THE EFFECTIVENESS OF FUNGAL BIOFERTILIZERS IN PRODUCING P AND K-RICH ORGANIC MINERAL MANURES FOR MAIZE

Rowndel Khwairakpam, Asst. professor, School of Agriculture, Graphic Era Hill University, Dehradun Uttarakhand India DOI:10.48047/ejmcm/v07/i04/386

Abstract

Solubilization and mineralization of soil nutrients for direct plant uptake, synthesis and regulation of hormones, production of volatile organic molecules, microbial enzyme, suppression of plant pathogens, amelioration of abiotic stress, and so on are all actions that contribute to PGPF's promotion of plant growth. Improvements in germination, vigour, growth, root development, photosynthetic rate, early blooming, and increased flowering have all been linked to PGPF, either directly or indirectly. The significance of PGPF in maintaining rhizosphere competence and stability is crucial. Most PGPF rely on successful colonisation as a prerequisite for displaying their positive features. When PGPF colonise a plant, they interact with the root system with a degree of specificity that has a positive impact on the crop.

Keywords: Fungal Biofertilizers, Producing P, K-rich, Organic, Manures for maize.

1. Introduction

Dwarf, high-yielding, and fertilizer-responsive cultivars have altered the national landscape, and intensive farming is now seen as a viable strategy for feeding a rapidly expanding population. Soil productivity has declined generally as a result of farmers' utter reliance on chemical fertilisers, herbicides, and other inputs. More food is produced by chemically centred agricultural cultivation, but farmland's output capacity is diminished and the agroecosystem is disrupted as a result of environmental contamination. Soil pollution, water pollution, and a disturbed native microbial population are all direct results of chemically intensive agriculture, which has also resulted in a number of other problems, such as a diminished response to fertiliser application, changes in soil pH (due to acidity or the deposition of salts, for example), a decrease in soil organic carbon, and a negative impact on the diversity of microflora and macroflora. Chemical fertiliser has not only harmed agriculture as a whole, but also human health and the economy. The high price of fertiliser is a burden on the government in the form of subsidies and on farmers in the form of higher production costs. Total fertiliser subsidies amount to roughly 23475 crores, or about 9296 crores for imported P and K and about 14179 crores for indigenous P & K. Farmers are put in a difficult financial position as a result of the high cost of production brought on by the use of chemical fertilisers. An alternative ecocentric strategy is needed to reduce the negative impacts of agrochemicals on the environment and the economy.[1-2]

Crop nutrient shortages often result from a lack of both macro and micro nutrients in the soil, despite the fact that plants need good nutrition for optimal production. When applied to soil, nutrients like phosphorus and potassium quickly get bound to other substances or transformed into inaccessible complexes. These deposited nutrients were either washed away by rainwater runoff or left in the soil in their unutilized state.[3]

Microbes on Earth have had intricate connections with plants for millennia; microbial communities in the rhizosphere promote plant vitality, growth, and development through altering plant metabolism. Rhizospheric fungi, which colonise plant roots and promote plant development, are a diverse collection of saprophytic, soil-borne, nonpathogenic fungus with the ability to improve plant health via the efficient use of soil-borne macro- and micronutrients. Endophytic rhizospheric fungi reside inside roots and directly exchange metabolites with plants, whereas epiphytic rhizospheric fungi reside freely on the root and benefit the plant indirectly. They are crucial to the processes of nutrient cycling and nutrient pool maintenance. In the present bio-intensive agriculture context, plant growth promoting fungi (PGPF) have the potential to emerge as an environmentally benign and effective biofertilizer choice for improving crop output, which might help meet the rising demand for agricultural products without negatively impacting soil ecology.[4-5]

