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RESEARCH ARTICLE

EVALUATION OF INTEGRATED NUTRIENT MANAGEMENT PACKAGES IN PADDY WITH ENDOPHYTIC DIAZOTROPHIC BACTERIA IN COASTAL SALINE, RED & LATERITE AND VINDHYAN ALLUVIAL ZONES OF WEST BENGAL FOR BETTER ECONOMICS

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ABSTRACT

Integrated Nutrient Management (INM) packages were designed and field tested for submerged paddy for Coastal Saline, Red &Laterite and Vindhyan Alluvial agro-climatic zones of West Bengal (India) with 3 promising endophytic diazotrophic bacteria previously selected through laboratory analysis, pot experiment and micro field experiment. The bacteria were isolated from roots of wild paddy, *Oryza rufipogon* from Sundarban area. The selected isolates were applied through seed to ensure endophytic association in controlled condition of seed bed and to avoid the inhibitory factors of field like presence of excess chemical nitrogen, residual pesticides *etc.*. The packages were evaluated on the basis of yield and profit. The result was compared with farmers' practice, and previously *Azospirillum* based INM package where this biofertilizer applied directly during field preparation along with phosphate solubilizing and potash solubilizing bacteria. Significant increase in production and economic gains were noted in this package as compared with farmers' practice as well as *Azospirillum* based previous practice.

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INTRODUCTION

During last four decades, a paradigm shift from agricultural to non-agricultural sector is observed in India (Roy Choudhury *et al,* 2020). From 1983-1984 to 2018-2019, dependence of rural households on agriculture declined from 77% to 50%, contribution of agriculture to GDP came down from 34% to 16% and its contribution to rural employment declined from 81% to 58%. Low return from agriculture is one of the key important factors for this decline, which will still continue. Cost for production, yieldand selling price are the important issues which determines the profit from agriculture (Salam, 2019) and the former two factors depends on sustainability of agricultural system which is the ability of a system to be maintained in steady state in time when all the factors affecting the system are constant. Pretty (2008) defined agricultural sustainability as constant production when all the inputs remain constant in time. He pinpointed four basic principles for agricultural sustainability as integrated biological &ecological process, minimal use of non-renewable inputs, productive use of knowledge & skills of farmers, and productive use of people's collective capacities. Integrated Nutrient Management (INM) in plants is one of the approaches that integrate biological and ecological processes (Batabyal, 2017). This is a management system that adjust plant nutrient supply from all possible sources. Selim (2021) pointed that Integrated Nutrient Management is a good option for economical choice for agricultural production. Biofertilizer is an efficient, reliable and economical component in INM where a portion of chemical fertilizer requirement is substituted by its application. Barraquio *et al.* (1997) from IRRI, Philippines, reported that wetland rice plants and its related genera harbour diazotrophic bacteria endophytically in their roots and culms. Recently, the role of endophytic bacteria in improving crop production is well documented by Sing *et al. (*2021). This is an alternative microbial resource for development of safe and sustainable rice production (Rangjaroen *et al.,* 2017). In addition to nitrogen contribution, some members of this bacterial type enhances plant growth through production of Indole Acetic Acid (IAA) & siderophore , and phosphate solubilization (Verma *et al.,* 2001; Ji *et al.* 2014;

