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Response of Soil Biological Functions to Different Row Ratios and Bio Stimulants in a Wheat Chickpea Intercropping System

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ABSTRACT

Background: Soil is a crucial natural resource and its health has been deteriorating at an increased pace. The application of biostimulants in different cropping systems could improve soil health by increasing the soil buffering capacity while also encouraging the proliferation of beneficial soil microbes. The soil microbial activity provides an indicative response to plant growth and yield while promoting soil health.

Methods: The experiment was performed in a spilt plot design with 3 replications and 16 treatment combinations. The main plots consist of M1: sole wheat, M2: sole chickpea, M3: wheat: chickpea (2:1), M4: wheat: chickpea (4:1) and subplot treatments includes S0: control, S1: Seaweed extract, S2: Humic and fulvic acid and S3: Amino acids.

Result: The main plot treatments received a boost in the microbial activities through foliar application of biostimulants with sole chickpea (M2) receiving a remarkable increase in SMBC (245.72, 269.09, 263.61 µg g^{-1}), DHA (12.71, 24.00, 19.46 µg TPF g^{-1} soil day^{-1}) and UEA (1.30, 4.64, 4.05 µg urea N g^{-1} soil min^{-1}) respectively at 45 DAS, 90 DAS and at harvest. Also, the seaweed extract treatment (S1) enumerated the highest SMBC (252.22, 276.27, 269.26), DHA (15.13, 25.97, 20.82) and UEA (1.32, 4.58, 4.24) respectively at 45 DAS, 90 DAS and at harvest. The other biostimulant treatments S2 (humic and fulvic acid) and S3 (amino acids) also performed well in comparison to the control. This study realises the importance of biostimulants for enhancing soil biological properties for better soil health.

Key words: Biostimulants, Dehydrogenase enzyme activity, Intercropping, Seaweed extract, Soil microbial biomass carbon, Urease enzyme activity.

INTRODUCTION

Wheat (*Triticum aestivum*), is one of the most important cereal crops in the world and it is essential to global agriculture since it supports millions of farmers and ensures food security. Another important crop is chickpea (*Cicer arietinum*), which is a highly significant legume and grown extensively in temperate, tropical and subtropical climates. Chickpea is a resilient crop to environmental stresses such as drought due to its deep root system, which enhances soil structure and water infiltration (Kumar *et al.,* 2019).

Cropping system plays a crucial role in soil biological properties. Cereal crops are exhaustive in nature as compared to the legume crops. For maximizing productivity and improving soil properties, optimum combination of crops in a proper ratio is a requisite. So, intercropping maximizes resource utilization, enhances biodiversity and improves soil health (Gupta *et al.,* 2025). Among intercropping systems, wheat (*Triticum aestivum)* and chickpea (*Cicer arietinum*) are highly compatible and mutually beneficial. It ensures efficient use of sunlight, water and nutrients while improving overall productivity (Banik *et al.,* 2006). The benefit of combining two crop species is more likely to occur when each of the components have distinct morphologies and growth cycles (Chandranath *et al*., 2020).

In agricultural systems, cropping pattern, management techniques and crop types all have an impact on soil microbial biomass carbon (SMBC). Soil microbial activity and the activity of other important enzymes help in growth and yield of crop (Naikoo *et al.,* 2025). SMBC is the living component of soil organic matter and it is a significant indicator of soil health, fertility and microbial activity (Yang *et al.,* 2024). According to Liu *et al.* (2024), intercropping systems like wheat and chickpea have substantial impact on microbial biomass due to increase in soil microorganisms. Additionally, Dehydrogenase enzyme activity (DHA) is a reliable

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biochemical indicator of soil biological activity and a crucial marker of microbial activity in cropping systems (Hao *et al.,* 2024). Intercropping systems can improve soil quality and crop yield by increasing microbial diversity and functional efficiency, which in turn can enhance dehydrogenase activity (Lai *et al.,* 2022). Similar to this, urease enzyme activity (UEA) is an essential soil enzyme that aids in nitrogen cycling (Raza *et al.,* 2024).

The fortification of crops with biostimulants could impart positive response to their development as well as amplify its effects in the soil system (Kocira *et al.,* 2018). Biostimulants, such as seaweed extracts and humic acids promote plant physiological processes, leading to improved yield and soil health (Du Jardin, 2015). Application of humic and fulvic acids induces root stimulation in wheat crop and further increase the biodiversity of soil microorganisms (Bezuglova *et al.,* 2017). When integrated with wheat-chickpea intercropping, it can amplify these benefits. This study explores the potential of wheat and chickpea intercropping supplemented with biostimulants to optimize soil health and sustainability. This approach could address sustainability and environmental conservation.

