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RESEARCH ARTICLE
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EXTRACTION OF HUMIC SUBSTANCES FROM PAKISTANI PRETREATED LIGNITE AND THEIR APPLICATIONS AS NUTRIENTS, ZN/MG FERRITE NANOCOMPOSITE AND PGPB FORTIFIED HUMIC SUBSTANCES ON WHEAT (*TRITICUM AESTIVUM L.*) AND RICE (*ORYZA SATIVA L.*) CROPS

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ABSTRACT
Traditional agriculture is the largest sector in the world that produces food. Yet, these agroecosystems are often and closely associated with rural poverty, reflecting the periphery countries' dual emphasis on subsistence farming and commodity production. Consequently, increasing the yield of subsistence crops with socially adapted technology may be a reliable and advantageous strategy. The application of fertilizer treatments was applied in two ways half applied by flood and other half through foliar spray on rice and wheat crops in field experiment. Results were shown positive response to all the treatments because pure humic substances were used as control and saw their effect on crops alternatively micro and macronutrients fortified HS, Zn/Mg Ferrite fortified HS, PGPB fortified HS and mixture of PGPB, Nutrients, and Zn/Mg Ferrite fortified HS. Out of all treatments, the treatment no.5 was shown the best positive response on both rice and wheat crops such as it was proved more effective for greater yield, greater protein and fat contents, greater nutrients amount and showed greater content of multivitamins in samples rather than other treatments and the trend was drawn for treatment as follows; **T5** **(Macro + Micro Nut + PGPB + ZnMg Ferrite + HS)** > **T4** **(PGPB + HS)** > **T3** **(ZnMg Ferrite + HS)** > **T2** **(Macro + Micro Nut + HS)** > **T1** **(HS = Control)**. The instrumental characterization was performed such as, FTIF for functional groups detection, HPLC for multivitamins and bioactive compounds diction, Flame Photometer and Atomic absorptions spectrophotometer for minerals detection, and grain analyser for moisture content, protein, fat detection and Aflatest Fluorometer for aflatoxin detection in samples in which the rice sample showed 5.6 ppb quantity of aflatoxins that range fall within acceptable range as below from 20 ppb and wheat sample showed 7.9 ppb quantity of aflatoxins that range fall within acceptable range as below from 20 ppb. All results were analyzed statistically using software statistix 8.1.

Keywords: Humic substances, Humic acid, Fulvic acid, Zn/ Mg Ferrite nano-composite, Micro and Macronutrients, PGPB, Rice crop, Wheat crop, Vitamins, HPLC, Aflatoxins

1. INTRODUCTION

The rapidly increasing rate of global population, which is assumed to exceed 9.4 billion in 2050 is one of the foremost and major factors causatives to the dire need for the advancement of efficient and effective agricultural tools to alleviate poverty and global hunger (Ndaba et al., 2022).

Among the most prevalent and significant fossil fuels in the world for the production of electricity is coal. The origin, content, and degree of coalification are just a few of the distinctive characteristics that distinguish the many varieties of coal. The low-grade metamorphism and short formation period of brown coal, sometimes referred to as lignite (Akimbekov et al., 2021). Lignite is an energy source as well as a big source of humic substances, accounting for 45% of reserves of low-rank coal globally. Coal-derived humic substances are big polymers of organic compounds that nourish soil microbes, enhancing their activity and diversity (Zykova, et al., 2024). Organic macromolecules with complicated structures include carbonyl, phenolic, hydroxyl, carboxylic, amine, amide, and aliphatic moieties are known as humic substances. Because of their unique chemical characteristics, humic substances can be used in agriculture, biomedicine, industry, and surroundings (Jarukas, et al., 2021). Humic substances increase microbial presence and help create a balanced ecosystem that positively impacts plant health (Turan, et al., 2022). They modify the soil environment, making it less hospitable to soil-borne diseases. The growth of beneficial microbes can suppress pathogens, consequently improving plant resilience. By boosting nutrient efficiency, humic substances reduce the dependency on synthetic fertilizers (Li, et al., 2022). The crop production of plants grown by subsistence farmers who do not participate in genetic breeding programs can be efficiently supported by the foliar application of a humic substance combined with PGPB mixed bio-stimulant (Canellas et al., 2022).

The soil has become deficient in both macro and micronutrients as a result of intensive agricultural practices. This has led to decrease in soil fertility and productivity rate (Dhayal et al., 2023). Continuously and inappropriate application of chemical fertilizers can significantly affect the soil pH, which has adverse impacts on the bacterial diversity and physicochemical properties of soil (Al-Saif et al., 2023). Because of their high pH, calcareous soils have limited macro and micronutrients availability. The excessive consumption of chemical fertilizers in recent decades has created multiple environmental problems all over the world (Rasouli et al., 2022).

Higher humic acid concentrations and micronutrient use resulted in higher plant yields. The detrimental effects of salinity stress were lessened by humic acid and micronutrients. In the case of humic acid 1000 mg/L + zinc spraying (681.15 g m^{-2}), the highest plant production (679.54 g m^{-2}) was associated with the interaction of humic acid 1000 mg/L + iron without salinity stress. Within the control and salinity conditions, the index value was the lowest (335.97 g /L). Humic acid 1000 mg/L + iron had the lowest proline level (0.088 mg gFW^{-1}), while salinity stress had the highest proline content (0.328 mg g FW^{-1}). Thus, humic acid and micronutrient spraying were suggested as ways to increase plant output and tolerance to salt stress (Korani et al., 2023).

Application of HS enhances plant height, spike density/m^2, grain yield, grain weight, protein content and gluten content. HS are used to control the amount of stress hormones that plants release in response to both abiotic and biotic stresses (Tahoun et al., 2022). The genera Pseudomonas, Enterobacter, Bacillus, Acinetobacter, Arthrobacter, Burkholderia, and Paenibacillus are home to the most prevalent plant growth-promoting bacteria (PGPB) (Balasjin et al., 2022). The use of Plant-Growth-Promoting Bacteria (PGPB) to aid in the development of agricultural food crops has grown significantly during the last 15 to 20 years. In addition to providing phytohormones, soluble phosphate, zinc, potassium, and nitrogen, these bacteria are known to strengthen plant protection against diseases (Reed and Glick, 2023).

PGPB improves plant resistance to drought and salinity through a number of mechanisms, such as: a) synthesis of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which promotes plant growth by

cleaving ACC and lowering ethylene in plants; b) improving nutrient availability through solubilization and mineralization; c) osmolyte accumulation through the production of soluble sugars and organic solutes (proline and glycine betaine); d) synthesis of phytohormones (ABA, GA, IAA, and cytokinins); e) generating volatile organic compounds by regulating the expression of genes linked to cell wall synthesis, choline and glycine betaine synthesis, and stomata closure; f) oxidative stress reduction through ROS scavenging mediated by antioxidant enzymes (SOD, CAT, APX, and Gr) and antioxidant metabolites (ascorbate and phenolic compounds); and ion homeostasis by raising K+ and maintaining K+/N+ ratio (Ma et al., 2022).
Several theories explain the variations that occur after HS is applied: composition of the HS may favor certain genera that can use these compounds, suggesting a direct nutritional impact; HS may alter plant metabolism, including root iron homeostasis, and favor particular phyla, such as Actinobacteria belonging to the genus Frigoribacterium, In response to mild stress caused by HS, certain microorganisms may be recruited, triggering plant defense systems. Additionally, HS may have effects that are unrelated to endophytic composition, which could benefit plants in a similar way and alter their microbial communities (Lengrand et al., 2024).