Patel and Archana, 2017*;* Shabanamol *et al.,* 2018; Wozniak *et al.*, 2019; Das *et al.,* 2021). As a nitrogen biofertilizer in paddy, endophytic diazotriphic bacteria is privileged during its growth and nitrogen fixation as it is endophytically lives within plant tissue and hence less affected by soil environmental condition like residual chemical fertilizer, residual pesticides and physical factors like temperature, rainfall & seasonal variation. Moreover, their niche inside plant tissue also gives lesser competition for food and shelter (Banik *et al.,* 2019). Application of chemical fertilizers is a common practice in agriculture. Application of nitrogen fertilizer may be beneficial (Kanungo *et al.,* 1997; Muthukumarasamy *et al.,* 2007) or harmful (Omar and Ismail, 1999: Wu *et al*., 2011) depending on the dose and combination with other fertilizers. Shresta and Maksey (2005) reviewed that profit from nitrogen fixation is limited by inhibitory effect of exogeneous nitrogen. This inhibitory effect may be minimised by alternate flooded and dry condition (Zhang *et al.,* 2021), but during the *monsoom* season of West Bengal (India) such condition cannot be available. Ayuni *et al*. (2015) reported significant decrease in of population size and nitrogen fixation of rhizosphere and endosphere population of diazotrophic bacteria *Stenotrophomonas maltophila* due to presence of excess chemical nitrogen. Lesser number of endophytic diazotrophic bacteria was observed in sugarcane roots highly fertilized with chemical nitrogen than poorly fertilized (Oliveira *et al.,* 2003). Endophytic colonization and reduction of nitrogenase activity by certain pesticides was also noted by several workers. Niewiadomska (2003) showed that pesticides, like, carbendazim, imazetapir and thiram, inhibit nodulation and nitrogen fixation by *Rhizobium leguminosarun*in red clover plant. Fungicides like, mancozeb, and copper sulphate, and insecticide fenvalerate is reported to inhibit growth, respiration and nitrogen fixation of some *Azospirillum* species (Omar and Alla, 1992). To avoid these inhibitory effects of soil environment an effort is undertaken to allow endophytic colonization during seedling production in controlled environment. In the previous work of the authors, 3 isolates of endophytic diazotrophs were selected for this purpose for Coastal Saline, Red and Laterite and Vindhyan Alluvial zones of West Bengal , India (Das *et al.,* 2021). On this basis INM package for paddy is designed and field evaluated on the basis of yield and economical aspect.

MATERIALS AND METHODS

Paddy Varieties *Pusa Sugandh-5* (PS-5), Shatabdi (IET 4786) and Swarna (IET 7029), were used for Coastal Saline (South 24 Parganas District), Red & Laterite (Bankura District) and Coastal saline (South 24 Parganas District), respectively. Previously selected isolates of Endophytic diazotrophic bacteria were included within INM package constituting 75% of soil analysis based recommended dose of chemical fertilizers and biofertilizers, endophytic diazotrophs, Phosphate Solubilizing Bacteria (PSB) and Potash Solubilizing Bacteria (KSB) . For coastal saline zone endophytic diazotrophic isolates VIBENB003 & VIBENB010 were tested. Two isolates VIBENB001 & VIBENB003 were tested for both Red & Laterite and Vindhyan Alluvial zones. Total 6 treatments were designed for each zone. T-1- Farmers' practice, T-2: Full NPK as per soil analysis-based recommendation, T-3: 75% NPK, T-4: INM with previously used *Azospirillum*as nitrogen biofertilizer. T-5 and T-6 were designed as INM with 2 selected isolates of endophytic diazotrophs. The plots were in Randomised Block Design. Sprouted seeds were treated with aqueous suspension of selected carrier based culture of endophytic diazotrophic bacteria (@ 20 g/L of culture having 10^8 cell count /g). The PSB and KSB was applied during field preparation (@ 2 Kg/ha of culture having 10^8 cell count /g), 72 h after application of chemical fertilizers. Data for yield and other yield attributing parameters were taken by 1 m^2 block cutting method and analysed statistically by ANOVA method.

