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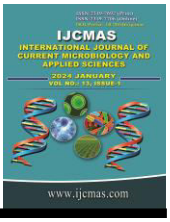

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Study on Enzyme Activities and Nutrient Availability in Calcareous Soil as Influence by Phytase and FYM Levels under Soybean Cultivation

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ABSTRACT

Study on Enzyme Activities and Nutrient Availability in Calcareous Soil as Influence by Phytase and FYM Levels under Soybean Cultivation" was conducted during *Kharif* 2016 at Division of Soil Science, College of Agriculture, Pune. The treatment consisted of four levels of each phytase (0, 1200, 2400 and 3600 IU) and FYM (0, 2.5, 5, 7.5 t ha^{-1}) replicated thrice in factorial randomized block design. Enzyme activities and nutrient availability in soil was assessed and 50 % flowering and harvest of soybean. Application of phytase @ 3600 IU or 7.5 t ha^{-1} FYM recorded significantly higher acid phosphatase, alkaline phosphatase and dehydrogenase activity at 50 % flowering and harvest of soybean. Significant increase in soil available phosphorus in calcareous soil at both the growth stages of soybean over initial was observed with the application of phytase and FYM in all the treatments. Application of 3600 IU phytase and 7.5 t ha^{-1} FYM recorded higher soil available nitrogen (245.65 and 180.08 kg ha^{-1}) and phosphorus (16.08 and 13.34 kg ha^{-1}) at 50 % flowering and at harvest. However, phytase application @ 2400 IU (252.62 and 243.36 kg ha^{-1}) and FYM application either @ 7.5 or 5 t ha^{-1} (253.96 and 239.72 kg ha^{-1}) were found effective for soil available potassium at both growth stages. Application of phytase @ 3600 IU recorded reduction magnitude from 13.31 to 12.43 % at 50 % flowering and from 12.29 to 10.93 % at harvest was obtained with the amendment of phytase. Similar trend was also recorded for 7.5 t ha^{-1} FYM. The magnitude of reduction in $CaCO_3$ content was noticed very less in no phytase and no FYM at 50% flowering 14.06 per cent and at harvest 12.60 per cent.

Keywords

Soybean, phytase, calcareous soil, soil enzymes, nutrient availability

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Introduction

Phosphorus is least mobile and is unavailable to plants in most soil conditions. The phosphorus content in average soil is about 0.05 % (W/W) but only 0.1% of the total phosphorus is available to plants because of its low solubility and its fixation in soil (Scheffer and Schachtschabel, 1992). Phosphorus is added in soil through application of chemical fertilizers but major part of it gets fixed in the soil, and only 15-20 % is utilized by the crop (Wani, 1980 and Gaur, 1985). Major limitation of adequate phosphorus supply to plant is phosphorus fixation. In neutral to alkaline calcareous soil, phosphorus retention is dominated by precipitation reaction of calcium and in acid soil it is fixed with iron and aluminum (Lindsay *et al.,* 1989) or on the surface of clay mineral (Devau *et al.,* 2010).

Soil phosphorus exists both in organic and inorganic form, the organic P compounds can be classified into i) the inositol phosphatase primarily of plant origin comprising up to 60% of soil organic P, ii) the nucleic acids and iii) phospholipids. In soils 20-85 per cent of the total P is in organic form but plants can only utilize this organic P after it's mineralization. The amount of organic P in soil is strongly correlated with total organic carbon and depth of soil.

The importance of soil organic P as a source of plant available P depends on its rate of solubilization and release of inorganic P. Several types of phosphatases like phytases are able to increase the rate of dephosphorylation (hydrolysis of organic P). The rate of N & C mineralization is positively correlated with that of organic P mineralization. The presence of C, N and P in the organic material like FYM with microbial activity, phosphatase and phytase enzyme activity proportionately regulate the mineralization of organic P, N & C. Phosphatase & phytase which catalyzes hydrolytic cleavage of the C-O-P ester bond of organic P present in soil and release plant available inorganic form (HPO_4^{2-} and $H_2PO_4^-$) (Yadav and Tarafdar, 2004). The C:P ratio of an organic manure like FYM determines the extent of which inorganic P is immobilized. Micro-organisms may produce both acid and alkaline phosphatase but plant can only secrete acid phosphatase. P mineralization is an enzymatic process which involves group of phosphstase enzyme catalyzes different soil biochemical reactions & release plant usable P (Tarafdar *et al.,* 1989).

