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Synergestic Effects of *Azotobacter*, Hairamine and Humic Acid on Plant Growth, Biomass and Grain Yield in Wheat (*Triticum aestivum* L.)

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ABSTRACT

Wheat (*Triticum aestivum* L.) is the most important cereal crop contributing to global food security. Global food security necessitates about 4% increase in grain yield in wheat annually. Bio stimulants offer great promise to increase plant growth, plant biomass, harvest index and hence grain yield. An experiment was conducted over two years using three genetically diverse three wheat varieties and three bio-stimulants; *Azotobacter,* hairamine and humic acid as well as their combinations to ascertain beneficial variety specific effects of bio-stimulants. Experiment was conducted in factorial randomized block design. By *Azotobacter* grain yield improved to 43.27 q/ha which was about 23.34 q/ha higher than control plots (without fertilizers). Likewise, hairamine and humic acid exhibited 46.74 and 56.28 q/ha grain yield, respectively, which was 26.81 and 36.35 q/ha, respectively, higher than control. The combined treatment of hairamine+*Azotobacter* exhibited added response for increase in grain yield which was higher than *Azotobacter* alone (35.48%) as well as hairamine alone (33.31%). Similarly, combination of hairamine and humic acid figured much better than their individual treatment for increase in grain yield (30.95%), respectively. This may be because of combination of high organic carbon in hairamine and humic acid, high organic nitrogen and calcium in hairamine and phytohormone and bioactive compounds in humic acid. This increase in biomass and grain yield could be attributed to higher carbon sequestration particularly in variety DBW-222 showing highest biomass (123.07 q/ha), straw yield (68.11 q/ha) and grain yield (54.96 q/ha) due to cumulative effect of bio-stimulants on plant canopy, chlorophyll content, stability and duration of activity as well as size of stomatal aperture, higher CO_2 capture and higher carbon accumulation in plant and soil.

Key words: *Azatobacter*, bio stimulants, hairamine, humic acid and wheat

INTRODUCTION

The wheat (*Triticum aestivum* L.) probably referred as a staff of life and king of cereals along with staple food for millions people worldwide. It leads in wide spectrum in global agriculture as well as food security. In India, wheat is the second most important cereal crop and contributes significantly to food and nutritional security, particularly in the northern regions of the country. Majority (20%) of the people derive nearly daily caloric intake. Owing to the unique functional properties of gluten, wheat-based products-especially baked goods–constitute a major component of diets in western countries. Nutritionally, wheat is a well-balanced food crop, providing approximately 13% protein, 1.8% lipids and 59.2% starch, along with essential nutrients such as manganese, phosphorus, niacin and B-complex vitamins. India ranks as the third-largest wheat producer globally, recording a production of 112.74 million metric tonnes during 2022–23 from a cultivated area of 31.87 million hectares. Despite this achievement, nutrient availability in wheat-growing soils often remains sub-optimal due to factors such as poor seed quality, water-logging, soil salinity, and inefficient fertilizer management practices. Furthermore, high soil pH and lime content exacerbate nutrient fixation, thereby limiting nutrient uptake by crops and ultimately reducing wheat productivity.

The application of Plant Growth-Promoting Rhizobacteria (PGPR) offers several advantages by increasing soil fertility and promoting plant growth by secreting different advantageous metabolites. PGPR application also enhances drought tolerance in plants through the production of exopolysaccharides and osmolytes, which help to maintain soil moisture, improve root–soil interactions, and protect plants against water stress (Azarmi *et al*., 2019), PGPR also contribute to the protection of plants against a wide range of pathogens by generating hydrolytic enzymes, hydrogen cyanide and antibiotics which suppress pathogen growth and enhance the plant's natural defence mechanisms (Patil *et al*., 2019). Moreover, it induces resistance to plants triggering different jasmonic acid and other pathways (Luh *et al*., 2020).

Humic acid (HA) is a comparatively stable product of organic matter which breakdown and accumulate in ecosystems (Pacuta *et al*., 2021). Humic acid reduces oxidative damage, enhances root water transport capacity, and facilitates the regulation of water movement between cell-to-cell and apoplastic and transport pathways (Quiroga *et al*., 2017).

Seaweed functions as an elevating bio stimulant to boost plant growth which serves soil amendment to increase yield, owing to its rich content of plant growth–promoting compounds. The macro and micronutrients, growth hormones, amino acids, vitamins, betaines, cytokinins and sterols even improve plant vigour, nutrient uptake and overall productivity (Singh *et al*., 2023). It is significant conversion in plant physiological and biochemical processes, leading to improved nutrient uptake and enhanced plant growth. In addition, seaweed improves the physico-chemical and biological properties of soil, thereby creating a more favourable environment for root development and overall crop productivity (Yuanyuan *et al*., 2020).