Many different processes are at work in PGPF to promote plant growth, such as the solubilization and mineralization of soil nutrients for direct plant uptake, the synthesis and regulation of hormones, the production of volatile organic molecules, microbial enzyme, the suppression of plant pathogens, the amendment of abiotic stress, and so on. Increases in germination, plant vigour, growth, root development, photosynthetic rate, and the number and timing of flowers produced by a plant are only some of the other benefits linked to PGPF. PGPF are crucial in maintaining the competence and stability of the rhizosphere. The positive characteristics of most PGPF depend on successful colonisation. The favourable impact on

crop is an outcome of the specificity with which PGPF colonise and interact with plant roots.[6-7]

2. Literature review

Abou El-Khai & Mohsen, A. A. M. (2020)Trichoderma was extracted from Amazonian soils and tested for its ability to solubilize phosphate and its impact on soybean growth. In a greenhouse setting, they used a combination of rock phosphate, triple superphosphate, and a few carefully chosen strains of Trichoderma to treat the plants. Nineteen percent of the isolates tested positive for phosphate solubilization and organic acid generation. P uptake efficiency was 141%, and the range of positive responses for stimulating soybean growth was 2.1% to 41.1%.[8]

Bashir, Z. & Rasool, F. (2019)discovered bacteria capable of breaking down rock phosphate and potassium feldspar into usable P2O5 and K2O. Aspergillus niger was determined to be the most effective microorganism to dissolve ground phosphate compared to the non-treated control after being infected with Aspergillus niger, Penicillium sp., Chaetomium sp., and Actinomycetes. The most efficient microbe at dissolving potassium feldspar and releasing the corresponding 'K' was Actinomycetes, followed by Chaetomium sp. Potassium feldspar that had been sterilised and cultured with actinomycetes was found to release substantially more potassium than the control.[9]

Aly, A. H. & Proksch, P. (2018)investigated the potential of rhizosphere fungi to mineralize insoluble potassium. The soil was mixed with feldspar and left to incubate at room temperature for a week before being sampled. Subsequently, 100 ml of liquid medium comprised of 1% glucose, 0.05% yeast extract, 0.5% feldspar was infected with 1 gramme of soil, and the mixture was cultured for a week at 37 °C on 120 rpm. After successive dilution, up to a dilution of 10-6, enriched samples were put into Aleksandrov agar medium. Candidate strains of the fungus for use as a potash solubilizer were chosen from dilutions of 10-4, 10-5, and 10-6 based on the presence of a clear zone surrounding the colonies. In the secondary screening, the fungal isolate (A. terreus) with the strongest solubilizing ability was determined based on the zone of clearance by isolates utilising Khandeparkar's selection ratio.[10]

Bhatnagar, R. & Talati, J. G., (2017) provided a procedure for making PROM and reported that, compared to a control group receiving 100% NPK, PROM increased groundnut yield by

an average of 10.52 percent. In comparison to the control group, plots that were treated with PROM resulted in a net gain of between Rs. 320 and Rs. 1850/ha. It was determined that PROM may be an appropriate alternative for DAP based on a comparison to a chemical fertiliser of the same grade. This research not only offers a replacement for DAP, but it also suggests ways to save money on other, more expensive chemical fertilisers. Native rock phosphate is an excellent phosphate resource that also improves soil biodiversity.[11]

De Vleesschauwer & Hofte, M. (2016)shown that alkaline soils may be fertilised just as well with a blend of fine-grained rock phosphate and organic manure as with di-ammonium phosphate (DAP). Sedimentary rock phosphates, which he employed as fertiliser, were put to acidic soils. When tested for their agronomic effectiveness as PROM, rock phosphate from Jhamarkotra, India (sedimentary origin), Egypt (sedimentary origin), and South Africa (volcanic origin) all fared well. Low-grade rock phosphate slimes are just as effective as high-grade phosphate concentrate when used in PROM, as shown by results of efficiency of PROM prepared using two grades (24% P2O5 and 34% P2O5) of rock phosphate mineral from Jordan. Despite DAP's utter failure in saline soils, they found PROM to have a lingering impact.[12]

3. Methodology

The purpose of this study was to investigate the potential benefits of using organic mineral enriched manures high in phosphorus and potassium in maize cultivation.