The application of organic compounds like amino acids, humic acids, fulvic acid, salicylic acid, plant extracts, and inorganic compounds like potassium, calcium, silica, zinc, selenium, and boron, as well as irrigation of crops below their full crop water requirements, have all been shown to improve plant tolerance and decrease the negative effects of water stress on plants (Forotaghe, et al., 2021).
Because of their small size, nanoparticles can transport foreign materials into plant cells while shielding them from deterioration. Diffusion, root hairs, stomata, and leaf surface fractures are some of the ways that nanoparticles can reach the plant system (Wang et al., 2023). This study's primary goal was to apply “nutrients, Zn/Mg Ferrite nanocomposite and PGPB fortified humic substances as fertilizers on Wheat and Rice crops” that are more efficient, non-toxic, environmentally friendly, soften and fertilize the soils, and increase yield of crops when compared to existing fertilizers. It is essential to analyze the wheat and rice crops to evaluate their nutritional value, including macronutrients, micronutrients, and bioactive compounds.

2. MATERIALS AND METHODS

2.1. Micro and macronutrients fortified humic substances (HS)

2.1.2. (a) Extraction of humic substances from bacterial pretreated coal

Extraction of humic substances from bacterial pretreated coal was prepared by using the method (Valero et al., 2014; Olawale et al., 2020).

2.1.2. (b) Micro and macronutrients fortified humic substances (HS)

Micro and macronutrients fortified humic substances (HS) complexed fertilizer was prepared by using this method (Ichwan et al., 2022).

2.2. PGPB-fortified humic substances (HS)

Isolation of plant growth promoting bacteria from soil and PGPB-fortified humic substances synthesis

Isolation of bacteria was done according i.e., potassium-solubilizing bacteria (Fatharani and Rahayu, 2018), nitrogen-fixing Azotobacter (Din et al., 2019), Phosphate solubilizing bacteria (Panhwar et al., 2012; Awais et al., 2019).

PGPB and humic substances are auspicious choices in agriculture for reducing the usage of mineral and pesticide fertilizers. PGPB-fortified humic substance fertilizer was prepared by using this method (Olivares et al., 2015; da Silva et al., 2021).

2.3. Magnesium Zinc Ferrite ($MgZnFe_2O_4$) nanocomposite fortified humic substances

2.3.1. (a) Green Synthesis of Magnesium Zinc Ferrite ($MgZnFe_2O_4$) Nanocomposite

Following the method outlined by Taha et al. (2018), the experimental procedure was modified by replacing Ni (Nickel) with Mg (Magnesium) as the metal component. Instead of bovine gelatin, moringa leaves extract was utilized as a natural stabilizing or reducing agent.

{[$(Mg(NO_3)_2 + Zn(NO_3)_2 + Fe(NO_3)_3 + H_2O$] + Moringa leaves extract} → Stir and heat
↓ **For Reaction Process**
add KOH to adjust pH =11 → [Stir + heat] for 5h allow ppts to age → ppt formed after 5h
↓**For the Drying and Calcination Process**
placed in microwave for 120 min at 120 °C for drying → Dried ppt washed at pH 7.00

2.3.1. (b) Magnesium Zinc Ferrite ($MgZnFe_2O_4$) nanocomposite fortified HS

The co-precipitation method was used to chelate nanocomposite with humic acid. The 300 mg/L of HS solution was prepared and then added 2.043 g of prepared nanocomposite into 50 mL HS solution and stirred for 48 h at 25 °C. Then precipitates were obtained by centrifuging the solution for 08 min at 600 rpm (Sable et al., 2022; Al-Hayani and Sallume, 2023).

2.4. Applications of micro and macronutrients, Zn/Mg Ferrite nanocomposite and PGPB fortified humic substances on Wheat and Rice crops

The field experiments were conducted at Shaheed Mahfouz Garrison Cantt Lahore, Punjab Province of Pakistan on two crops wheat and rice during the three consecutive years 2022 to 2024 (**Table: 1**).

Table: 1. Treatment of HS amendments as fertilizers

Treatments		Composition													
		Macronutrients (kg ha^{-1})				Micronutrients (kg ha^{-1})						Bio-stimulant (L ha^{-1})			
		For K	For P and N			For Mg, Zn, Fe, Cu and S				For Mn	For B	Liq HA	Liq FA	PGPB	ZnMg Ferrite
		KOH	$Ca_3(PO_4)_2$	DAP	Urea	$MgSO_4$	$ZnSO_4$	$FeSO_4$	$CuSO_4$	MnH_4PO_4	H_3BO_3				
T1	HS as Control	0	0	0	0	0	0	0	0	0	0	24.7	25	0	0
T2	Macro + Micro Nut + HS	4.94	4.94	4.94	9.88	1.85	1.85	1.85	1.2	1.24	1.85	24.7	25	0	0
T3	ZnMg Ferrite + HS	0	0	0	0	0	0	0	0	0	0	24.7	25	0	1
T4	PGPB + HS	0	0	0	0	0	0	0	0	0	0	24.7	25	1	0
T5	Macro + Micro Nut + PGPB + ZnMg Ferrite + HS	4.94	4.94	4.94	9.88	1.85	1.85	1.85	1.2	1.24	1.85	24.7	25	1	1

2.4.1. Soil preparation

In this study, the soil was mixed and passed through a 2mm sieve before being analysed for physiochemical characteristics. The soil was calcareous lithosol with a loamy texture and pH of 8.5. To make the soil soft and suitable for seed sowing, the field was ploughed three to four times. After that, all fertilizer treatments were applied through the flood, and after one week, both garlic varieties' cloves were sowed in the field.

Micro and macronutrients, Zn/Mg Ferrite nanocomposite and PGPB fortified humic substances were synthesized at PCSIR Lahore which were used as bio-stimulant active ingredients, and macro and micronutrients were purchased from Annar Kali Bazar Abkari Road Lahore (**Fig. 1**).

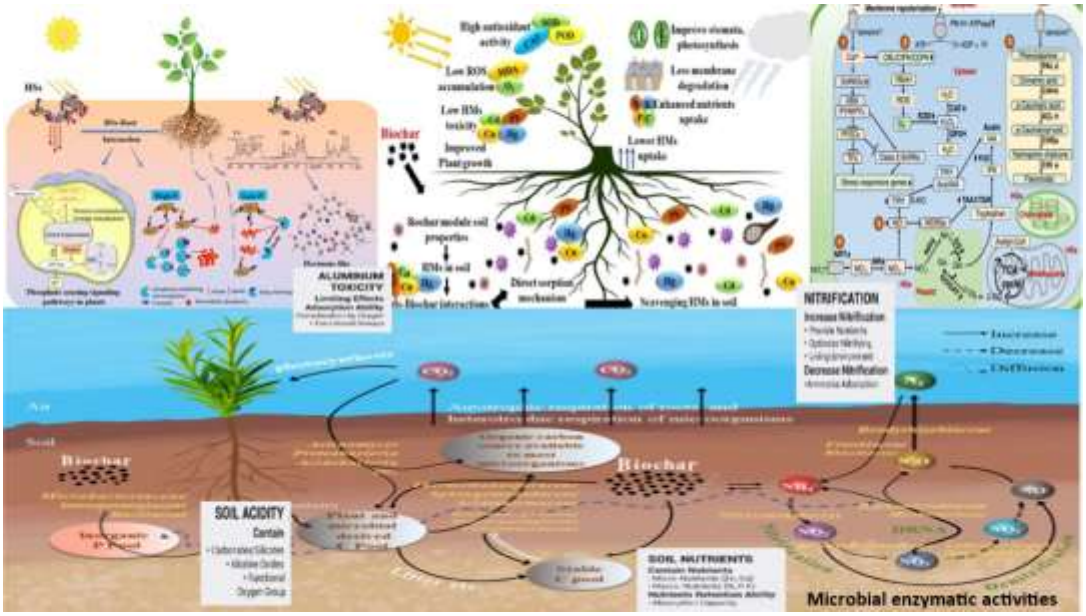


Fig. 1 The impact of fertilizer-amendments on the conceivable processes of trace element mobility in the rhizosphere: ion flow interactions and elemental sequestration (Hasnain et al., 2023).

The applications of all treatment fertilizers (made of seven equal parts) were conducted in two ways firstly, treatments were applied through flooding directly to the soil to enhance nutrient availability and promote root development, secondly, treatment fertilizers were applied through foliar sprays during critical growth stages, such as vegetative growth, to improve nutrient absorption at seven distinct periods (**Table: 2)**.

