
5	K (me.100 g ⁻¹ tanah)	0,12	0,21	0,19	0,17	0,22	0,24	0,25
6	CEC (me.100 g ⁻¹ tanah)	12,70	16,19	15,04	11,81	14,69	14,79	14,76
7	Base Saturation (%)	4,88	5,25	5,65	5,82	5,72	5,34	5,42

Intensification of paddy soils that have been treated by giving indigenous *Azotobacter* isolates the chemical nature of the soil has been changed. Low soil pH conditions at the beginning of 5.25 (Table 1) increased mainly in treatments I₃, I₄ and I₅ to the range of 6.02-6.08 (medium criteria). Moderate soil pH conditions will increase the availability of macronutrients such as nitrogen, phosphorus, and potassium making it easier for plants to absorb it. This increase is due to the activity of indigenous *Azotobacter* isolates, especially the I₄ treatment. This has a positive impact on soil nutrient absorption, especially nitrogen (N), phosphorus (P). The availability of N and P is still increasing after being utilized by plants, the results of soil analysis after harvest are used more for the energy process in rice plants. Furthermore, rice production increased (Table 2 and 3). Increasing the pH value after harvest, this is more due to the base saturation value rising around 130.33% from the initial conditions (Table 1). The increase in soil pH value is the impact of the process of soil bacterial community [29]. In Fig.2 the pH, C-organic and Base Saturation values are presented.

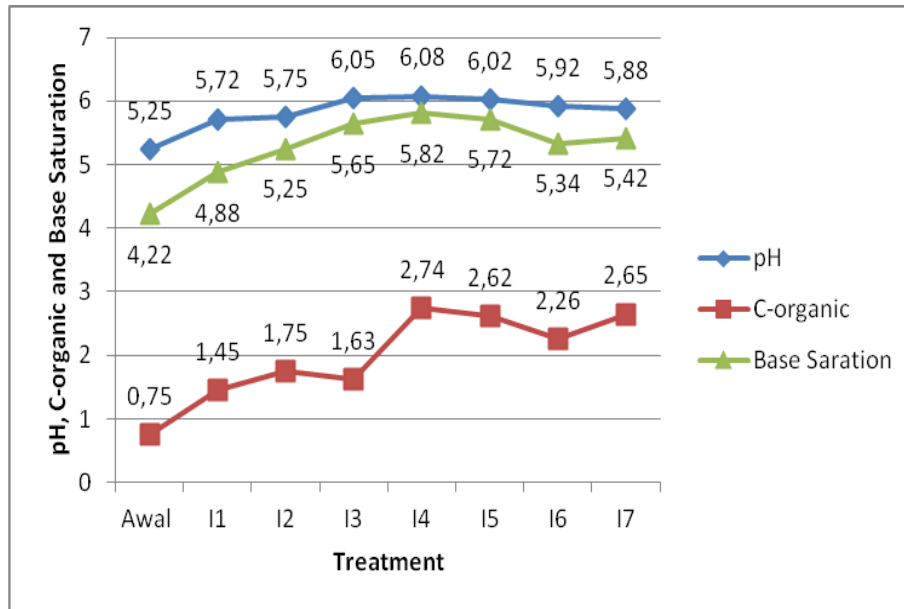


Fig. 2:- pH value, C-organic, base saturation at the beginning and end of the study in each treatment.

Fig. 2. The organic C content of soil illustrates the soil organic matter content which is a parameter of soil fertility. Initial analysis of the C-organic content of the study site was very low at <1%, indicating very little organic material production at the study site. The value of C-organic is very low because the location of the study of the humidity of the surroundings often floods when heavy rains, so that the existing organic material is carried away by erosion. After the application of indigenous *Azotobacter* isolates in all treatments an increase in soil C-organic value >2% ranged from 1.45 to 2.74 (moderate). On the same soil, the growth of different microbial communities is influenced by temperature and vegetation so that the C-organic value of the soil is different [30].

Based on the content of the content, N, P, K is classified as moderate and organic C is classified as low, as well as medium saturation which is classified as moderate, characterizing intensification of paddy soils after being given indigenous *Azotobacter* isolates categorized as moderate fertility. This shows that the inoculation of indigenous *Azotobacter* isolates can increase soil nutrient availability. According to [31] states, the use of free nitrogen-fixing biological fertilizers such as *Azotobacter* sp can increase nitrogen fixation, reduce the use of urea, prevent the reduction of soil organic matter and reduce environmental pollution.

Conclusions:-

The ability of *Azotobacter* single isolate in N fixation is still low as indicated by observations of plant height, number of tillers, number of productive tillers number of grain/panicle and weight of 1000 seeds are not significantly different. Single isolate I₃ on the observation of the amount of filled grain and production ha⁻¹ showed significant differences with other single isolates. The best *Azotobacter* combination isolates obtained in isolate I₄ (A₁.b.k + A₂.bb.p) were able to increase vegetative and generative plants per plant. The response of *Azotobacter* isolates to the growth and rice production of the SRI method was determined by the type and combination of

Azotobacter isolates. The ability of Azotobacter isolates for N fixation is influenced by environmental growth factors, which have an impact on soil nutrient content. The stronger the survival ability of Azotobacter isolates the better the N fixation and soil nutrient content also increases. The results of the analysis of soil nutrients at the end of the study showed an increase in the main soil nutrients at pH, N, P, K, base saturation, which showed the characteristics of moderate soil.

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