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Effect of indigenous microorganism extended solution (IMO-ES) on basmati rice

Sanchez, R. G.[1], Barrientos, D. S.[1*] and Galindez, J. L.[2]

[1]Department of Crop Science, College of Agriculture, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines; [2] Ramon Magsaysay Center for Agricultural Resources and Environment Studies, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines.

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Abstract The findings showed that application of 1L of Indigenous Microorganism Extended Solution (IMO-ES) every four weeks in Basmati rice has significantly improved the plant height, productive tiller that results to higher panicle and also improved the yield components such as number of filled grains per panicle and harvest yield per hectare. Application of IMO-ES once a month in rice plant to supply nutrients for growth and yield showed significant interaction indicating appropriate frequency application of IMO-ES is needed. The presence of *Bacillus pumilus, Bacillus cereus* and *Bacillus thuringiensis* bacteria in the solution enhance pest and disease resistance for better growth and development of Basmati rice.

Keywords: Bacteria, Basmati rice, Indigenous Microorganism Extended Solution, yield components

Introduction

Water from washed rice will always be for domestic and household use. And the domestic use of washed rice is a good way to recycle water in the household rather than just discarding it. Watering our household plants with water from our washed rice is effective, as good as or even better than using fertilizers because it contains water-soluble vitamins like protein, crude fiber and amino acid. It also contains minerals like calcium, phosphorus, iron, zinc and potassium (Juliano, 1993).

Indigenous Microorganisms (IMO) is a large group of microorganisms that contribute beneficial soil effects such as nitrogen fixation, mineralization, humus formation, disease suppression and decomposition. Indigenous microorganisms such as photosynthetic bacteria, lactic acid bacteria, fermenting fungi *(Aspergillus, Penicillium, Trichoderma)* promotes plant growth and

[*]**Corresponding Author**: Barrientos, D. S.; **Email**: dioniebarrientos@clsu.edu.ph

development, increases rapid decomposition of organic matter, suppress odors and prevent infestation of harmful insects and maggots (Lim, 2006).

Indigenous Microorganisms Extended Solution (IMO-ES) is a mixture of IMO concentrate, rice wash that have valuable nutrients and muscovado. IMO-ES contains beneficial microorganisms that promote plant growth, increase yield and serves as decomposers of biodegradable wastes (Tumaca *et. al.*, 2010). The new solution was not been tested in rice production.

Rice (*Oryza sativa*) is one of the most important and widely used cereals in the Philippines because this is the number one staple food. It is a rich source of carbohydrates. Basmati is an aromatic rice variety with a potential yield of 4-5 t/ha during dry season and commercially produced in the research area of Central Luzon State University. The production includes intensive application of inorganic fertilizer. Hence, the potential of declining soil fertility in the future, alternative solution shoud be done. With the good benefits of IMO-ES, this study was conducted that aims to determine the ideal frequency of application and identify the microorganism present on the Indigenous Microorganisms Extended Solution responsible for the growth and yield of rice.

Materials and Methods

The study was conducted from January 2018 to May 2018 at the Ramon Magsaysay Center for Agricultural Resources and Environment Studies (RM-CARES), Central Luzon State University, Science City of Muñoz, Nueva Ecija. The area was certified organic by the Bureau of Agriculture and Fisheries Standards. The total area of 399 sq.m was divided into three equal blocks to represent replication. Each block was subdivided in four plots. Every plot measures 4x5 sq. m and 1 m distance between plots and block. The field experiments were laid out in a Randomized Complete Block Design (RCBD) with the following treatments replicated three times. No application (control)**,** 1L of IMO-ES applied every week, 1L of IMO-ES applied every two weeks and 1L of IMO-ES applied every four weeks.