3.1 Location

Anand Agricultural University in Anand was the site of the study; it is located at an elevation of 45 metres above sea level in latitude of 220-350 and a longitude of 720-550.

3.2 Materials

3.2.1 Glass-Wares and Chemicals

Schott Duran, Borosil, J-Sil, Polylab, and Eppendorf glasswares and plastics were utilised in the experiment. In this study, we only utilised high-quality chemicals and reagents from reputable companies like Sigma, Merck, HiMedia, etc. that are suitable for use in analytical and molecular biology.

All necessary equipment, including glassware, plasticware, and reagents, were autoclaved and properly stored before use.

3.2.2 Media, Chemicals, Stains and Reagents

Materials used in experiments, including as media, stains, buffers, reagents, and chemicals

3.3 Methodology

Soil samples were taken at a depth of 5-12 centimetres in cultivated soil. At three locations around the campus of Anand Agricultural University, eight soil samples were taken to serve as a representative cross-section.

Several HDPE bags of soil were frozen at 4 degrees Celsius after being gathered from three separate farms at AAU (the farm, the organic patch on the agronomy farm, and the Regional Research Station). Isolated fungi were maintained on potato dextrose agar (PDA) slants at 4-8 °C after being isolated using the serial dilution technique. Isolates were sub-cultured once per month.

Sr.No.	Sites	Cultivated/Non-cultivated
	tyFarms	
1.	Agronomyfarm(Organicfields)	Cultivated
2.	Agronomy farm(Conventionalfields)	Cultivated
3.	RRSfarm(Conventionalfields)	Cultivated

Table 3.1: List of sampling sites

3.4 Preparation of prom, krom and their combined preparation using native pgpf

3.4.1 Phosphate Rich Organic Manure

Specifically, one litre of T. aggressivum AAU PRM 17 as P solubilizing fungal culture (108 cfu/ml) was added to the mixture to increase the microbial activity, and 200 kilogrammes of high grade rock phosphate (containing about 30% P2O5) was mixed with five tonnes of cow dung and crop residue mixture. After 90 days, the combination had decomposed completely. The procedure was carried out at a constant moisture level of 55%-65%. In 90 days, the manure was ready for collection.

3.4.2 Potash Rich Organic Manure

One litre of A. flavipes AAU KRM 3 as K solubilizing fungal culture (108 cfu/ml) was added to the mixture, along with 355 kilogrammes of high-grade feldspar (containing about 17% K2O) and 5 tonnes of cow dung and crop residue mixture. This yielded KROM sufficient for one hectare of land. It took the combination 90 days to disintegrate. The relative humidity was maintained between 55% and 65% during the operation. Manure was collected after 90 days.

3.4.3 Combined preparation of PROM and KROM

PROM & KROM were prepared by combining 5 tonnes of cow dung and agricultural leftovers with 200 kilogrammes of high-grade rock phosphate (containing approximately 30% P2O5) and 355 kilogrammes of high-grade feldspar (containing about 17% K2O). T. aggressivum AAU PRM 17 (108 cfu/ml) was added to the combination as a P solubilizing fungus, and A. flavipes AAU KRM 3 (108 cfu/ml) was added as a K fungal culture. It took the combination 90 days to disintegrate. Manure was collected after 90 days.

3.5 Field efficacy of prom, krom and their combined preparation on maize

3.5.1 Experimental Details

During the rabi seasons of 2019 and 2020, a randomised block experiment with five treatments and four replications was carried out in the field.

Design		RandomizedBlockDesign
Treatments		5
Replication	1	4
Plotsizo	Gross	$4.5 \text{ m} \times 5.0 \text{ m}$
I IUISIZC	Net	$3 \text{ m} \times 4.2 \text{ m}$
Cropandvariety		Maize(GujaratAnand YellowMaizeHybrid-3, GAYMH-3)
Recommended doseofFertilizer		150 kg N-60 kgP ₂ O ₅ -60 kg K ₂ O
Croppingseason		<i>Rabi</i> – 2019-20 and 2020-21
Methodofsowing		Dibbling

Table 3.6: Details of experiment

Spacing	75cm×20-25cm
Seedrate	20-25kg/ha

3.6 Statistical Analysis

Completely Randomised Design (CRD) methodology was applied to laboratory trial data, while randomised block design and pooled analysis were used to analyse field trial data.