The information about enzymatic mineralization of phosphorous along with other nutrients in calcareous soil for soybean through phytase enzyme in presence of FYM is not available. Hence a pot culture experiment was planned to assess the effect of phytase and FYM levels on soil enzyme activities and nutrient availability of black calcareous soil under soybean crop.

Materials and Methods

A pot culture experiment was conducted at the Division of Soil science and Agriculture Chemistry, College of Agriculture, Pune, during *Kharif* 2016. The experiment consisted of sixteen treatment combinations based on four levels of phytase (0, 1200, 2400 and 3600 IU) and FYM (0, 2.5, 5.0 and 7.5 t ha^{-1}) replicated thrice in Factorial Completely Randomized Design. The enzyme unit was expressed as IU which is the amount of enzyme that liberates 1μmol of inorganic orthophosphate from phytin per minute at pH 5.5 and 37 °C (Zyla *et al.,* 1995). The required quantity of black calcareous soil was collected from Agronomy farm, College of Agriculture, Pune. The quantity of FYM required for the experiment was procured from Division of Animal Husbandry and Diary Science, College of Agriculture, Pune.

The phytase isolated from *Aspergillus niger* was obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune, India. The initial chemical and biochemical properties of soil were analyzed by using standard methods. Forty eight earthen pots with 10 Kg black calcareous soil were used during the experiment. Further another set of sixteen pots were maintained up to 50 % flowering of soybean. Treatment wise required quantity of phyatse and FYM were thoroughly mixed with soil and sowing of soybean carried out. The recommended dose of nitrogen @ 50 kg ha^{-1} was supplied through urea and no phosphorus and potassium given. At 50 % flowering, extra set of 16 pots were broken down carefully without disturbing the soil-root biomass. The same root biomass was shaken to collect root adhered and nearby rhizosphere soil and immediately processed for analysis of enzymes and nutrients. The acid and alkaline phosphatase activity in soil was assessed by using the method advocated by Tabatabai and Bremner (1969). However, activity of soil dehydrogenase was assessed by using methods recommended by Casida *et al.,* (1964). The soil $KMnO_4$-N, Olsen's-P and N *N* NH_4OA-K was analyzed by methods given by Subbia and Asijia (1956); Olsen *et al.,* (1954) and Knudsen *et al.,* (1982) respectively. The soil $CaCO_3$ content was assessed by rapid titration method prescribed by Jackson (1973). The data generated during the course of investigation were tabulated and statistically processed as per Panse and Sukhatme (1962).

Results and Discussion

Soil enzyme activities

Acid phosphatase, alkaline phosphatase and dehydogenase activity in calcareous soil was significantly influenced by phytase and FYM levels imposed to soybean. Application of phytase @ 3600 IU recorded significantly higher acid phosphatase (16.09

and 12.87 µM 'P' g^{-1} soil hr^{-1}), alkaline phosphatase (28.16 and 24.17 µM 'P' g^{-1} soil hr^{-1}) and dehydrogenase (13.74 and 10.56 µg TPF g^{-1} soil hr^{-1}) activity at 50 % flowering and harvest of soybean. (Table 1,2 and 3). In case of FYM, 7.5 t ha^{-1} recorded significantly higher acid phosphatase (15.46 and 11.47 µg TPF g^{-1} soil hr^{-1}), alkaline phosphatase (27.44 and 22.16 µg TPF g^{-1} soil hr^{-1}) and dehydrogenase (12.73 and 9.66 µg TPF g^{-1} soil hr^{-1}) activity at both the growth stages of soybean. Soil acid phosphatase, alkaline phosphatase and dehydrogenase activities were consistently increased with successive levels of phytase. Consistent increase in the activities of soil alkaline phosphatase and dehydrogenase at both the growth stages of soybean was noticed with the successive levels of FYM further, higher activity was recorded with 7.5 t ha^{-1} FYM. However, soil acid phosphatase activity was increased with 5.0 t ha^{-1} FYM. The magnitude of increase in all the enzyme activities were more pronounced with the application of phytase than FYM.