Table: 2 Pattern of treatment applications

Treatments Application	Wheat Crop (2022-2024)	Rice Crop (2022-2024)
1st application of fertilizer treatments was after ploughing as flooded with water in the field	treatments were applied after ploughing as flooded with water in the field before sowing the wheat seeds	treatment was applied before planting the laab of rice
2nd application was after 1 week	treatments were applied after 1 week from sowing the seeds in the soil as a flood with water	treatments were applied on paddy rice as a flood with water
3rd application was after one month	treatments were applied after one month of sowing as a flood with water	Application was after one month of plantation of paddy rice as a foliar spray under sunlight during day time.
4th time treatments were applied after 2nd month	treatments were applied after 2nd month of sowing as foliar application under sunlight	treatments were applied 2nd month of plantation of paddy rice as a foliar spray under sunlight during day time.
5th application was after 3rd month	treatments were applied after 3rd month as half flood with water and half foliar spray under sunlight	treatments were applied after 3rd month as half flood with water and half foliar spray under sunlight
6th treatments were applied after 3rd and half month	treatments were applied after 3rd and half month as half flood with water and half foliar spray under sunlight and	treatments were applied after 3rd and half month as half flood with water and half foliar spray under sunlight and
7th treatments were applied after 4th month	treatments were applied after 4th month as a flood with water.	treatments were applied after 4th month as a flood with water.

2.4.1. Characterization for determination of grain quality parameters

(a) UV-Vis Spectrophotometry and FTIR Analysis

UV-Vis and FTIR analysis were used to characterize the samples (Sharif et al., 2022; Zykova et al., 2024).

(b) Protein, Fat and Fiber analysis

Crude protein, fat, and fiber contents of both crops were determined according to the procedure mentioned in the methodology (Thiex, 2009; Nikoogoftar-Sedghi et al., 2024).

(c) Grain Analysis through Grain analyzer

Grain analysis of the samples, was performed according to the methodology (Hilliard and Daynard, 1976; Downey and Byrne 1983).

(d) For Flame Photometer and Atomic Absorption Analysis:

Using a flame photometer and atomic absorption spectrophotometer different metals (Sodium, potassium, calcium, magnesium, manganese, copper, and iron) of soil as well as wheat and rice samples were analysed according to the procedure mentioned in the methodology of (Ahmad et al., 2017: Banerjee and Prasad, 2020).

(e) For HPLC analysis:

In the HPLC analysis, of samples were analysed according to the methodology of (Debska et al., 2010; Zdzieblo and Reuter, 2015; Silveira et al., 2016; Chroho et al., 2022).

(f) For Aflatoxins detection

In the aflatoxin's detection analysis, of samples were analysed according to the methodology of (Kumar et al., 2022; Kousar et al., 2024).

2.5. Statistical analysis of data

Data analysis of wheat and paddy rice yield response to fertilizer treatments were performed by analysis of variance (RCBD) and means of comparison were performed using a Tukey test at a 95% confidence level using Statistix 8.1 software (Xia and Yan, 2011).

3. Results and discussion

3.1. Foliar application effects of all treatments on production and grain quality of wheat and rice crops

The type of treatments used, such as nutrients that improve chlorophyll content, and photosynthesis, and bio-stimulants that improve growth, stress resistance, and nutrient uptake, all impact on both crops production and their grain quality. Foliar and flood fertilizer treatments can enhance grain quality and nutritional content. Results from various treatments on both crops have demonstrated that nutrients and bio-stimulants increase the height of the plants, and stimulate the development of leaf area, which is necessary for photosynthesis as shown in **Tables 3, 4 and 5 (a) and (b)** for rice and wheat crops and in flowsheet diagrams of rice and wheat crops, the step-by-step initial to final stages till harvest of both crops were shown in **Fig. 2. (a)** rice crop and **(b)** wheat crop, the application of the treatments.

(a)

(b)

Fig. 2. Flowsheet diagrams of (a) Rice crop and (b) wheat crop at site where the experiments were carried out

After application of different composition of fertilizers treatments, the results have been drawn such as treatment no.5 showed the best positive response on both rice and wheat crops in which they showed greater yield, greater protein and fat contents, greater nutrients amount and showed greater content of multivitamins in samples rather than other treatments and the trend has been drawn for treatment as follows; **T5** **(Macro + Micro Nut + PGPB + ZnMg Ferrite + HS)** > **T4** **(PGPB + HS)** > **T3** **(ZnMg Ferrite + HS)** > **T2** **(Macro + Micro Nut + HS)** > **T1** **(HS = Control)** as shown in **Tables 3, 4 and 5 (a) and (b)** for rice and wheat crops.

Table: 3. (a) The effect of treatments for three Consecutive Financial Years's mean plant height, plant biomass, and grain yield in Rice crop

Treatments (for FY 2022-2024 of Rice Crop)	**Tukey HSD All-Pairwise Comparisons**			
	Plant Height (cm)	**Plant Biomass (Kg/Hec)**	**Grain Yield (Kg/Hec)**	**No. of Tillers per plant**
T5 **(Macro + Micro Nut + PGPB + ZnMg Ferrite + HS)**	124.86^{A}	21434^{A}	5549.0^{A}	17.373^{A}
T4 **(PGPB + HS)**	117.73^{B}	19287^{B}	5261.7^{B}	16.177^{B}
T3 **(ZnMg Ferrite + HS)**	115.53^{C}	18415^{C}	4967.3^{C}	14.347^{C}
T2 **(Macro + Micro Nut + HS)**	107.67^{D}	17856^{D}	4877.3^{D}	12.253^{D}
T1 **(HS = Control)**	102.23^{E}	16530^{E}	4681.0^{E}	10.143^{E}
Result:	All 5 means at alpha 0.05 are significantly different from one another.			

Table: 3. (b) The effect of treatments for three Consecutive Financial Years's mean plant height, plant biomass, and grain yield in Wheat crop

Treatments (for FY 2022-2024 of Wheat Crop)	**Tukey HSD All-Pairwise Comparisons**			
	Plant Height (cm)	**Plant Biomass (Kg/Hec)**	**Grain Yield (Kg/Hec)**	**No. of Tillers (per m2)**
T5 **(Macro + Micro Nut + PGPB + ZnMg Ferrite + HS)**	107.78^{A}	15849^{A}	5987.3^{A}	565.33^{B}
T4 **(PGPB + HS)**	104.19^{B}	14582^{B}	5674.0^{B}	551.00^{D}
T3 **(ZnMg Ferrite + HS)**	97.82^{C}	14149^{C}	5509.0^{C}	504.00^{E}
T2 **(Macro + Micro Nut + HS)**	97.27^{D}	13959^{D}	5367.3^{D}	571.67^{A}
T1 **(HS = Control)**	96.43^{E}	13148^{E}	5106.7^{E}	555.00^{C}
Result:	All 5 means at alpha 0.05 are significantly different from one another.			

Crude protein, fat, and fiber contents of both crops were determined according to the procedure mentioned in the methodology (Thiex, 2009; Nikoogoftar-Sedghi et al., 2024). The results were shown in **Table 4. (a) and (b)** of rice and wheat samples.

Table: 4. (a) Quality parameters of Rice Crop for protein, fat, and fiber % content

Treatments (for FY 2022-2024 of Rice Crop)	**Tukey HSD All-Pairwise Comparisons**					
	Ash%	**Fat%**	**Fiber**	**Starch%**	**Protein%**	**Moisture%**
T5 **(Macro + Micro Nut + PGPB + ZnMg Ferrite + HS)**	1.1167^{A}	2.9633^{A}	5.8267^{C}	59.953^{B}	15.620^{A}	14.520^{E}
T4 **(PGPB + HS)**	1.0800^{AB}	2.9167^{A}	8.1933^{A}	57.880^{D}	14.960^{C}	14.970^{D}
T3 **(ZnMg Ferrite + HS)**	1.0633^{B}	2.8533^{A}	5.5933^{C}	59.783^{B}	15.137^{BC}	15.573^{C}
T2 **(Macro + Micro Nut + HS)**	1.0600^{B}	$.8300^{AB}$	6.2500^{B}	58.683^{C}	15.303^{B}	15.870^{B}
T1 **(HS = Control)**	0.8600^{C}	2.7000^{B}	3.3267^{D}	62.387^{A}	13.547^{D}	17.180^{A}
Result:	The means are not significantly different from one another					All 5 means are significantly different from one another.