4. Results

We set out to see how effective natural fungal biofertilizers, particularly those high in phosphorus and potassium, would be in growing maize.

4.1 Preparation of prom, krom and their combined preparation using native PGPF

Based on the principle of compositional reciprocal contents, enough T. aggressivum AAU PRM 17 as P solubilizing fungal culture (108 cfu/ml) for one hectare of land was added to five tonnes of cow dung and crop residue mixture to create phosphate-rich organic manure. There was a 90-day period for decomposition. After 90 days of decomposition, the PROM was collected for its total organic carbon (29.5%), phosphorus (1.6%), and potassium (0.7%). Composition Similar components were used to create potash-rich organic manure, which included the addition of 355 kilogrammes of high-grade feldspar (carrying around 17% K2O) to a combination of 5 tonnes of cow dung and crop debris. K solubilizing fungal cultures of A. flavipes AAU KRM 3 (108 cfu/ml) were added to the mixture, bringing the total volume to 10 litres. There was a 90-day period for decomposition. With a total organic carbon content of 28.9%, 0.6% total P, and 0.9% total K, the well-decomposed PROM was collected after 90 days.

It takes 5 tonnes of cow manure and crop residue, 355 pounds of high-grade feldspar, and 10 litres of T. aggressivum AAU PRM 17 and A. flavipes AAU KRM 3 as P and K solubilizing fungal cultures (108 cfu/ml) to make enough PROM and KROM to cover an acre. The mixture broke down in the plastic container after being there for 90 days. The allotted time for decay was 90 days. After 90 days, the PROM had degraded to the point where it could be collected; it had 20.9% total organic carbon, 1.3% total P, and 0.8% total K.

Table 4.1: Preparation properties of PROM, KROM, and their mixtures

Particula r	Organiccarbon(%)	Tota l P(%)	P ₂ O ₅ (%)	Total K(%)	K ₂ O(%)
FYM	36.2	0.4	0.9	0. 6	0.7
PROM	29.5	1.6	3.6	0. 7	0.8
KROM	28.9	0.6	1.3	1. 8	2.1
PROM+ KROM	20.9	1.3	2.9	1. 5	1.8

4.2 Field efficacy of prom, krom and their combined preparation on maize

This article presents a pooled analysis in a randomised block design using plant height (cm) data collected at 30, 60, and 90 DAS during Rabi 2018–19 and 2019–20. It seems that adequate availability of necessary nutrients was present in all the treatments, as the maximum mean plant height was reported in T1 (62 cm) at 30 DAS, followed by T4. However, there was no significant difference between any treatment for both years and in pooled analysis. There was no statistically significant difference in plant height between the PROM and KROM groups and the T1 treated check groups at either 60 or 90 DAS, which is consistent with the findings shown at 30 DAS (Table 4.2).

T5, which included 14 Recommended NK, 14 Recommended P, and ALL RECOMMENDED P from PROM, resulted in the tallest plants, whereas treatments T1 (14 Recommended NPK) and T3 (14 Recommended NK, 12 Recommended P, and HALF RECOMMENDED P from PROM) had similar results. Similarly, Bairwa et al. (2019) found that the recommended doses of NPK (T2), NK (T3), and PROM (T4) resulted in significantly taller plants than the absolute control (T1), as did the RDs of NK (T3) and PROM (T4), RDs of NK (T6) and PROM (T7) and PROM (T8).