The culture of Indigenous Microorganisms (IMO) was done using 2500g steamed rice and 2500g of muscovado. The steamed rice was placed in wooden boxes covered with unprinted Manila paper and plastic to protect it from rain. The wooden box was buried in the soil, under the bamboo ecosystem. After three days the wooden boxes with steamed rice was checked the presence of white molds. On the same day, this was placed in a clay jar, mixed the muscovado and fermented for seven days. IMO was harvested after seven days, placed in a plastic bottle ready for liquid culture. Rice wash from NSIC 216 rice was collected and one liter of each was mixed with 100ml IMO concentrate.

The Indigenous Microorganisms Extended Solution (IMO-ES) were fermented anaerobically in sealed plastic bottle containers for seven days after which IMO-ES were ready for use. One (1) liter of IMO-ES were mixed in 16 liters of water and a knapsack sprayer was used as the instrument to apply the IMO-ES directly on the rice at the end of the day (4:00pm) when microbes are most active (Teodosio and Abayon, 2008). The first application of IMO-ES in the assigned treatments were seven days after transplanting when the rice plant were fully recovered and the application of IMO-ES in assigned treatment were continuous until grain filling.

The described procedure by Ahmed *et al.* (2013) was done for primary cell culture of bacteria by ten-fold dilution was used following the spread plate technique. The pipette was used to get one hundred microliters of dilution sample unto the surface agar. The sterile spreader was used to spread the sample on the entire agar surface. The plates were labelled and incubated at 37 degree celcius for 72 hours in a bacteriological incubator. The isolation of pure bacterial cultures described by Cheesbrough (2000) was used for the streak plate technique. The sterile inoculation loop was used to pick out small amounts of bacteria. This was used to inoculate sterile nutrient agar surfaces by streaking. Plates were inoculated and incubated for 24 to 48 hours at 37 degree celcius in a bacterial incubator. Based on colony and cellular morphology as described by Cheesbrough (2000) isolated bacteria were characterized. The different bacteria strains were sent to the University of the Philippines-Natural Science Research Institute for identification using BIOLOG GEN III.

All the data gathered was analyzed in Statistical Tool for Agricultural Research (STAR) using Analysis of Variance (ANOVA) in Randomized Complete Block Design (RCBD). Significant result were further tested using the Least Significant Difference Test.

Results

Plant height (cm)

The Basmati rice plant applied with 1L IMO-ES every four weeks gave the highest plant height with a mean of 88.22cm while rice applied with 1L of IMO-ES every two weeks with a mean of 77.75cm was comparable with the rice plant applied with 1L of IMO-ES every week with a mean of 76.87cm. And the Basmati rice plants with no application of IMO-ES were the lowest plant height recorded with a mean of 71.88cm (Table 1). Analysis of variance showed that at 5% level of significance there was a highly significant difference between treatments.

Table 1. Plant height at maturity of Basmati rice variety as influenced by different treatment

Treatment	Replication			Mean[1/]
	I	II	II	
No application	68	74.05	71.75	71.88[c]
1L of IMO-ES applied every week	74.45	68.8	75.65	76.87[b]
1L of IMO-ES applied every two weeks	76.3	74.35	75.8	77.75[b]
1L of IMO-ES applied every four weeks	86.2	86.4	82.95	88.22[a]

1/: Means not showing letter in common differ significantly by LSD at 5% level

Productive tiller

Result showed that Basmati rice plants applied with 1L of IMO-ES every four weeks have the highest number of productive tiller with a mean of 13.40 followed by the Basmati rice plants treated with 1L of IMO-ES applied every two weeks with a mean of 11.70 which is also comparable to 1L of IMO-ES applied every week with a mean of 11.37 (Table 2). The lowest number of productive tiller was observed on the untreated plants. Analysis of variance showed that there was highly significant difference among treatments.