Table: 4. (b) Quality parameters of Wheat Crop for protein, fat, and fiber % content

Treatments (for FY 2022-2024 of Wheat Crop)		**Tukey HSD All-Pairwise Comparisons**					
	Ash%	**Moisture%**	**Starch%**	**Wet Gluten%**	**Fat%**	**Protein%**	**Fiber**
T5 **(Macro + Micro Nut + PGPB + ZnMg Ferrite + HS)**	1.1600^{A}	11.020^{D}	62.707^{A}	27.800^{A}	4.5600^{A}	18.237^{A}	10.607^{A}
T4 **(PGPB + HS)**	1.1500^{A}	11.767^{C}	58.750^{D}	24.350^{B}	3.6200^{CB}	14.653^{B}	10.060^{B}
T3 **(ZnMg Ferrite + HS)**	1.1033^{B}	13.867^{A}	61.667^{C}	24.240^{B}	3.5167^{D}	12.237^{D}	7.610^{C}
T2 **(Macro + Micro Nut + HS)**	1.0467^{C}	13.600^{B}	62.070^{B}	23.633^{C}	3.6800^{B}	13.820^{c}	5.783^{D}
T1 **(HS = Control)**	0.9800^{D}	11.140^{D}	62.653^{A}	22.297^{D}	2.7700^{E}	11.850^{E}	2.203^{E}
Result:	The means are not significantly different from one another				All means are significantly different from one another.		

Using a flame photometer and atomic absorption spectrophotometer different metals (Sodium, potassium, calcium, magnesium, manganese, copper, and iron) of soil as well as wheat and rice samples were analysed according to the procedure mentioned in the methodology of (Ahmad et al., 2017: Banerjee and Prasad, 2020) and results were shown in **Table 5. (a) and (b)** of both rice and wheat samples.

Table: 5. (a) The effect of treatments on nutrients content on rice crop

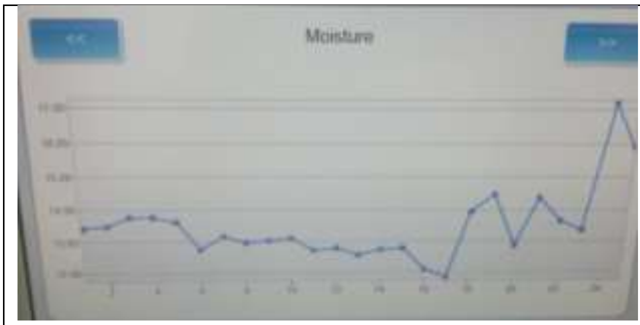
Treatments (for FY 2022-2024 of Rice Crop)	**Tukey HSD All-Pairwise Comparisons**								
	Cu (ppm)	**Mn (ppm)**	**Fe (ppm)**	**Zn (ppm)**	**K (ppm)**	**Na (ppm)**	**Ca (ppm)**	**P (ppm)**	**Mg (ppm)**
T5 (Macro + Micro Nut + PGPB + ZnMg Ferrite + HS)	4.7667 ^{A}	17.333 ^{A}	17.667 ^{A}	17.667 ^{A}	297.67 ^{A}	7.8667 ^{A}	15.667 ^{A}	94.000 ^{A}	191.67 ^{A}
T4 (PGPB + HS)	4.5333 ^{A}	12.667 ^{B}	15.000 ^{AB}	14.667 ^{AB}	254.00 ^{B}	7.0667 ^{B}	14.000 ^{A}	86.333 ^{B}	184.00 ^{B}
T3 (ZnMg Ferrite + HS)	2.4000 ^{B}	14.667 ^{AB}	18.000 ^{A}	16.000 ^{A}	244.33 ^{B}	6.2333 ^{C}	15.333 ^{A}	77.000 ^{C}	172.33 ^{C}
T2 (Macro + Micro Nut + HS)	1.1333 ^{C}	13.667 ^{AB}	15.000 ^{AB}	15.000 ^{AB}	232.67 ^{BC}	5.8000 ^{CD}	13.333 ^{A}	63.667 ^{D}	165.00 ^{D}
T1 (HS = Control)	0.6667 ^{D}	11.000 ^{B}	12.667 ^{B}	12.000 ^{B}	208.33 ^{C}	5.3667 ^{D}	11.667 ^{A}	54.667 ^{E}	154.00 ^{E}
Result:	The means are not significantly different from one another.							All 5 means are significantly different from one another.	

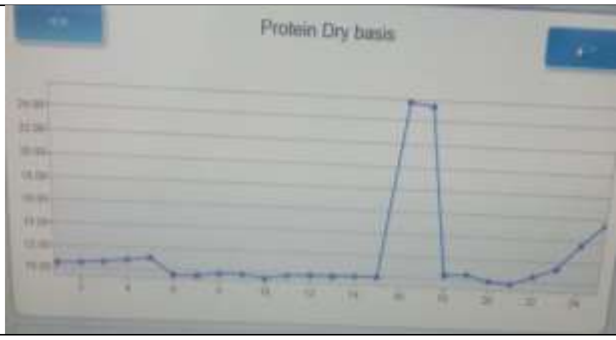
Table: 5. (b) The effect of treatments on nutrients content on wheat crop

Treatments (for FY 2022-2024 of Wheat Crop)	**Tukey HSD All-Pairwise Comparisons**								
	Cu (ppm)	**Fe (ppm)**	**P (ppm)**	**K (ppm)**	**Na (ppm)**	**Mg (ppm)**	**Ca (ppm)**	**Mn (ppm)**	**Zn (ppm)**
T5 (Macro + Micro Nut + PGPB + ZnMg Ferrite + HS)	8.133 ^{A}	51.33 ^{A}	498.67 ^{A}	1343.3 ^{A}	13.96 ^{A}	294.00 ^{A}	126.00 ^{A}	43.66 ^{A}	30.33 ^{A}
T4 (PGPB + HS)	5.366 ^{B}	29.00 ^{B}	414.67 ^{B}	864.3 ^{B}	9.367 ^{B}	216.00 ^{C}	108.00 ^{B}	33.00 ^{B}	107.3 ^{A}
T3 (ZnMg Ferrite + HS)	3.433 ^{C}	33.33 ^{B}	385.6 ^{C}	392.0 ^{C}	5.867 ^{C}	235.33 ^{B}	88.67 ^{C}	25.00 ^{C}	30.00 ^{A}
T2 (Macro + Micro Nut + HS)	2.833 ^{C}	13.33 ^{C}	378.33 ^{CD}	392.0 ^{C}	4.700 ^{C}	177.33 ^{D}	60.33 ^{D}	19.00 ^{D}	13.67 ^{A}
T1 (HS = Control)	00.50 ^{D}	4.533 ^{D}	351.00 ^{D}	359.0 ^{C}	2.700 ^{D}	140.67 ^{E}	35.00 ^{E}	3.867 ^{E}	3.03 ^{A}
Result:	The means are not significantly different from one another.					Means are significantly different from one another.			There are no significant pairwise differences among the means.

3.2. Grain Analysis of Rice and Wheat grains with UV-Visible Spectra using Grain Analyzer Perten Instrument

Grain analysis of the rice and wheat samples, were performed according to the methodology (Hilliard and Daynard, 1976; Downey and Byrne 1983). The results were shown in **Fig. 3 (a) and (b)** of rice and wheat samples.