Table 4.2: Height of plants at 30, 60, and 90 DAS as a result of using PROM, KROM,and a combination of the two

		Plantheight(cm)	
IKEAIWENIS	30DAS	60DAS	90DAS

	YEA	YEA	Pool	YEA	YEA	Pool	YEA	YEA	Pool
	R-I	R-II	ed	R-I	R-II	ed	R-I	R-II	ed
T1(RDF)	59	64	62	196	199	198	204	207	206
T2(100%RDNK+50%RDP+ 50%RDP byPROM)	58	63	60	192	197	195	204	206	205
T3(100%RDNP+50%RDK+ 50%RDKbyKROM)	57	63	60	191	195	193	202	204	203
T4(100%RDN+50%RDPK+50%RDPbyP ROM+50%RDK byKROM)	59	63	61	193	199	196	206	207	207
T5(100%RDN+50%RDPK+ 50% RDPbyRockphosphateandKbyfeldspar+N ativePGPF mixture1 lit / ha+5 t FYM)	56	62	59	190	195	192	201	203	202
S.Em.±	3.37	3.16	2.31	8.05	10.43	6.59	11.40	11.34	8.04
C.D.at5%	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV%	11.6	10.1	10.9	8.34	10.7	9.6	11.1	11.0	11.1

Table 4.3: Chlorophyll content at 30, 60, and 90 DAS as affected by PROM, KROM,

and their combined preparation

	Chlorophyll(µg/cm ²)									
TREATMENTS	30DAS			60DAS			90DAS			
	YEA R-I	YEA R-II	Poole d	YEA R-I	YEA R-II	Poole d	YEA R-I	YEA R-II	Poole d	
T1(RDF)	55.79	54.33	55.06	63.40	57.18	60.29	54.15	52.61	53.38	
T2(100%RDNK +50%RDP + 50%RDP byPROM)	54.87	52.53	53.70	62.91	56.17	59.54	52.30	50.24	51.27	
T3(100%RDNP +50%RDK+ 50%RDKbyKROM)	54.60	53.43	54.01	60.80	55.74	58.27	52.23	50.02	51.12	
T4 (100%RDN+50%RDPK + 50% RDP by PROM + 50% RDKbyKROM)	55.43	54.03	54.73	62.15	57.03	59.59	53.25	51.80	52.52	

T5 (100%RDN+50%RDPK + 50%RDP byRock phosphate and	54.58	53.13	53.85	59.43	52.29	55.86	52.20	50.88	51.54
Kbyfeldspar+NativePGPFmixt									
ure1 lit / ha+5t FYM)									
S.Em.±	1.48	1.78	1.16	1.77	1.39	1.13	1.86	1.35	1.15
C.D.at5%	NS								
CV%	5.40	6.67	6.05	5.73	5.02	5.42	7.06	5.29	6.26

The chlorophyll content of the leaves was measured at 30, 60, and 90 DAS using a spade metre, and while T1 and T4 had the highest chlorophyll content, there were no statistically significant differences between the treatments, indicating that the treatment was having no effect on chlorophyll content. Since there was no discernible difference between the treatments, it's possible that nitrogen, which has a disproportionately large effect on leaf chlorophyll, was the culprit.

The height of the plants in each treatment's plot was recorded. There was no statistically significant difference in plant density between years one and two, or between the two years in the pooled analysis (Table 4.4).

	No.ofplants/plot(Net plot)				
TREATMENTS	YEAR-I	YEAR-II	Pooled		
T1(RDF)	76.3	77.5	76.9		
T2(100%RDNK +50%RDP + 50%RDP	75.5	77.0	76.3		
byPROM)					
T3(100%RDNP +50% RDK+50%RDK	74.0	77.3	75.6		
byKROM)					
T4 (100%RDN+50%RDPK+50%RDP	76.8	79.3	78.0		
byPROM+50% RDKbyKROM)	70.0	17.5	70.0		
T5(100% RDN+50% RDPK +50% RDP					
by Rock phosphate and K by feldspar	72.3	76.8	74.5		
+NativePGPFmixture1lit/ha+5tFYM)					

Table 4.4: Each plant on its own plot

S.Em.±	1.56	2.04	1.29
C.D.at5%	NS	NS	NS
CV%	4.16	5.27	4.77

Measurements of 100 seeds from each treatment revealed that T4 had the greatest test weight, followed by T1, but that treatment differences were not statistically significant (Table 4.5).