MATERIALS AND METHODS

Site description and experimental design

This study was conducted at Agronomy research farm, LPU, Phagwara during *rabi* season of 2022-2023. Co-ordinates of the study area (Fig 1) are 31°14′39.10″N and 75°41′51.67″ E. The experiment was implemented in the split plot design with three replications. Main plot consisted of M1: sole wheat, M2: sole chickpea, M3: wheat: chickpea 2:1 and M4: wheat: chickpea 4:1; the sub plot treatments consisted of S0: no biostimulant, S1: seaweed extract, S2: humic acid + fulvic acid and S3: amino acid. The biostimulants were applied through foliar application. Recommended dosage of NPK were also applied. Standard analysis of variance techniques was used to assess the data and mean comparisons based on the least significant differences test at 0.05 probability were carried out.

Soil sampling method

Soil samples were collected and a portion of it was kept aside in a freezer (44°C) for analysis of soil microbial biomass carbon (SMBC). The rest were airdried, passed through a 2 mm sieve and kept aside for further chemical and enzymatic analyses. Soil samples were collected before sowing (initial sample), at 45 days after sowing (DAS), 90 DAS and at time of harvest. The pH of soil was estimated from a soil: water suspension ratio of 1: 2.5 through the pH meter as given Jackson (1973). The available nitrogen (Av. N) was estimated through a micro-Kjeldahl distillation unit. The available phosphorus (Av. P) was estimated through Bray's method (Bray and Kurtz, 1945) and available potassium (Av. K) in a flame photometer (Table 1).

SMBC and enzymatic activities

The soil microbial biomass carbon (SMBC) was estimated through the chloroform fumigation extraction method (Witt *et al.,* 2000). The dehydrogenase enzyme activity (DHA) was analysed through the TPF reduction method (Casida, 1964). The urease enzyme activity was determined using Tabatabai and Bremner's buffer method (Tabatabai and Bremner, 1972).

RESULTS AND DISCUSSION

Effect of row ratios and foliar biostimulants application on soil microbial biomass carbon

The results indicated that intercropping systems (M3 and M4) exhibited higher SMBC (in µg g^{-1}) than sole wheat (M1) but slightly lower than sole chickpea (M2). At 45 DAS, M3

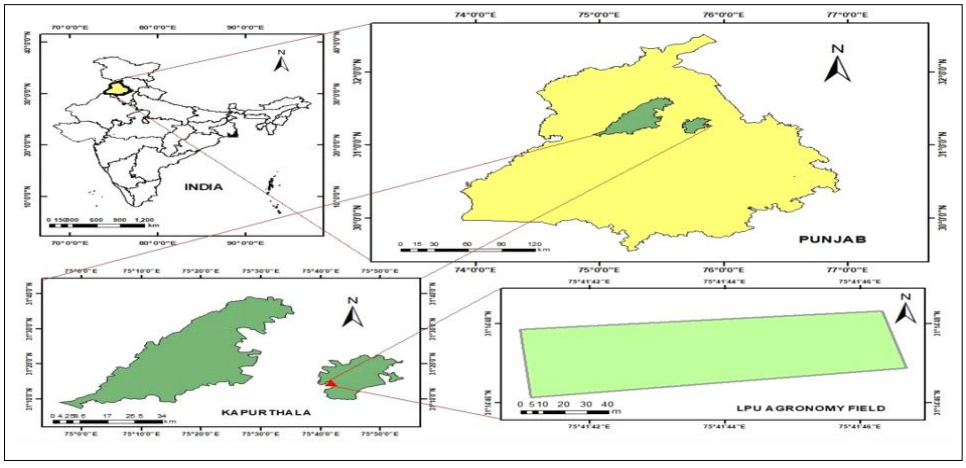


Fig 1: Study area of the experiment.

Table 1: Initial values of soil samples.

Parameters	pH	EC (dS m^{-1})	Av. N (kg ha^{-1})	Av. P (kg ha^{-1})	Av. K (kg ha^{-1})	SMBC (µg g^{-1})	DHA (µg TPF g^{-1} soil day^{-1})	UEA (µg urea N g^{-1} soil min^{-1})
Value (Initial)	8.02	0.42	150.26	11.64	273.75	209.43	11.06	1.1

recorded values of 241.73, while M4 had 236.92 (Table 2). These values were higher than M1 (233.02) but lower than M2 (245.72). At 90 DAS, M2 (269.09) maintained the highest SMBC closely followed by M3 (266.11). M4 (263.92) was subsequently at par with M1 (261.02). A similar trend was observed at harvest, with intercropping treatments maintaining intermediate SMBC levels between the two sole treatments.