Hairamin is a novel product derived from hydrolyzed waste human hair and is rich in organic carbon, organic nitrogen, amino acids and amides. Owing to these properties, hairamin functions as an effective plant growth promoter. Foliar application of hairamin in cereals such as bread wheat, triticale and *durum* wheat, as well as in plantation crops like banana and fiber crops such as cotton, has been reported to enhance biomass production, plant height, leaf number and leaf area, ultimately resulting in higher economic yields across these crops.

The intensive application of chemical fertilizers has resulted in multiple environmental concerns, including deterioration of soil fertility and organic matter, decrease water-holding capacity and impaired nutrient mobilization. Therefore, the application of bio stimulants, particularly through foliar sprays, plays a crucial role in improving soil health and enhancing plant growth. Additionally, bio stimulants are increasingly used in cultivation of crop to promote plant development and optimize the efficiency and utilization of plant nutrients (Yakhin *et al*., 2017). The study investigates the ways to lower chemical fertilizers use without sacrificing wheat yield potential (Jag Mohan *et al*., 2024). Therefore, study evaluated the influence of bio stimulants applied through seed dressing and foliar spray on yield related attributes of wheat.

MATERIALS AND METHODS

A field experiment was carried out during season 2023-24 of *rabi* and 2024-25 at the Research Farm of the Department of Agriculture, Maharishi Markandeshwar University, Mullana, Ambala, Haryana. Before sowing, the soil samples were drawn from the soil profile to specify the physico-chemical characteristics of the soil. The soil contained a sandy loam texture, and well-drained, possessing an alkaline reactivity (pH 7.23), respectively. However, soil was found low in nitrogen and medium in phosphorus, with a conductivity of 0.89 d/Sm.

The present study consisted of 11 treatments and three varieties (DBW 222, DBW 303 and WH 1270), the treatments were T_1: Control, T_2: 100% RDF, T_3: Seed treated with *Azotobacter* + RDF, T_4: Humic acid + RDF, T_5: Seaweed extract + RDF, T_6: Organic carbon + RDF, T_7: Hairamin + RDF, T_8: Hairamin + humic acid + RDF, T_9: Hairamin + seaweed extract + RDF, T_{10}: Hairamin + organic carbon + RDF and T_{11}: Hairamin + seed treated with *Azotobacter* + RDF. Observations were taken on number of tillers, peduncle length, number of ears per meter row length, number of spikelets per ear, number of grains per ear, test weight, grain yield, straw yield, biological yield and harvest index. The data were analyzed by using suitable OP STAT.

RESULTS AND DISCUSSION

Wheat variety DBW 303 performed best for all yield attributes (Tables 1 and 2) like numbers of tillers per meter row length (88.35), peduncle length (33.59), number of ears per meter row length (90.85), spikelets per ear (20.85), number of grains per ear (36.43) and 1000 grain weight (37.08).

Table 1. Synergestic effects of *Azotobacter*, hairamine and humic acid on tillers per running meter row length, peduncle length and number of ears per running meter row length

Characters	Tillering per running meter length			Peduncle length			No. of ears/running meter row length		
Treatments	2023-24	2023-24	2023-24	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled
Varieties									
V_1	85.09	27.52	27.52	27.52	28.59	28.05	67.97	68.64	68.30
V_2	83.55	30.28	30.28	30.28	31.36	30.82	69.79	70.46	70.12
V_3	87.64	33.06	33.06	33.06	34.13	33.59	73.06	73.73	73.39
S. E(m)±	1.51	0.73	0.73	0.73	0.76	0.75	1.05	1.08	1.06
C. D. (p=0.05)	NS	2.07	2.07	2.07	2.16	2.11	2.98	3.05	3.01
Factors									
T_1	55.00	7.63	7.63	7.63	8.63	8.13	47.44	48.11	47.78
T_2	69.22	20.02	20.02	20.02	21.02	20.52	61.56	62.22	61.89
T_3	73.78	22.82	22.82	22.82	23.82	23.32	64.01	64.67	64.33
T_4	91.33	34.19	34.19	34.19	35.19	34.69	72.99	73.67	73.33
T_5	87.56	31.98	31.98	31.98	32.98	32.48	70.98	71.67	71.33
T_6	82.22	28.64	28.64	28.64	29.64	29.14	67.99	68.67	68.33
T_7	77.56	25.58	25.58	25.58	26.58	26.08	65.99	66.67	66.33
T_8	107.56	45.07	45.07	45.07	46.89	45.98	85.00	85.67	85.33
T_9	102.11	42.01	42.01	42.01	43.01	42.51	82.07	82.67	82.33
T_{10}	98.44	38.70	38.70	38.70	39.70	39.20	78.99	79.67	79.33
T_{11}	94.89	36.49	36.49	36.49	37.49	36.99	76.00	76.67	76.33
S. E(m)±	2.89	1.40	1.40	1.40	1.46	1.43	2.02	2.06	2.04
C. D. (P=0.05)	8.19	3.96	3.96	3.96	4.14	4.05	5.71	5.84	5.77

Where, wheat varieties V_1: DBW 222, V_2: WH 1270, V_3: DBW 303, treatments T_1: Control, T_2: 100% RDF, T_3: RDF + Seed treated with *Azatobacter*, T_4: RDF+Foliar spray of humic acid, T_5: Foliar spray of sea weed extract, T_6: Foliar spray of organic carbon, T_7: RDF+Foliar spray of Hairamin, T_8: RDF+Combined foliar application of hairamin and humic acid, T_9: RDF + Combined foliar application of hairamin and seaweed extract, T_{10}: RDF+Combined foliar application of hairamin and organic carbon and T_{11}: RDF+Seed treated with *Azatobacter* and foliar spray of hairamin. NS–Not Significant.