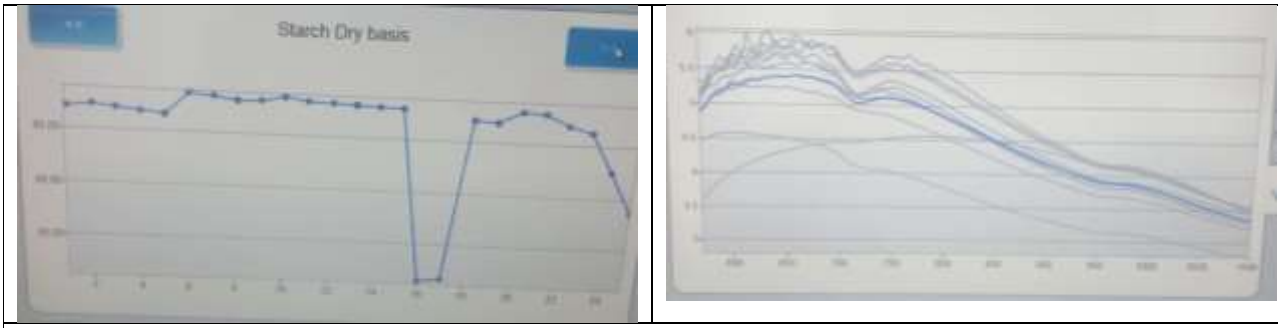


Fig. 3 (a) Results of Moisture %, Starch%, Protein% and UV specta of rice samples (Grain Analyzer-Perten instrument IM 9500)

Fig. 3 (a) Results of Moisture %, Starch%, Protein%, Oil%, Wet Gluten% and UV specta of wheat samples (Grain Analyzer-Perten instrument IM 9500)

3.3. FTIR Analysis of samples

The infrared spectrum is crucial for figuring out the molecules' structural details. The following groups were present in the region of the FTIR spectra: 3666 cm^{-1} of OH phenolic, 3300–3700 cm^{-1} of OH in hydrogen boning form, 3550 cm-1 of NH, 2892–2823 cm^{-1} of CH aliphatic, 2137 cm^{-1} of carbon, 1633 cm^{-1} of C=C in conjugated form, 1019 cm^{-1} of C-O, and 751 cm^{-1} of C-Cl. Coal, HA, and FA spectra are displayed in Fig. 4 (a). The OH group is represented by the broadband at 3430 cm^{-}

[1] in all of these spectra, whereas the presence of CH and CH2 groups is shown by the absorption band around 2900-3300 cm^{-1}. The absorbance band at 1700 cm^{-1} is caused by a carboxylic acid's C=O group. A prominent band between 1500 cm^{-1} and 1650 cm^{-1} represents the C=C group. The stretching of the Si-O and C-O atoms is shown by a few peaks between 1100 cm^{-1} and 1200 cm^{-1}. Since coal is treated with HCl to preserve pH, peaks between 450 cm^{-1} and 600 cm^{-1} showed the presence of the C-Cl group. The -SO3 and -CN stretching vibrations can be attributed to the bands centered on 1234 cm^{-1} and 1080 cm^{-1}, respectively (Saikia et al., 2007; Eshwar et al., 2017; Hansima et al., 2022)
The results of FT-IR characterization as shown in **Fig. 4 (a, b, c, d, e, and f).** These include the aromatic C=C double bond, hydrogen-bonded carbonyl-C=O- at 1670–1530 cm−1; fertilizer minerals, like kaolinite, at 1050–970 cm−1; and Si-O- vibrations at 1330–1480 cm−1; quartz mineral (-Si-O-) vibration at 920–900 cm−1; 1H-atom out-plane deformation vibration on aromatic nucleus at 890–820 cm−1; 2H-atoms out-plane deformation vibration on aromatic nucleus at 810–790 cm−1; 4H-toms out-of-plane deformation vibration on aromatic nucleus at 755–745 cm−1; 710–695 cm−1 is a benzene ring folding vibration; and 595–420 cm−1 is an inorganic mineral (Feng et al., 2024).