	Testweight(g)			
TREATMENTS	YEAR-I	YEAR-II	Pooled	
T1(RDF)	24.97	22.53	23.75	
T2(100%RDNK+50%RDP+50%RDP byPROM)	24.93	22.18	23.55	
T3(100%RDNP+50%RDK+50%RDK byKROM)	24.87	21.86	23.37	
T4(100%RDN+50%RDPK+50%RDP byPROM+50% RDKbyKROM)	25.27	23.09	24.18	
T5(100%RDN+50%RDPK+50%RDP byRockphosphateandKbyfeldspar+NativePGPFmi xture1lit/ha+5 tFYM)	24.75	21.57	23.16	
S.Em.±	0.33	0.37	0.25	
C.D.at5%	NS	NS	NS	
CV%	2.76	3.35	3.04	

Table 4.5: Test weight

There was no statistically significant difference between the two groups in the first year of the research, however in the second year, T2 had the greatest P content (0.048%) and T4 had the lowest (0.047%). The pooled analysis revealed that T2 (0.046%) and T4 (0.044%) both had the highest P. Since the experiment was repeated on the same plots, the increased plant P might be the consequence of the treatment's residual impact.

Table 4.6: Plant P

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TREATMENTS	PlantP(%)		
	YEAR-I	YEAR-II	Pooled
T1(RDF)	0.040	0.044	0.042
T2(100%RDNK +50%RDP + 50%RDP byPROM)	0.045	0.048	0.046
T3(100%RDNP +50% RDK+50%RDK byKROM)	0.041	0.042	0.041
T4 (100%RDN+50%RDPK + 50%RDP byPROM+50% RDKbyKROM)	0.042	0.047	0.044
T5(100% RDN+50%RDPK +50%RDP by Rock phosphate and K by feldspar +NativePGPFmixture1lit/ha+5tFYM)	0.039	0.040	0.039
S.Em.±	0.001	0.001	0.0009
C.D.at5%	NS	0.004	0.001
CV%	5.73	7.19	6.55

Grain production in T4 was significantly greater than in T1 and T2 in all three years (6095 kg/ha, 6305 kg/ha, and 6200 kg/ha, respectively; Tale 4.7). The associative impact of organic preparation of given PROM, KROM with fungus, which released nutrients and continually delivered to crop for a longer time up to grain formation, may account for the better yield in the research.

Table 4.7: Crop production

TREATMENTS	Grain yield(kg/ha)		
	YEAR-I	YEAR-II	Pooled
T1(RDF)	5336	5618	5477
T2(100%RDNK +50%RDP + 50%RDP byPROM)	5242	5543	5393
T3(100%RDNP+50%RDK+50%RDK byKROM)	4959	5281	5120

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T4 (100%RDN+50%RDPK +50%RDP byPROM+50% RDKbyKROM)	6095	6305	6200
T5 (100%RDN+50%RDPK +50%RDP			
by Rock phosphate and K by feldspar	4656	4858	4757
+NativePGPFmixture1lit/ha+5tFYM)			
S.Em.±	296.2	266.8	199.3
C.D.at5%	912.8	822.0	581.8
CV%	11.27	9.67	10.46

5. Conclusion

The effectiveness of organic mineral manures high in phosphorus and potassium on maize (Zea mays L., GAYMH-3) was tested in this experiment. The Agronomy farm (both organic and conventional) and the RRS farm at Anand Agricultural University provided soil samples for the study. The 28 fungal cultures were selected for their capacity to grow and soluble mineral P and K on solid medium. Seed germination, the seedling vigour index, and the percent discolouration index were used to further screen isolates for pathogenicity. Based on their superior seed germination and seedling vigour with less PDI and greater P and K solubilizing ability according to FCO, T-3 and T-17 isolates were selected for the further plant growth stimulating investigations.

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