The foliar application of different biostimulants developed significant synergistic association with the soil biota at different DAS (45, 90 and at harvest). As such, significant variations were observed in the soil microbial biomass carbon. At 45 DAS, foliar application of seaweed extract (S1: 252.22) dominated the other treatments. It was closely followed by S2 (244.41) Ã S3 (235.25) in comparison to S0 (225.50). A sharp increase in SMBC was observed at 90 DAS which could be explained by the increase in biomass of the root and shoot system. At 90 DAS, the value of S1 (276.85) demonstrated a strong bond between seaweed extract treatment and SMBC (Table 2). The other biostimulants also improved SMBC, with S2 showcasing a value of (270.51) and S3 (264.62) when compared to control S0 (248.75). A slight decline was observed amongst the treatments at harvest. However, there was no significant changes in S0 at 90 DAS and at harvest. This suggests that the inclusion of chickpea in the cropping system improves microbial biomass, likely due to the beneficial rhizospheric interactions and organic matter addition from leguminous root exudates (Liu *et al.,* 2024). Since the biostimulants are hydrophilic colloids, they may have an impact on the physical, chemical and biological characteristics of soil (Van *et al.,* 2017).

The interaction effects (M × S) were also significant, indicating that microbial biomass responded positively to both intercropping and biostimulants application. In all the subsequent days, M2S1, a combination of sole chickpea and seaweed extract retained maximum values (256.87, 280.75 and 276.39) respectively at 45 DAS, 90 DAS and at harvest (Table 3). Similar findings have been reported by Wadduwage *et al*. (2023), emphasizing the role of organic stimulants in enhancing soil microbial activities.

Effect of row ratios and foliar biostimulants application on dehydrogenase enzyme activity

Dehydrogenase enzyme activity (DHA) is an essential parameter reflecting the overall microbial metabolic activity in the soil. The results demonstrated that among the main plot treatments, M2 exhibited the highest DHA (in µg TPF g^{-1} soil day^{-1}), with values of 12.71 at 45 DAS, 24.00 at 90 DAS and 19.46 at harvest. However, M1 exhibited reduced enzyme activity at the corresponding phases, with values of 11.46**,** 20.68 and 17.18 respectively. Dehydrogenase activity was intermediate in M3 and M4. M3 recorded values of 12.24**,** 23.08 and 18.78 respectively at 45 DAS, 90 DAS and harvest. A sharp increase was observed in the enzymatic activities at 90 DAS (Table 2). The DHA for M4 at 45 DAS, 90 DAS and harvest showed a similar pattern, with values of 11.85, 21.86 and 17.96 respectively. Since M4 contains less chickpea, there may be less nitrogen fixation and organic carbon inputs, which may explain the slightly decreased enzyme activity in M4 compared to M3. This suggested that the presence of legumes enhances microbial enzymatic activity due to the higher nitrogen fixation potential and organic matter decomposition (Gayan *et al.,* 2023).

In comparison to the other biostimulants, the seaweed extract treatment S1 remarkably maximized DHA at 45 DAS, 90 DAS and at harvest (15.13, 25.97 and 20.82 respectively). S0 showed the lowest results (Table 2). These findings align with the study of Chen *et al.* (2020), indicating that seaweed extracts can enhance microbial enzyme activities

Table 2: Effect of different row ratios and biostimulants on the soil biological properties.