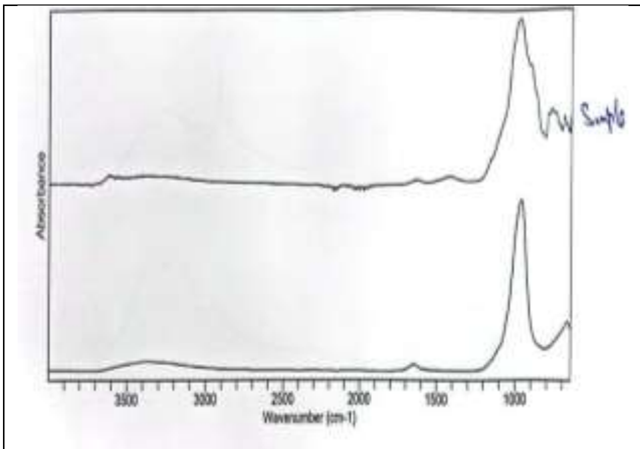


Fig. 4. (a) Soil sample

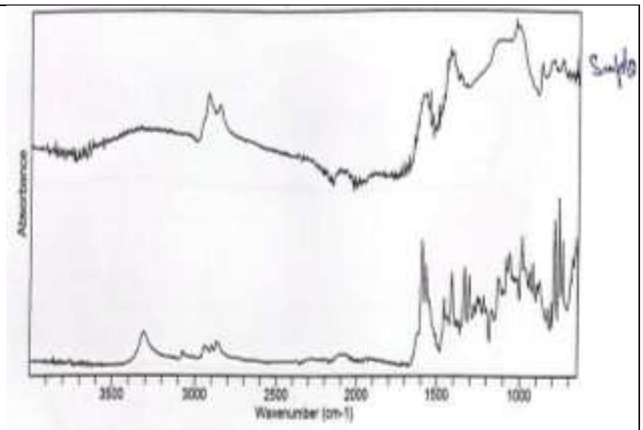


Fig. 4. (b) Natural Coal sample

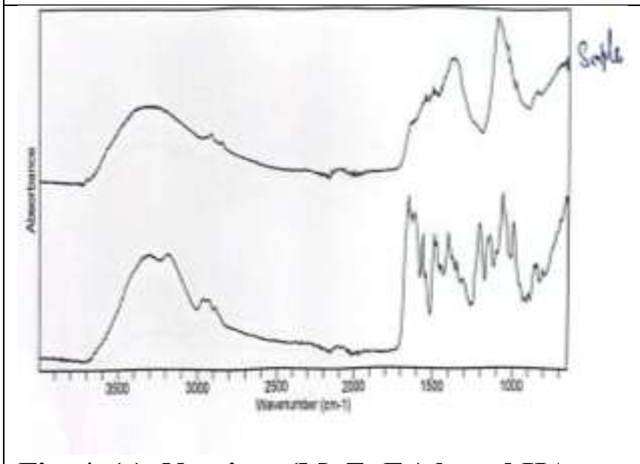


Fig. 4. (c) Nutrient (MgZnFe) based HA

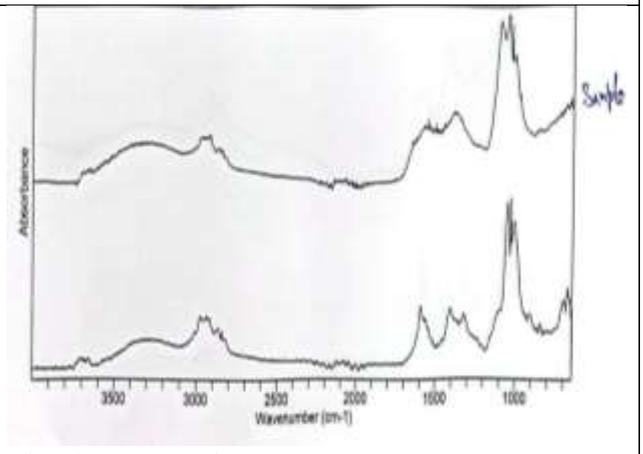


Fig. 4. (d(Nutrient (MgZnFe) based FA

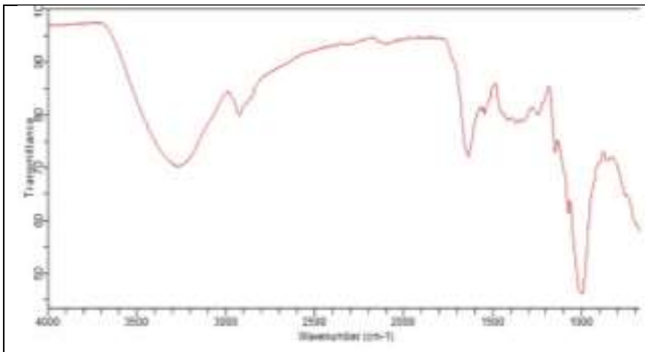


Fig. 4. (e) Wheat sample

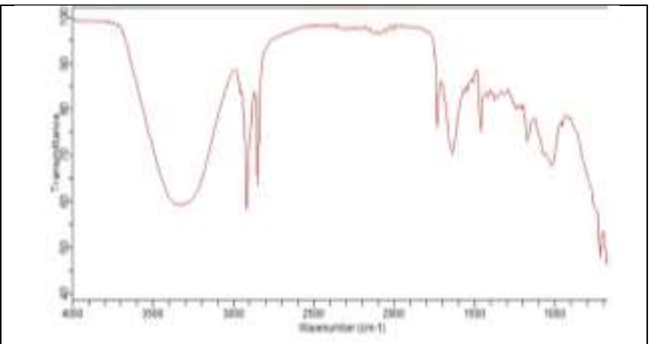


Fig. 4. (f) Rice sample

3.4. HPLC analysis of samples

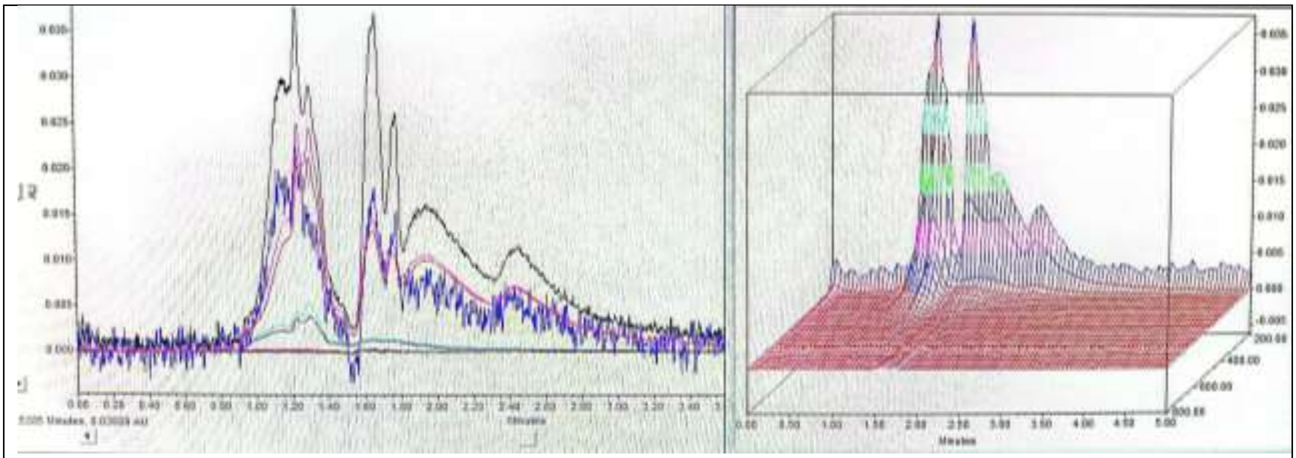
When HS, nanocomposite and biofertilizers are analysed using HPLC (High-Performance Liquid Chromatography), the results usually show how complicated and heterogeneous they are as shown in (**Fig. 5)**. Humic compounds have broad, poorly defined peaks because they are mixes of a wide range of organic components. Their overlapping molecule sizes and polydisperse nature are the causes of this. Because humic substances contain conjugated double bonds, aromatic compounds, and other chromophores, they substantially absorb visible and ultraviolet light, especially in the 200–400 nm range. Due to differences in the components' charge density, hydrophobicity, or molecule sizes, the chromatogram may display a distribution of retention durations (López-Martínez et al., 2021; Ghani et al., 2021; Lu et al., 2022; Stefan-van Staden et al., 2024).

Maximum retention of humic substances occurred at pH 5, specifically when the stationary phase attained positive charges by the vicinal silanol groups. Therefore, it is considered that the positively charged stationary phase interacts through attractive electrostatic forces with the humic substances. Thus, the attractive electrostatic forces are vital in the separation of the humic substances in this case (Debska et al., 2010; Zdzieblo and Reuter, 2015; Wang et al., 2015)

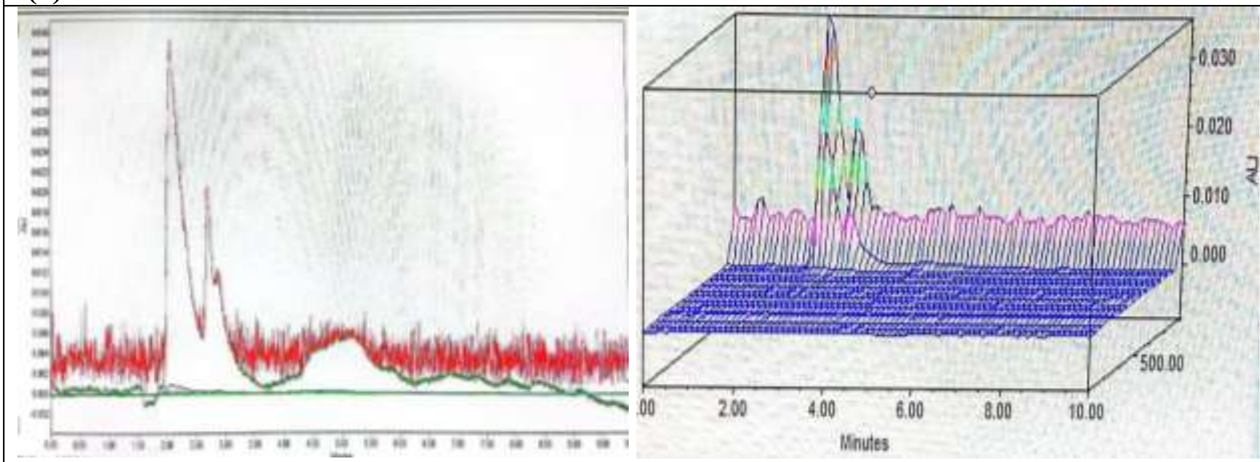
peak A and B fractions had distinct UV–vis absorbance spectra **(Fig. 5 (a, b and c),** possibly indicating their different physical-chemical properties. In addition, the major peak of the isolated HA corresponded to pre-dominant peak A, while that of the isolated FA corresponded to pre-dominant peak B (Wu et al., 2007).

Hydrophilic fractions correspond to peaks within the 2.50–4.60 min range, whereas hydrophobic fractions are within the 12.80–17.60 min range. The greatest proportion of hydrophilic fractions and the lowest proportion of hydrophobic fractions were recorded for HA and FA with nutrients additives. Introducing organic fertilizers into the soil increased the proportion of hydrophobic fractions and decreased in the proportion of hydrophilic fractions (Silveira et al., 2016; Chroho et al., 2022).