RESULTS AND DISCUSSION

The selected isolates of endophytic diazotrophic bacteria contributed positive effects on yield and yield attributing parameters, like plant height, number of tiller per stock, number of panicle per stock and 1000 seed weight (Table 1, Table 2 and Table 3). Although both the isolates VIBENB003 and VIBENB010 showed significant positive effect on yield and yield attributing parameters in Coastal saline zone, VIBENB010 showed better result (Table 1). Also 2 tested isolates in Red & Laterite zone showed significant positive impact, but isolates VIBENB003 imparted better results. (Table 2). In Vindhyan Alluvial zone, isolates VIBENB001 showed better result although the other tested isolates VIBENB003 also showed significant positive effects (Table 3). The economics of the INM package was also analysed on the basis of total cost, cost for fertilizer, cost for pesticides, cost per unit of production, fertilizer cost per unit of production, pesticide cost per unit of production, gross return, net return, return from unit of production and benefit: cost ratio. Benefit was compared with farmers' conventional practice and also with previously used INM package where *Azospirillum* was used as nitrogen biofertilizer. In coastal saline zone (Table 4) an increase in net return of INR 12495 (21.14%) could be achieved applying INM with endophytic diazotrophs (VIBENB010), whereas this increase in INM with normal *Azospirillum* was INR 4795(8.11%). The B:C ration was 2.4, 2.91 and 3.41 for farmers' practice, INM with *Azospirillum,* and INM with ENBVIB010, respectively. Same observation was in Red & Laterite zone (Table 5) an increment of net return of INR 8014 (30.96%) with ENBVIB003 whereas this is only INR3994 (15.31%) with *Azospiriullum*The B:C ratios are 1.72, 1.89 and 2.36 accordingly. In Vindhyan Alluvial zone (Table 6), the isolates VIBENB001 showed an increase in return of INR 6995 (44.60%). This increase with *Azospirillum*is INR 3580(22.82%). This figure is also reflected in B:C ratio- 1.34, 1.44 and 1.53 for farmers practice, INM with *Azospirillum*and INM with VIBENB001, respectively. The analysed data clearly showed that application od endophytic diazotrophic bacteria as nitrogen biofertilizer in INM package of paddy impart positive result in farm economy when total cost, fertilizer cost & pesticide cost per unit production in addition to net return and B:C ratio. A remarkable observation is decrease in pesticide cot in all the fields. Our results clearly showed that the application of selective endophytic diazotrophic bacteria through seed allows their endophytic association with paddy roots in seedling condition. Thus, the inhibitory effects in the soil of main field due to presence of excess chemical nitrogen and residual pesticides are mitigated. Jha *et al.* (2013) explained that reduction of colonisation by endophytic bacteria with host root is due to reduced carbohydrate concentration by presence of excess chemical nitrogen.

Table 01. Effect of Endophytic Diazotrophs based INM on yield and yield attributing parameters of paddy in Coastal Saline agro climatic zone of West Bengal

Treatment	Description	No. of tiller per stock	Plant Height (cm)	No. of panicle per ctock	1000 grain weight (g)	Total yield (t/ha)
T1	Farmers' Practice	12.3	109.33	11.67	26.67	4.70
T2	Full NPM (recommended)	12.3	109.00	12.00	27.17	4.81
T3	75%NPK	10.7	108.00	10.67	26.50	4.60
T3	75% NPK + PSB +KSB + Asospirillum	12.3	109.00	11.33	27.43	4.81
T5	75% NPK + PSB +KSB + VIB-ENB003	15.0	110.00	14.67	28.20	5.57
T6	75% NPK + PSB +KSB+ VIB-ENB010	12.67	108.67	12.33	27.00	5.01
	CD0.05	0.99	1.95	0.79	0.99	0.17
	CD0.01	1.65	3.25	1.31	1.65	0.28

Table 02. Effect of Endophytic Diazotrophs based INM on yield and yield attributing parameters of paddy in Red & Laterite agro climatic zone of West Bengal

Treatment	Description	No. of tiller per stock	Plant Height (cm)	No. of panicle per ctock	1000 grain weight (g)	Total yield (t/ha)
T1	Farmers' Practice	14.7	87.67	10.67	20.87	3.73
T2	Full NPM (recommended)	15.3	86.00	11.00	21.51	3.83
T3	75%NPK	12.7	81.67	8.67	19.63	3.48
T3	75% NPK + PSB +KSB + Asospirillum	16.3	87.67	11.67	21.58	3.82
T5	75% NPK + PSB +KSB + VIB-ENB001	17.0	89.33	13.33	22.33	3.93
T6	75% NPK + PSB +KSB+ VIB-ENB003	19.0	90.33	15.00	22.57	4.12
	CD0.05	0.93	1.01	0.79	0.42	0.05
	CD0.01	1.55	1.69	1.31	0.71	0.08