Treatments	SMBC (µg g^{-1})			DHA (µg TPF g^{-1} soil day^{-1})			UEA (µg urea N g^{-1} soil min^{-1})		
	45 DAS	90 DAS	At harvest	45 DAS	90 DAS	At harvest	45 DAS	90 DAS	At harvest
M1: Sole wheat	233.02	261.02	252.25	11.46	20.68	17.18	1.14	2.91	2.73
M2: Sole chickpea	245.72	269.09	263.61	12.71	24.00	19.46	1.30	4.64	4.05
M3: Wheat : Chickpea (2:1)	241.73	266.11	260.01	12.24	23.08	18.78	1.22	4.15	3.90
M4: Wheat : Chickpea (4:1)	236.92	263.92	254.97	11.85	21.86	17.96	1.26	3.97	3.65
SEd (±)	0.31	0.31	0.24	0.04	0.13	0.05	0.01	0.01	0.01
C.D (P =0.05)	0.76	0.75	0.59	0.09	0.31	0.11	0.02	0.03	0.03
Sub-plots									
S0: No biostimulant	225.50	248.75	246.52	9.01	18.44	16.28	1.08	2.62	2.41
S1: Seaweed extract	252.22	276.27	269.26	15.13	25.97	20.82	1.32	4.58	4.24
S2: Humic acid + Fulvic acid	244.41	270.51	262.54	13.08	23.74	18.87	1.29	4.35	3.96
S3: Amino acid	235.25	264.62	252.53	11.03	21.45	17.42	1.24	4.11	3.71
SEd (±)	0.53	0.51	0.64	0.05	0.13	0.12	0.01	0.02	0.01
C.D (P =0.05)	1.10	1.05	1.32	0.10	0.27	0.25	0.01	0.04	0.03
Interaction (M × S)									
SEd (±)	0.92	0.88	1.11	0.09	0.22	0.21	0.01	0.04	0.02
C.D (P =0.05)	1.90	1.88	2.29	0.18	0.46	0.43	0.02	0.07	0.04

Table 3: Interaction of different row ratios and biostimulants on SMBC at increasing number of days after sowing.

Treatments	Soil microbial biomass carbon (µg g^{-1})														
	45 DAS					90 DAS					At harvest				
Sub plots → Main plots ↓	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean
M1	223.20	247.97	236.17	224.72	233.02	245.91	272.15	265.17	260.86	261.02	244.04	262.14	257.83	245.00	252.25
M2	227.37	256.87	253.04	245.58	245.72	256.59	280.75	273.53	265.50	269.09	246.43	276.39	270.29	261.37	263.62
M3	226.60	252.71	249.42	238.20	241.73	247.08	277.05	273.42	266.91	266.11	248.79	270.30	263.40	257.57	260.02
M4	224.82	251.31	239.02	232.51	236.92	245.43	275.12	269.91	265.22	263.92	246.81	268.23	258.66	246.18	254.97
Mean	225.50	252.22	244.41	235.25		248.75	276.27	270.51	264.62		246.52	269.26	262.54	252.53	
M at the same		SEd (±)		CD (P =0.05)			SEd (±)		CD (P =0.05)			SEd (±)		CD (P =0.05)	
level of S		0.92		1.90			0.88		1.82			1.11		2.29	
S at the same or different levels of M		0.66		1.80			0.62		1.36			0.74		1.56	

Table 4: Interaction of different row ratios and biostimulants on DHA at increasing number of days after sowing.

Treatments	Dehydrogenase enzyme activity (µg TPF g^{-1} soil day^{-1})														
	45 DAS					90 DAS					At harvest				
Sub plots → Main plots ↓	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean
M1	8.13	14.55	12.79	10.35	11.46	17.55	23.62	21.20	20.33	20.68	16.23	19.01	16.99	16.50	17.18
M2	9.85	15.60	13.59	11.78	12.71	19.21	28.26	26.77	21.74	24.00	16.28	22.56	20.48	18.54	19.46
M3	9.48	15.32	12.74	11.42	12.24	18.59	26.79	24.29	22.65	23.08	16.91	20.93	19.14	18.16	18.78
M4	8.56	15.05	13.19	10.58	11.85	18.43	25.19	22.71	21.10	21.86	15.70	20.79	18.87	16.49	17.96
Mean	9.01	15.13	13.08	11.03		18.44	25.97	23.74	21.45		16.28	20.82	18.87	17.42	
M at the same		SEd (±)		CD (P =0.05)			SEd (±)		CD (P =0.05)			SEd (±)		CD (P =0.05)	
level of S		0.09		0.18			0.22		0.46			0.21		0.43	
S at the same or different levels of M		0.07		0.14			0.19		0.42			0.14		0.29	

Table 5: Interaction of different row ratios and biostimulants on UEA at increasing number of days after sowing.