Table.1 Effect of phytase and FYM levels on acid phosphatase activity on calcareous soil under soybean cultivation**.**

a. At 50% flowering of soybean

FYM (F) Phytase (P)	**Acid phosphatase (µM PNP g-1 soil h-1)**				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	10.26	10.59	11.77	10.87	10.87
1200 IU	11.40	12.65	12.95	15.55	13.14
2400 IU	13.09	15.70	15.49	16.49	15.19
3600 IU	13.37	15.00	17.07	18.94	16.09
Mean	12.03	13.48	14.32	15.46	
	P		F	P x F	
S.E. ±	0.151		0.151	0.302	
CD at 5%	0.438		0.438	0.876	

Initial acid phosphatase activity: 9.87 µM 'P' g^{-1} soil hr^{-1}

b. At harvest of soybean

FYM (F) Phytase (P)	**Acid phosphatase (µM PNP g-1 soil h-1)**				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	8.25	8.80	9.55	7.71	8.58
1200 IU	7.85	8.93	8.44	10.76	8.99
2400 IU	9.03	9.22	11.31	12.45	10.50
3600 IU	10.00	11.10	15.42	14.97	12.87
Mean	8.78	9.51	11.18	11.47	
	P		F	P x F	
S.E. ±	0.168		0.168	0.335	
CD at 5%	0.486		0.486	0.973	

The increase in alkaline phosphatase over acid phosphatase may be due to the higher pH of black calcareous soil. Similar results were reported by Kramer and Green (2000); Wang *et al.,* (2011) that activity of acid phosphatase is predominant in acid soils whereas alkaline phosphatase is in alkaline soil. Adnane Bargaz *et al.,* (2012) reported significant increase in acid phosphatase and phytase activity in rhizospheral soil of

Phaseolus vulgaris under low soil phosphorus situations as against soil with sufficient phosphorus. Yadav *et al.,* (2009) reported 15 % increase in acid phosphatase, 12 % increase in alkaline phosphatase, 71% increase in soil phytase activity in soil due to inoculation of *Chaetomium globosum* with organic matter compared to application of only organic matter by conducting an experiment on phosphorus mobilization and yield of cluster bean under arid ecosystem. The activity of acid and alkaline phosphatase was found to correlate with organic matter in various studies (Guan, 1989; Jordan and Kremer, 1994; Aon and Colaneri, 2001). Tarafdar and Rao (1996) studied dehydrogenase and phosphatase activity as influenced by inoculation with *Aspergillus* in rhizosphere and non rhizosphere soils of cluster bean, chickpea and wheat and they concluded higher dehydrogenase, acid phosphatase and alkaline phosphatase activities in rhizospheral soil than non rhizospheral soil.

Table.2 Effect of phytase and FYM levels on alkaline phosphatase activity on calcareous soil under soybean cultivation.

a. At 50% flowering

FYM (F) Phytase (P)	**Alkaline phosphatase (µM PNP g-1 soil h-1)**				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	21.67	23.58	23.76	24.78	23.45
1200 IU	24.83	24.32	25.32	27.09	25.39
2400 IU	26.72	25.50	27.08	28.07	26.84
3600 IU	27.47	27.04	28.33	29.81	28.16
Mean	25.17	25.11	26.12	27.44	
	P	F		P x F	
S.E. ±	0.210	0.210		0.419	
CD at 5%	0.608	0.608		N.S.	