Table 2. The number of productive tiller of Basmati rice variety as influenced by different treatment

Treatment	Replication			Mean[1/]
	I	II	II	
No application	10.3	10.3	9.9	10.7[c]
1L of IMO-ES applied every week	11.8	10.5	11.8	11.37[bc]
1L of IMO-ES applied every two weeks	11.9	11.8	11.4	11.70[b]
1L of IMO-ES applied every four weeks	14.7	12.2	13.3	13.40[a]

1/: Means not showing letter in common differ significantly by LSD at 5% level

Days to maturity

Among the treatments the Basmati rice applied with 1L of IMO-ES, every four weeks had earlier to mature with 103 DAS, followed by the Basmati rice plant applied with 1L of IMO-ES every two weeks with 104 DAS and untreated Basmati rice plant with 105 DAS. While Basmati rice plant treated with 1L of IMO-ES every week had the longest number of days to mature with 106 DAS (Table 3). Analysis of variance showed the days to maturity were not significant differences among treatments.

Table 3. Days to maturity of Basmati rice variety as influenced by different treatment

Treatment	**Replication**			**Mean**
	I	**II**	**II**	
No application	106	103	106	105
1L of IMO-ES applied every week	106	106	106	106
1L of IMO-ES applied every two weeks	106	103	103	104
1L of IMO-ES applied every four weeks	103	103	103	103

Table 4. Panicle length of Basmati rice variety as influenced by different treatment

Treatment	**Replication**			**Mean**
	I	**II**	**II**	
No application	19.94	23.07	23.54	22.18
1L of IMO-ES applied every week	22.23	23.81	23.94	23.16
1L of IMO-ES applied every two weeks	24.19	24.56	22.56	23.77
1L of IMO-ES applied every four weeks	26.73	25.77	24.5	25.52

Panicle length (cm)

Basmati rice applied with 1L of IMO-ES every four weeks had a mean of panicle length 25.52. And the Basmati rice treated with 1L of IMO-ES every weeks and every two weeks had closely measured panicle length. The shortest panicle length was observed in control. Statistically there were no significant differences among treatments (Table 4).

Number of filled grains per panicle

Result revealed the highest number of filled grains per panicle was the Basmati rice applied with 1L of IMO-ES every four weeks had the mean number of filled grains per panicle of 100 compared to other treatments (Table 5). Basmati rice applied with 1L of IMO-ES every week, every two weeks and no application have comparable number of filled grains per panicle. Result of analysis of variance revealed the number of filled grains had significant differences among treatments.

Table 5. Number of filled grains per panicle of Basmati rice variety as influenced by different treatment

Treatment	Replication			Mean[1/]
	I	II	II	
No application	44	76.2	67	62[b]
1L of IMO-ES applied every week	61	75.7	68.2	68[b]
1L of IMO-ES applied every two weeks	84	80.2	70.9	78[b]
1L of IMO-ES applied every four weeks	101	100	98.6	100[a]

1/: Means not showing letter in common differ significantly by LSD at 5% level

Table 6. 1000 grain weight of Basmati rice variety as influenced by different treatment

Treatment	Replication			Mean
	I	II	II	
No application	17.71	22.50	21.65	20.62
1L of IMO-ES applied every week	23.58	20.53	21.95	22.02
1L of IMO-ES applied every two weeks	22.51	22.19	21.86	22.19
1L of IMO-ES applied every four weeks	23.36	23.62	24.16	23.71

1000 grain weight (g)

Among the treatments, the Basmati rice applied with 1-liter of IMO-ES every four weeks had the heaviest 1000 - grain weight of 23.71g followed by Basmati rice applied with 1L of IMO-ES applied every two weeks with a mean of 22.19g and Basmati rice applied with 1L of IMO-ES every week with a mean of 22.02g. While the untreated Basmati rice plant was the lowest 1000-grain weight with 20.62g. The analysis of variance showed there was no significant difference among treatments (Table 6).

Harvest yield per hectare (tons)

Basmati rice plant applied with 1L of IMO-ES every four weeks had the highest yield with a mean of 2.90 tons per hectare was shown in Table 7. While the Basmati rice with no application and the Basmati rice applied with 1L of IMO-ES every week and every two weeks had comparable yield per hectare. The untreated Basmati rice plant had the lowest yield per hectare with a mean of 1.78 tons. Statistically there were significantly difference among treatments.