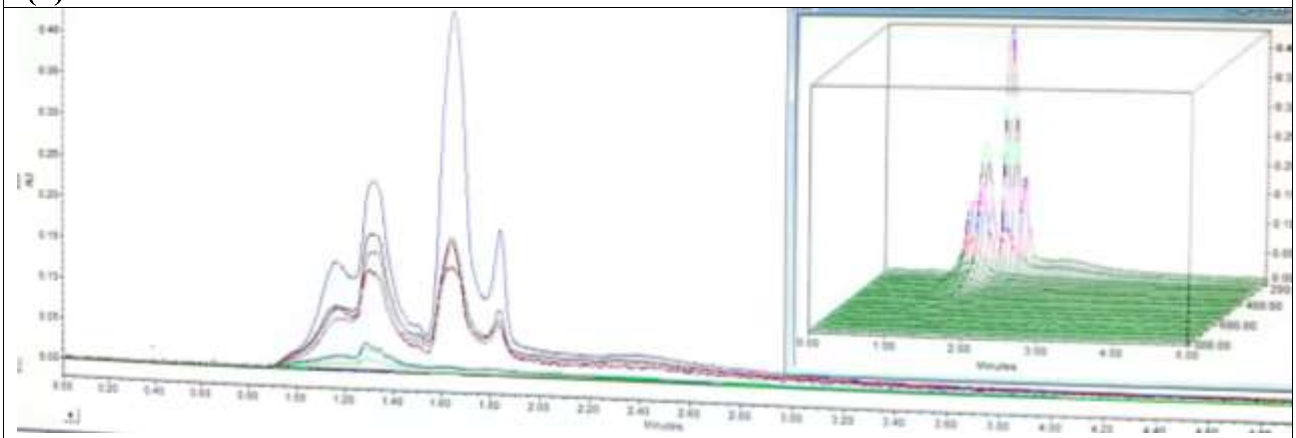
Using the optimized chromatographic conditions, as shown in **Fig. 5 (d, e, f, g, and h)** the HPLC separated multivitamins such as B complex vitamins as well as vitamin C and folic acid in both rice and wheat samples (Zdzieblo and Reuter, 2015**).** Folic acid reference standard peak at Rt 2.802 while in both samples, it peak at Rt 2.749 **(**Silveira et al., 2016).



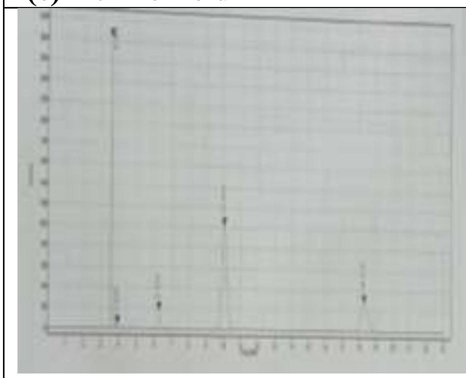
(a) Coal



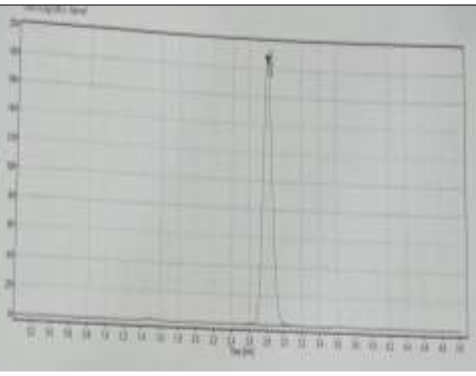
(b) Fulvic acid



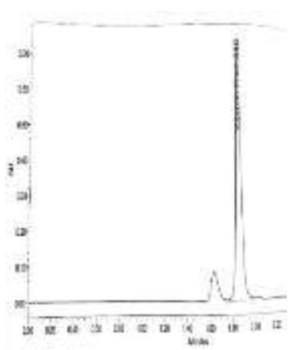
(c) Humic Acid



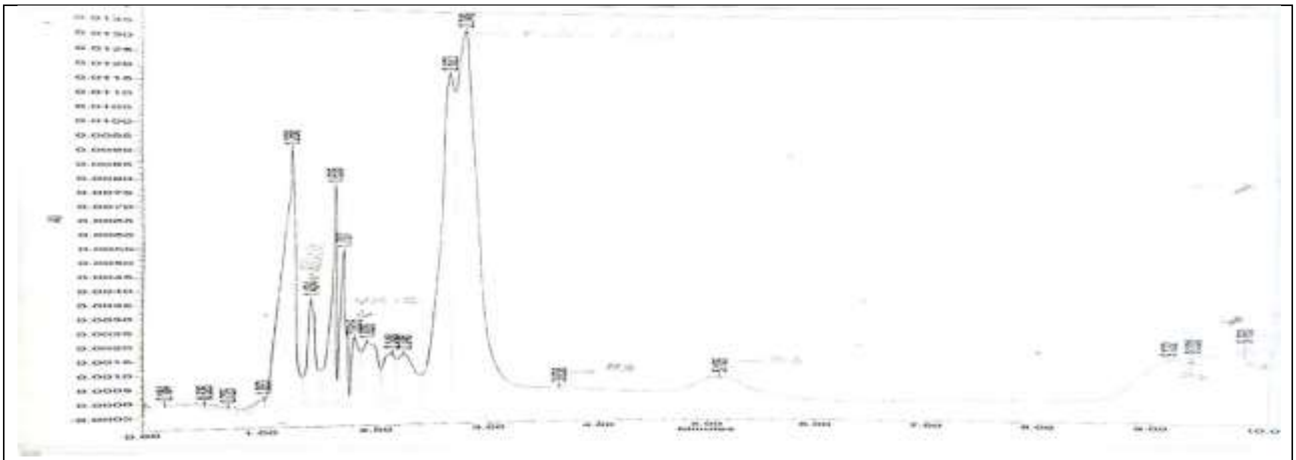
(d) Vitamin B_1, B_2, B_3 and B_6 std



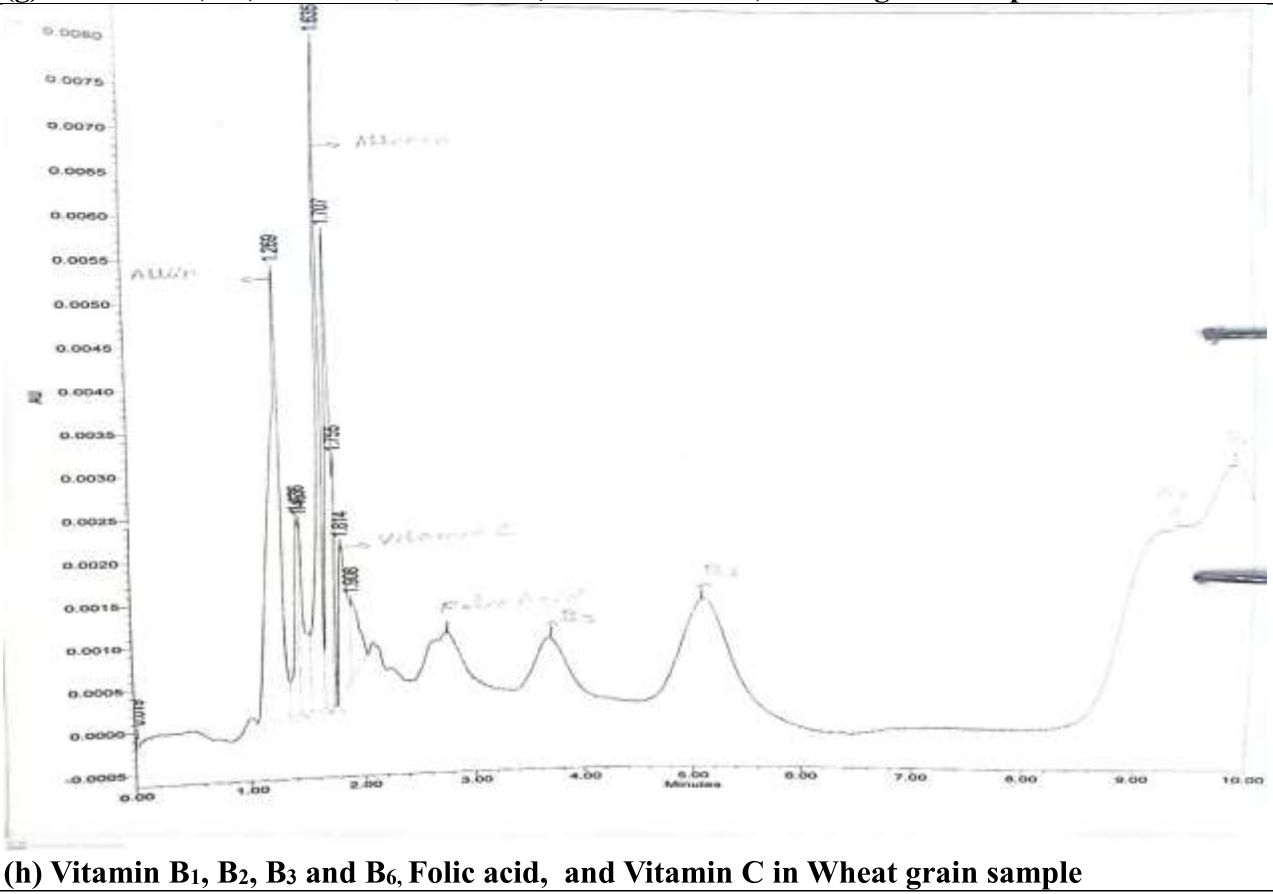
(e) Folic Acid std



(f) Vitamin C std



(g) Vitamin B_1, B_2, B_3 and B_6, Folic acid, and Vitamin C, in Rice grain sample