Initial alkaline phosphatase activity: 18.43 µM ‘P’ g^{-1} soil hr^{-1}

b. At harvest

FYM (F) Phytase (P)	**Alkaline phosphatase (µM PNP g-1 soil h-1)**				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	17.05	17.57	18.61	19.40	18.16
1200 IU	18.88	20.85	20.74	20.00	20.11
2400 IU	22.16	23.12	24.56	23.58	23.35
3600 IU	22.80	24.14	24.07	25.68	24.17
Mean	20.22	21.42	21.99	22.16	
	P		F		P x F
S.E. ±	0.301		0.301		0.602
CD at 5%	0.873		0.873		N.S

Nutrient availability

Application of 3600 IU phytase and 7.5 t ha^{-1} FYM recorded higher soil available nitrogen (245.65 and 180.08 kg ha^{-1}) and phosphorus (16.08 and 13.34 kg ha^{-1}) at 50 % flowering and at harvest (Table 4, 5 and 6). Soil available nitrogen in calcareous soil was found reduced over initial (263.42 kg ha^{-1}) at both the stages of analysis (i.e. 50 % flowering and harvest stage of soybean) in all the treatment combinations except application of 3600 IU enzymes along with 7.5 t ha^{-1} FYM (266.78 kg ha^{-1}) at 50% flowering. The magnitude of reduction in soil available nitrogen was more pronounced with no FYM level and lower levels of FYM application at both stages i.e. 50% flowering and harvest.

However, reduction in soil available nitrogen at 50 % flowering and harvest in all treatment combinations might be due to the crop uptake, leaching losses and evaporative losses. The available nitrogen content of soil in all treatment combinations was considerably reduced during 50% flowering (48-52 DAS). Reduction in soil available nitrogen may be due to microbial immobilization, denitrification losses and leaching losses. Similar results were also reported regarding FYM application on lower soil available nitrogen (Suresh and Surya Prabha, 2005).

Table.3 Effect of phytase and FYM levels on dehydrogenase activity on calcareous soil under soybean cultivation.

a. At 50% flowering

FYM (F) Phytase (P)	**Dehydrogenase (µgTPF g^{-1} soil hr^{-1})**				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	8.54	9.61	10.44	10.48	9.76
1200 IU	10.78	11.95	11.66	11.85	11.56
2400 IU	12.91	12.27	12.42	13.40	12.75
3600 IU	12.39	13.25	14.11	15.21	13.74
Mean	11.16	11.77	12.16	12.73	
	P		F		P x F
S.E. ±	0.179		0.179		0.359
CD at 5%	0.521		0.521		1.042

Dehydrogenase (µg TPF g^{-1} soil hr^{-1}) = 6.08

b. At harvest

FYM (F) Phytase (P)	**Dehydrogenase (µgTPF g^{-1} soil hr^{-1})**				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	6.62	7.47	7.74	7.93	7.44
1200 IU	8.33	8.69	9.45	9.56	9.01
2400 IU	9.01	10.42	9.94	9.75	9.78
3600 IU	9.24	10.49	11.13	11.39	10.56
Mean	8.30	9.27	9.56	9.66	
	P		F		P x F
S.E. ±	0.112		0.112		0.225
CD at 5%	0.326		0.326		0.652

Significant increase in soil available phosphorus in calcareous soil at both the growth stages of soybean over initial was observed with the application of phytase and FYM in all the treatments. The combine effect of phytase @ 3600 IU along with 7.5 t ha^{-1} FYM as an interaction effect reported significantly higher soil available phosphorus at 50 % flowering (19.09 kg ha^{-1}) and at harvest (14.90 kg ha^{-1}) of soybean than rest of the treatment combinations. The increase in soil available phosphorus may be due to presence of soil microorganisms which plays a key role in soil phosphorus dynamics and subsequently availability of phosphate to plants. Release of organic anions and production of siderophores and acid phosphatase by plant roots and microbes (Yadaf and Tarafdar, 2001) or alkaline phosphatase (Tarafdar and Claassen, 1988) enzymes hydrolyze the soil organic phosphorus and split phosphorus from organic residues. The largest portion of extracellular phosphatase is derived from the microbial population (Dodor and Tabatabai, 2003).