Table 03. Effect of Endophytic Diazotrophs based INM on yield and yield attributing parameters of paddy in Vindhyan Alluvial agro climatic zone of West Bengal

Treatment	Description	No. of tiller per stock	Plant Height (cm)	No. of panicle per ctock	1000 grain weight (g)	Total yield (t/ha)
T1	Farmers' Practice	11.3	94.67	10.67	28.67	3.97
T2	Full NPM (recommended)	11.3	95.00	11.00	27.33	4.13
T3	75%NPK	10.7	93.67	8.67	25.33	3.83
T3	75% NPK + PSB +KSB + Asospirillum	14.3	98.33	11.67	30.00	4.07
T5	75% NPK + PSB +KSB + VIB-ENB001	14.7	99.33	13.33	31.33	4.22
T6	75% NPK + PSB +KSB+ VIB-ENB003	17.0	98.33	15.00	31.00	4.17
	CD0.05	1.45	1.61	0.79	1.79	0.23
	CD0.01	2.42	2.69	1.31	2.98	0.39

Table 04. Cost benefit analysis of application of Endophytic Diazotrophs based INM in Coastal Saline zone of West Bengal

Criteria	Farmers Practice	INM with *Azospirillum*	Change	Change (%)	INM with VIB ENB010	Change	Change (%)
Total Cost (Rs/ha)	36000	33500	-2500	-6.94	33450	-2550.00	-7.08
Cost for fertilizer (Rs/ha)	8500	7900	-600	-7.06	8450	-50.00	-0.59
Cost for pesticides	4500	2500	-2000	-44.44	2500	-2000.00	-44.44
Production (Kg/ha)	3730	3820	90	2.41	4120	390.00	10.46
Cost per unit production (Rs)	9.65	8.77	-0.88184	-9.14	8.12	-1.53	-15.88
Fertilizer cost per Kg of product (Rs)	2.28	2.07	-0.21076	-9.25	2.05	-0.23	-10.00
Pesticide cost per Kg of product (Rs)	1.21	0.65	-0.55198	-45.75	0.61	-0.60	-49.70
Gross return (Rs/ha)	95115	97410	2295	2.41	105060	9945.00	10.46
Net return (Rs/ha)	59115	63910	4795	8.11	71610	12495.00	21.14
Return from unit of production (Rs/ha)	15.85	16.73	0.881841	5.56	17.38	1.53	9.67
B:C Ratio	2.64	2.91			3.14		

Table 05. Cost benefit analysis of application of Endophytic Diazotrophs based INM in Red & Laterite zone of West Bengal

Criteria	Farmers Practice	INM with *Azospirillum*	Change	Change (%)	INM with VIB ENB010	Change	Change (%)
Total Cost (Rs/ha)	36000	33500	-2500	-6.94	34450	-1550.00	-4.31
Cost for fertilizer (Rs/ha)	8500	7900	-600	-7.06	8450	-50.00	-0.59
Cost for pesticides	4500	2500	-2000	-44.44	2500	-2000.00	-44.44
Production (Kg/ha)	3730	3820	90	2.41	4120	390.00	10.46
Cost per unit production (Rs)	9.65	8.77	-0.88184	-9.14	8.36	-1.29	-13.36
Fertilizer cost per Kg of product (Rs)	2.28	2.07	-0.21076	-9.25	2.05	-0.23	-10.00
Pesticide cost per Kg of product (Rs)	1.21	0.65	-0.55198	-45.75	0.61	-0.60	-49.70
Gross return (Rs/ha)	61918	63412	1494	2.41	68392	6474.00	10.46
Net return (Rs/ha)	25918	29912	3994	15.41	33942	8024.00	30.96
Return from unit of production (Rs/ha)	6.95	7.83	0.881841	12.69	8.24	1.29	18.56
B:C Ratio	1.72	1.89			1.99		