Treatments	Urease enzyme activity (µg urea N g^{-1} soil min^{-1})													
	45 DAS					90 DAS					At harvest			
Sub plots → Main plots ↓	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean
M1	1.06	1.22	1.17	1.12	1.14	2.50	3.36	3.07	2.69	2.91	2.35	3.16	2.84	2.58	2.73
M2	1.10	1.42	1.37	1.32	1.30	2.76	5.46	5.23	5.09	4.64	2.46	4.93	4.62	4.25	4.06
M3	1.08	1.32	1.26	1.23	1.22	2.68	4.83	4.64	4.44	4.15	2.44	4.63	4.38	4.17	3.91
M4	1.07	1.33	1.36	1.28	1.26	2.54	4.66	4.46	4.22	3.97	2.44	4.29	4.08	3.85	3.67
Mean	1.08	1.32	1.29	1.24		2.62	4.58	4.35	4.11		2.42	4.25	3.98	3.71	
M at the same level of S		SEd (±)			CD (P =0.05)		SEd (±)			CD (P =0.05)		SEd (±)			CD (P =0.05)
		0.01			0.02		0.04			0.07		0.01			0.03
S at the same or different levels of M		0.01			0.02		0.02			0.05		0.01			0.02

by providing essential bioactive compounds and improving microbial habitat conditions. In addition to having an abundance of microbial biomass, soils supplemented with organic nutrient sources and biostimulants increased the enzyme production, which ultimately contributed to the higher dehydrogenase enzyme activity (Khursheed *et al.,* 2012).

The interaction effect (M × S) was also statistically significant higher in M2S1 (sole chickpea treatment in combination with seaweed extract) at 45 DAS, 90 DAS and at harvest (Table 4), further validating the role of biostimulants in enhancing microbial activity across different cropping systems. This indicates that optimal microbial enzymatic function can be achieved by integrating appropriate intercropping systems with biostimulants (Ocwa *et al.,* 2024). Enzymatic activity in soil is typically correlated with the amount of organic matter present. The dehydrogenase enzyme's activity was directly related to soil organic matter and microbial biomass (Jat *et al.,* 2022).

Effect of row ratios and foliar biostimulants application on urease enzyme activity

The biological characteristics of the soil are the primary determinants of soil quality. The actions of the enzymes are given particular attention (Bhattacharyya *et al.,* 2008). Urease enzyme activity (UEA) is a key indicator of soil nitrogen cycling and availability. The data show that sole chickpea (M2: 1.22, 4.60 and 4.05) at 45 DAS, 90 DAS and at harvest respectively, recorded the highest UEA values (in µg urea N g^{-1} soil min^{-1}), followed by wheat-chickpea (2:1) intercropping (M3). The lowest values were consistently found in sole wheat (M1: 1.14, 2.91, 2.73 at 45 DAS, 90 DAS and at harvest) (Table 2). The transformation and availability of soil nutrient is decided by the increase in soil enzyme activity from crop developing till maturity (Siwik-Ziomek *et al.,* 2019).

Among the biostimulants, seaweed extract (S1: 1.32, 4.58 and 4.24) at 45 DAS, 90 DAS and at harvest respectively, again outperformed the other treatments, with significantly higher UEA values at all stages. The lowest values were recorded in S0 (Table 2). S2 also showed notable improvements compared to S0. A study by McLeod *et al.* (2016) suggested that wheat and maize exhibited a decrease in UEA during the early reproductive period and an increase during the mid-to-late reproductive period with the application of biostimulants which is in tandem with our results.

The interaction effects (M × S) (M2S1: 1.42, 5.46 and 4.93) at 45 DAS, 90 DAS and at harvest, sole chickpea treatment in combination with seaweed extract was recorded significantly higher as compared with other treatments (Table 5), demonstrating that the combined effects of intercropping and biostimulants application play a crucial role in enhancing soil enzymatic functions. These results reinforce the notion that sustainable cropping practices and the use of organic biostimulants can significantly improve soil health and nutrient cycling.

CONCLUSION

Amongst the row ratios, the sole chickpea treatment continually improved the soil biota through introduction of higher SMBC and the other enzymatic activities. The application of biostimulants emanated significant effects on the biological functions of soil. Among the biostimulants, seaweed extract emerged as a potent and natural stimulus for augmenting soil microbes. A significant increase in SMBC, DHA and UEA was observed among the treatments with the application of seaweed extract and it was more pronounced in sole chickpea as compared with intercropped treatments. The other biostimulants *viz*. humic-fulvic acid and amino acids also performed proficiently, establishing a prominent place for enhancing soil microbiome. Optimizing soil microbial community and enzymatic activities is one of the most important mechanisms to improve soil health and agricultural sustainability. Therefore, this study could be a turning point whereby the inclusion of biostimulants in agriculture could potentially develop a more sustainable future.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions.

Informed consent

The experiment was approved by the Lovely Professional University.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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