Vibha *et al.,* (2014), reported significant improvement in soil phosphorus availability under mung bean cultivation in sandy loam soil with inoculation of *Aspergillus niger* + *Penicillium citrinum* and *Aspergillus niger 2* + *Aspergillus niger* 3. Ramesh *et al.,* (2011), observed higher soil available phosphorus due to inoculation of Bacillus isolates.

Table.4 Effect of phytase and FYM levels on available nitrogen on calcareous soil under soybean cultivation.

a. At 50% flowering

FYM (F) Phytase (P)	Available N (Kg ha^{-1})				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	150.08	154.96	162.73	171.74	159.88
1200 IU	184.50	183.78	203.65	225.54	199.37
2400 IU	193.64	213.24	221.60	246.69	218.79
3600 IU	221.60	246.69	247.54	266.78	245.65
Mean	187.45	199.67	208.88	227.69	
	P	F	P x F		
S.E. ±	1.899	1.899	3.798		
CD at 5%	5.512	5.512	11.023		

b. At harvest

FYM (F) Phytase (P)	Available N (Kg ha^{-1})				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	149.61	143.72	151.26	156.52	150.27
1200 IU	157.36	185.14	177.39	182.70	175.65
2400 IU	178.40	172.77	183.40	178.90	178.87
3600 IU	169.81	184.72	185.84	179.94	180.08
Mean	164.30	171.58	174.47	174.51	
	P	F	P x F		
S.E. ±	0.822	0.822	1.643		
CD at 5%	2.384	2.384	4.769		

Table.5 Effect of phytase and FYM levels on available phosphorus on calcareous soil under soybean cultivation.

a. At 50% flowering

FYM (F) Phytase (P)	**Available P (Kg ha^{-1})**				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	10.27	11.63	11.88	12.38	11.54
1200 IU	12.02	11.10	12.21	14.08	12.35
2400 IU	13.37	15.21	15.80	16.26	15.16
3600 IU	13.15	15.85	16.26	19.09	16.08
Mean	12.20	13.44	14.03	15.45	
	P		F		P x F
S.E. ±	0.047		0.047		0.094
CD at 5%	0.136		0.136		0.273

b. At harvest

FYM (F) Phytase (P)	**Available P (Kg ha^{-1})**				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	9.01	9.27	9.42	11.22	9.73
1200 IU	9.12	9.52	10.79	12.28	10.43
2400 IU	10.41	11.27	11.18	14.30	11.79
3600 IU	12.65	13.53	12.29	14.90	13.34
Mean	10.30	10.90	10.92	13.17	
	P		F		P x F
S.E. ±	0.065		0.065		0.130
CD at 5%	0.189		0.189		0.377

Table.6 Effect of phytase and FYM levels on available potassium on calcareous soil under soybean cultivation

a. At 50% flowering

FYM (F) Phytase (P)	**Available K (Kg ha^{-1})**				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	249.37	251.25	251.41	254.13	243.86
1200 IU	239.03	241.15	245.42	249.87	251.54
2400 IU	248.58	249.39	254.19	258.32	252.62
3600 IU	249.43	248.48	256.45	253.54	251.97
Mean	246.60	247.56	251.87	253.96	
	P		F		P x F
S.E. ±	0.708		0.708		1.417
CD at 5%	2.055		2.055		N.S.

b. At harvest

FYM (F) Phytase (P)	**Available K (Kg ha^{-1})**				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	232.83	238.52	236.07	242.16	237.40
1200 IU	244.55	234.54	238.89	240.24	240.55
2400 IU	243.12	246.20	245.11	239.00	243.36
3600 IU	233.82	234.77	238.80	235.89	235.82
Mean	238.58	238.51	239.72	239.32	
	P		F		P x F
S.E. ±	0.497		0.497		0.994
CD at 5%	1.442		N.S.		2.885