Table 06. Cost benefit analysis of application of Endophytic Diazotrophs based INM in Vindhyan Alluvial zone of West Bengal

Criteria	Farmers Practice	INM with *Azospirillum*	Change	Change (%)	INM with VIB ENB010	Change	Change (%)
Total Cost (Rs/ha)	45850	43820	-2030	-4.43	42730	-3120.00	-6.80
Cost for fertilizer (Rs/ha)	8400	7850	-550	-6.55	7750	-650.00	-7.74
Cost for pesticides	4500	2500	-2000	-44.44	2500	-2000.00	-44.44
Production (Kg/ha)	3970	4070	100	2.52	4220	250.00	6.30
Cost per unit production (Rs)	11.55	10.77	-0.78253	-6.78	10.13	-1.42	-12.33
Fertilizer cost per Kg of product (Rs)	2.12	1.93	-0.18712	-8.84	1.84	-0.28	-13.20
Pesticide cost per Kg of product (Rs)	1.13	0.61	-0.51925	-45.81	0.59	-0.54	-47.74
Gross return (Rs/ha)	61535	63085	1550	2.52	65410	3875.00	6.30
Net return (Rs/ha)	15685	19265	3580	22.82	22680	6995.00	44.60
Return from unit of production (Rs/ha)	3.95	4.73	0.782534	19.81	5.37	1.42	36.03
B:C Ratio	1.34	1.44			1.53		

As colonisation process was executed in controlled condition in seed bed, such inhibition was successfully avoided. Several workers reported from their laboratory studies that endophytic diazotrophic bacteria promote plant growth through production of IAA (Verma *et al.,* 2001; Ji *et al.* 2014; Patel and Archana, 2017*;* Shabanamol *et al.* 2018; Wozniak *et al.*, 2019) The same authors have also worked out that these bacteria also promote plant growth by producing siderophore which inhibit the growth of plant pathogenic microbes. In our previous report (Das *et al.,* 2021), it was shown that all the 3 test microbes are efficient siderophore producers and this property is reflected in reducing the cost of pesticides. In the same endeavour, it was also reported that these 3 microbes also affect positively on some vegetative plant characters as plant height, fresh and dry weight of whole plant and root and root length. Thus, it may be said that our tested bacteria impart positive effects on the yield attributing parameters through these vegetative characters. Kanbar *et al.* (2009) reported that root biomass as dry weight has great effect on plant height, shoot dry weight and grain yield in paddy in well-watered condition. Jian Chang *et al.* (2012) demonstrated that root system plays significant role for development of shoot and other yield attributing character of paddy plant. Higher root biomass has higher oxidation activity and cytokinin content which are manifested to higher number of panicles, more spiklets per panicle, greater grain filling percentage and higher yield. Paddy traits of higher root biomass with deeper root distribution shows higher nitrogen use efficiency and consequently higher yield (Ju *et al.,* 2015).
Higher yield in INM may be due to increased fertilizer use efficiency as also reported by Kumar *et al.* (2017). Increased nutrient uptake by rice plant in INM has also been reported by Wolie and Admassu (2016). Mohanty *et al.* (2020) demonstrated that INM reduces emission of green house gas like N2O to 11-24% and NH3 to 13-27%. This results in fertilizer use efficiency reducing cost of fertilizers. Siderophore production by the tested isolates reduces the disease incidence and the cost for pesticides is also reduced. All these phenomena are manifested to increase income and higher B:C ratio. Thus it is recommended that nitrogen biofertilizer may be produced using isolates VIBENB010, VIBENB003 and VIBENB001 for Coastal saline zone, Red & Laterite zone and Vindhyan Alluvial zone of West Bengal (India), respectively, and farmers may be encouraged to use it for long term benefit.

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