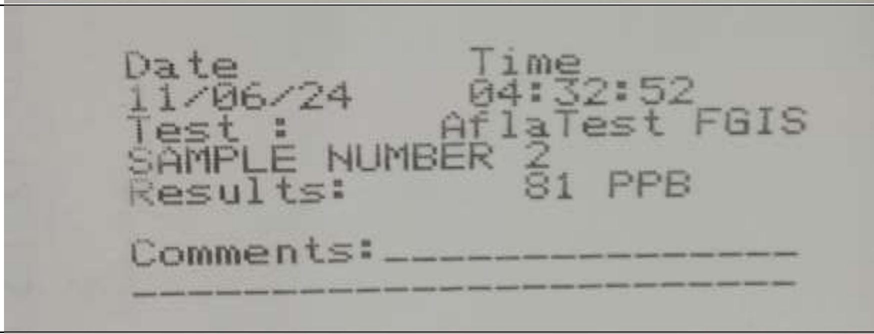
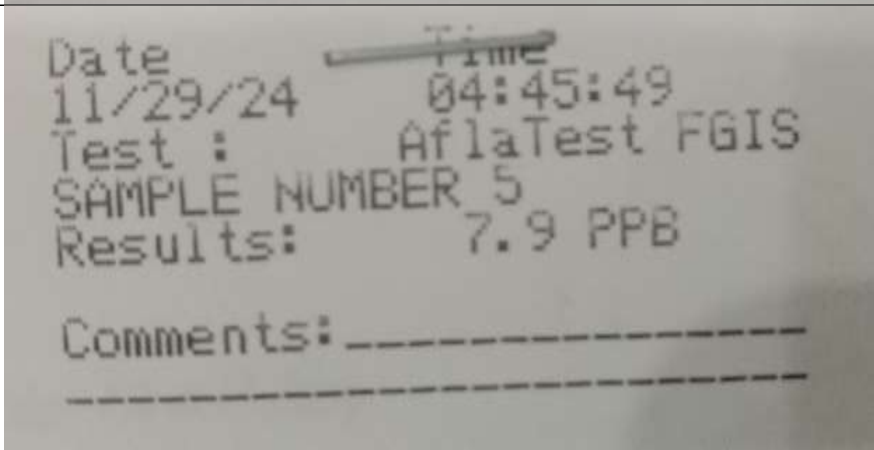
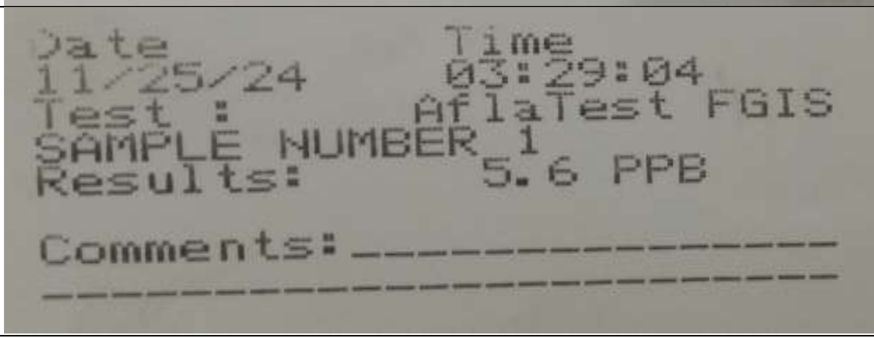
(h) Vitamin B_1, B_2, B_3 and B_6, Folic acid, and Vitamin C in Wheat grain sample

Fig. 5 (a,b,c,d,e,f,g and h) HPLC of soil, coal, nutrints based HS, rice and coal samples for bioactive compounds.

3.5. Aflatoxin Analysis in both Rice and Wheat crops

In the aflatoxin's detection analysis, of samples were analysed according to the methodology of (Kumar et al., 2022; Kousar et al., 2024) on aflatest fluorometer instrument. Results of aflatoxins were shown in **Table: 4** in which the rice sample showed 5.6 ppb quantity of aflatoxins that range fall within acceptable range as below from 20 ppb and wheat sample showed 7.9 ppb f quantity of aflatoxins that range fall within acceptable range as below from 20 ppb and while on the other hand soil has greater quantity of aflatoxins as soil showed results 81 ppb which is exceeds limit from 20 ppb (Kumar et al., 2022; Kousar et al., 2024).

Table: 4. Aflatoxin results of Soil, Rice and Wheat samples

Samples	**AflaTest Fluorometer results**
Blank Sample	 <<< VICAM >>> INSTRUMENT CALIBRATION Date Time 11/06/24 04:30:05 Test : AflaTest FGIS CALIBRATOR BLANK 160 PPB -2.0 PPB
Soil Sample:	 Date Time 11/06/24 04:32:52 Test : AflaTest FGIS SAMPLE NUMBER 2 Results: 81 PPB Comments:
Wheat Grain Sample	 Date Time 11/29/24 04:45:49 Test : AflaTest FGIS SAMPLE NUMBER 5 Results: 7.9 PPB Comments:
Rice grain sample	 Date Time 11/25/24 03:29:04 Test : AflaTest FGIS SAMPLE NUMBER 1 Results: 5.6 PPB Comments:

4. CONCLUSION

The world's largest food production industry is traditional agriculture. However, these agroecosystems are frequently and intimately linked to rural poverty, which reflects the peripheral nations' dual focus on commodity production and subsistence farming. Therefore, using socially adapted technology to increase the output of subsistence crops could be a dependable and beneficial approach. In the field experiment, treatments were given to rice and wheat crops in two ways: half by foliar spray and the other half by flood. Because humic substances were employed as a control and their effects on crops were observed, the results showed a good response to all treatments. Other treatments included micro and macronutrient fortified HS, Zn/Mg Ferrite fortified HS, PGPB fortified HS, and a combination of PGPB, nutrients, and Zn/Mg Ferrite fortified HS. Treatment number five demonstrated the best positive response of all the treatments on both rice and wheat crops. It was found to be more effective in increasing yield, protein and fat contents, nutrient amounts, and multivitamin content in samples compared to other treatments, and a trend was identified for treatment as follows; T5 $_{\text{(Macro + Micro Nut + PGPB + ZnMg Ferrite + HS)}}$ > T4 $_{\text{(PGPB + HS)}}$ > T3 $_{\text{(ZnMg Ferrite + HS)}}$ > T2 $_{\text{(Macro + Micro Nut + HS)}}$ > T1 $_{\text{(HS = Control)}}$. Instrumental characterization was carried out using techniques like FTIF for the detection of functional groups, HPLC for the prediction of multivitamins and bioactive compounds, Flame Photometer and Atomic Absorption Spectrophotometer for the detection of minerals, Grain Analyzer for the detection of moisture content, protein, and fat, and Aflatest Fluorometer for the detection of aflatoxin in samples. The rice sample contained 5.6 ppb of aflatoxins that ranged below 20 ppb in an acceptable range, and the wheat sample contained 7.9 ppb of aflatoxins that ranged below 20 ppb. Software called Statistix 8.1 was used to statistically examine all of the results.

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