Table 7. Harvest yield per hectare (tons) of Basmati rice variety as influenced by different treatment

Treatment	**Replication**			**Mean[1/]**
	I	**II**	**II**	
No application	1.80	1.67	1.87	1.78[b]
1L of IMO-ES applied every week	2.00	2.14	2.57	2.24[b]
1L of IMO-ES applied every two weeks	2.51	2.28	1.59	2.13[b]
1L of IMO-ES applied every four weeks	3.03	2.92	2.75	2.90[a]

1/: Means not showing letter in common differ significantly by LSD at 5% level

Pearson's Correlation Analysis

Basmati rice plant height has significant strong linear relationship (0.60-0.79) on harvest yield per hectare, and there is strong linear relationship (0.80-1.00) in terms of number of filled grains per panicle and total grain yield per panicle. There were very strong linear relationship (0.80-1.00) between productive tiller and harvest yield per hectare and there is strong linear relationship (0.69-0.790) in terms of number of filled grains per panicle and total grain yield per panicle (Table 8).

It revealed that the IMO-ES has the presence of bacteria (Fig.1 and Table 9). A total of three distinct bacterial isolates were obtained in the solution. The isolates were distinguished based on their colonial morphology. The identified bacteria were *Bacillus pumilus, Bacillus cereus* and *Bacillus thuringiensis*.

Table 8. Correlation of coefficients of growth and yield components

CHARACTERS	DM[1/]	PL[2/]	NOFG[3/]	1000 GW[4/]	HYPH[5/]	PH[6/]
PT[7/]	-0.47	0.69	0.72**	0.63	0.81**	0.83
DM		-0.48	-0.64	-0.49	-0.23	-0.61
PL			0.89	0.70	0.79	0.72
NOFG				0.75	0.77	0.83**
1000 GW					0.52	0.78
HYPH						0.78**

[1]/Days to Maturity ,[2]/ Panicle Length,[3]/ Number of Filled Grain, [4]/ 1000 Grain Weight,[5]/ Harvest Yield Per Hectare, [6]/ Plant Height,[7]/ Productive Tiller,**highly significant

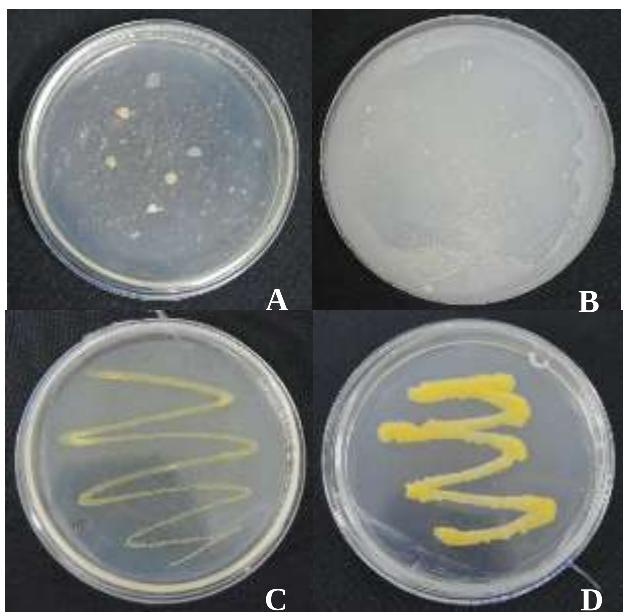

Figure 1. Bacteria culture (A and B) and isolation (C and D)

Table 9. Identified species of bacteria based on Biolog Gen III system

	Organism Type	Species
ISOLATE (C)	GP-Rod-SB	Bacillus pumilus
ISOLATE (D)	GP-Rod-SB Bacillus	Bacillus cereus Bacillus thuringiensis