Table.7 Effect of phytase and FYM levels on calcium carbonate on calcareous soil under soybean cultivation.

a. At 50% flowering

FYM (F) Phytase (P)	**$CaCO_3$ (%)**				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	14.06	13.33	13.13	12.73	13.31
1200 IU	13.63	13.06	13.33	12.83	13.21
2400 IU	12.56	12.90	12.63	12.00	12.52
3600 IU	12.46	12.63	12.56	12.06	12.43
Mean	13.18	12.98	12.91	12.40	
	P		F		P x F
S.E. ±	0.049		0.049		0.098
CD at 5%	0.143		0.143		0.286

b. At harvest

FYM (F) Phytase (P)	**$CaCO_3$ (%)**				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	12.60	11.40	12.40	10.63	11.75
1200 IU	11.93	12.73	11.26	11.46	11.85
2400 IU	12.16	11.33	10.86	10.93	11.32
3600 IU	11.66	12.40	10.53	10.20	11.20
Mean	12.09	11.96	11.26	10.80	
	P		F		P x F
S.E. ±	0.152		0.152		0.304
CD at 5%	0.442		0.442		0.884

The increase in phosphatase and phytase activity with inoculation of Bacillus isolates might be due to phosphorus mobilization and acquisition by plants. This is in consonance with the study revealing that inoculation of Aspergillus strains significantly improved phosphorus uptake by plants and extractable phosphorus status in soil. Similar results were reported by Tarafdar and Rao (1996). P availability is frequently greater in manured soils and with the addition of humic substances in lime-rich soil (Leytem and Mikkelsen, 2005). In case of soil available potassium, application of phytase @ 2400 IU (252.62 and 243.36 kg ha^{-1}) and FYM application either @ 7.5 or 5 t ha^{-1} (253.96 and 239.72 kg ha^{-1}) were found effective at both growth stages (Table 7). Reduction in soil potassium was recorded in all the treatment combinations over initial. Lower potassium availability was noticed in calcareous soil where no phytase enzyme was applied at 50 % flowering (243.86 kg ha^{-1}). Basal dose of FYM application for soybean in calcareous soil did not affect significantly for potassium availability in soil.

The $CaCO_3$ content in soil was decreased significantly in all the treatment combinations of FYM and phytase imposed to soybean. Reduction trend obtained for calcium carbonate content from 13.31 to 12.43 % at 50 % flowering and from 12.29 to 10.93 % at harvest was obtained with the amendment of phytase @ 3600 IU. Similar trend was also recorded for 7.5 t ha^{-1} FYM. The magnitude of reduction in $CaCO_3$ content was noticed very less in no phytase and no FYM at 50% flowering 14.06 per cent and at harvest 12.60 per cent. (Table 7).

The decrease in $CaCO_3$ content may be attributed to dissolution of $CaCO_3$ content with organic acids released during decomposition of organic matter added to soil (Zheng *et al.,* 2005). Anning Guo *et al.,* (2019) reported that higher microbial population in soil decrease $CaCO_3$ content due to secretion of organic acid and respiration of CO_2 leading to formation of carbonic acid. The reduction in $CaCO_3$ might also be due to phytate acting as a chelating agent and having the ability to hydrolyze the phosphorus from calcium salts and clay as inorganic PO_4. Similar results were also obtained by Gujar *et al.,* (2013) due to application of phytase.

Author Contribution

A. B. Jadhav: Investigation, formal analysis, writing—original draft. Barbie Taggu: Validation, methodology, writing—reviewing. Rahul Suradhkar:—Formal analysis, writing—review and editing. A. V. Patil: Investigation, writing—reviewing. J. M. Khire: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

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Ethical Approval: Not applicable.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

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