Discussion

The crop growth and yield of Basmati rice was greater in the treated soil with Indigenous Microorganism Extended Solution (IMO-ES) applied once every four weeks. The plant height, number of productive tillers, number of filled grains per panicle and harvest yield per hectare were higher in IMO-ES applied once every four weeks compared to IMO-ES applied once and twice a week and no application. The significant increase in plant height that implies the plant and microorganism live in symbiotic relationship. Prell (2010) pointed that beneficial microorganisms in IMO were indigenous to the soil and environmental conditions of the farm and could easily adapt. On the other hand, Alam *et al.* (2017) revealed higher plant height of paddy rice treated with IMO and SRI Anak formulation indicated that application of organic enhancer (IMO and SRI Anak formulation) will increase the soil fertility. Similar result was also reported by Samin (2011) that the plant and microorganism live symbiotically in natural environment. Beneficial microorganisms produce nutrients, hormones and antibiotics in small quantities and plant absorb it. The productivity of rice plant is greatly dependent on the number of productive tiller (tillers which bears panicle) rather than the total number of tillers. In present investigation maximum number of fertile tillers and spikelet per panicle were observed in natural farming in rice (Hasanuzzaman *et al.,* 2010). Tillering capacity is an important trait of plant architecture for grain yield because tiller number per plant determines panicle number and affects the ultimate production directly (Zhang *et. al.,* 2011). Natural farming with indigenous microorganisms (IMO) have good benefits such as higher yield and better quality crops. This was proven by the rice farmers in South Korea with bigger yields than usual, save money on inputs and sold the rice in a premium price (Prell (2010). IMO has been also effective in Irish potato by producing more number and weight of tubers compared to other treatments (Desire *et al.*, 2018). The present findings revealed that IMO with rice wash or IMO-ES could be applied in rice that resulted to the highest harvest yield per hectare. According to Babu *et al.* (2012) the number of productive tiller exhibited positive and significant association with harvest yield per hectare, number of filled grain per panicle and total grain yield per panicle. Rajput *et al.* (1996) reported that plant height and productive tiller was observed to be positively and significantly associated to the yield component such as number of filled grains per panicle, total grain yield per panicle and harvest yield per hectare. *Bacillus pumilus* is used for fungicide in treating seedling disease and it colonizes on plant root and competes and antibiosis (Umesha *et al.,* 2018). *Bacillus cereus* is frequently isolated from a diversity of habitats including soil and plants (Altayar and

Sutherland, 2006). It has a strong proteolytic activity that can liquefy gelatin in paddy fields (Watanabe and Hayano, 1993) and also been found to promote plant growth (Jetiyanon *et al.,* 2008). *Bacillus thuringiensis* is a species that produce multi-insecticidal metabolites like vegetative insecticidal protein (Estruch *et al.,* 1996), insecticidal crystal proteins (Vachon *et al.,* 2012), secreted insecticidal protein (Donovan *et al.,* 2006), cytotoxin (Soberon *et al.,* 2013) and thuringiensin (Levinson *et al.,* 1990) which is toxic for a number of lepidopterous and dipterous insects (Bulla *et al.,* 1980). Thuringiensin is a nucleotide antibiotic of *B. thuringiensis* and has insecticidal activity against a wide range of insects (Liu *et al.,* 2010). The identified microorganisms helps the Basmati rice plants to improve plant growth and development with resistance to pest and diseases.

It concluded that the application of 1L of IMO-ES every four weeks in Basmati rice has significantly improved the plant height, productive tiller that results to higher panicle and also improved the yield components such as number of filled grains per panicle and harvest yield per hectare. Application of IMO-ES once every four weeks in Basmati rice plant to supply nutrients for growth and yield showed significant interaction indicating appropriate frequency application of IMO-ES is needed. *Bacillus pumilus, Bacillus cereus* and *Bacillus thuringiensis* were identified species of bacteria present to IMO-ES that helps repel the pest and diseases to improve the Basmati rice growth and development. The result of this study is very promising to improve growth and yield of rice. However it is recommended to repeat the same research experiment for wet season in order to confirm the present findings.

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