Table 2. Synergistic effects of *Azotobacter*, hairamine and humic acid on spikelets/ear, number of grains/ear and 1000-grain weight

Characters	Spikelets/ear			No. of grains/ear			1000-grain weight		
Treatments	2023-24	2023-24	2023-24	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled
Varieties									
V_1	16.99	18.04	17.52	27.77	28.56	28.05	31.92	32.69	32.31
V_2	18.48	19.53	19.00	31.75	32.57	30.82	34.33	35.09	34.71
V_3	20.26	21.35	20.80	36.04	36.83	33.59	36.69	37.46	37.08
S. E(m)±	0.54	0.54	0.54	0.81	0.85	0.75	1.43	1.46	1.45
C. D. (p=0.05)	1.51	1.53	1.52	2.29	2.41	2.11	NS	NS	NS
Factors									
T_1	2.91	3.97	3.44	10.48	11.28	10.88	13.83	14.59	14.21
T_2	10.73	11.79	11.26	20.64	21.44	21.04	25.83	26.59	26.21
T_3	13.46	14.53	14.00	22.18	22.98	22.58	28.23	28.99	28.61
T_4	21.04	22.11	21.58	34.82	35.62	35.22	37.64	38.41	38.02
T_5	20.26	21.33	20.80	32.17	32.97	32.57	35.39	36.16	35.78
T_6	18.57	19.64	19.11	28.18	28.98	28.58	32.96	33.73	33.34
T_7	16.76	17.83	17.30	25.19	25.99	25.59	30.58	31.35	30.96
T_8	27.38	28.45	27.92	49.74	50.54	50.14	47.03	47.79	47.41
T_9	25.35	26.41	25.88	46.30	47.15	46.73	44.38	45.14	44.76
T_{10}	24.73	25.80	25.26	42.34	43.14	42.74	41.93	42.70	42.32
T_{11}	23.11	24.20	23.65	38.32	39.12	38.72	39.67	40.43	40.05
S. E(m)±	1.02	1.04	1.03	1.55	1.63	1.59	2.74	2.80	2.77
C. D. (P=0.05)	2.90	2.93	2.91	4.39	4.62	4.50	7.77	7.93	7.85

Varieties and treatments details are given in Table 1.
NS–Not Significant.

In treatments, the best treatment was T_8: RDF + combined foliar application of hairamin and humic acid with numbers of tillers per meter row length (88.35), peduncle length (45.98), number of ears per meter row length (109.94), spikelets per ear (27.92), number of grains per

Table 3. Synergistic effects of *Azotobacter*, hairamine and humic acid on grain yield (q/ha), straw yield (q/ha), biological yield (q/ha) and harvest index

Characters	Grain yield (q/ha)			Straw yield (q/ha)			Biological yield(q/ha)			Harvest index		
Treatments	2023-24	2023-24	2023-24	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled
Varieties												
V_1	51.34	52.11	51.73	64.34	65.28	28.05	115.68	117.39	116.54	43.83	43.91	43.87
V_2	48.76	49.52	49.14	61.76	62.79	30.82	110.51	112.31	111.41	43.45	43.41	43.43
V_3	54.58	55.35	54.96	67.58	68.64	33.59	122.16	123.99	123.07	44.27	44.30	44.29
S. E(m)±	1.32	1.36	1.34	1.31	1.36	0.75	1.97	2.04	2.00	1.03	1.01	1.02
C. D. (p=0.05)	3.74	2.61	3.80	3.71	3.86	2.11	5.58	5.77	5.67	NS	NS	NS
Factors												
T_1	19.54	20.31	19.93	32.54	33.58	33.06	52.08	53.88	52.98	37.30	37.65	37.48
T_2	39.54	40.31	39.93	52.54	53.58	53.06	92.08	93.88	92.98	42.85	42.84	42.84
T_3	42.88	43.65	43.27	55.88	56.92	56.40	98.76	100.57	99.66	43.31	43.31	43.31
T_4	55.89	56.66	56.28	68.89	69.93	69.41	124.79	126.58	125.68	44.75	44.75	44.75
T_5	52.79	53.56	53.18	65.79	66.83	66.31	118.59	120.39	119.49	44.45	44.43	44.44
T_6	49.42	50.19	49.81	62.42	63.46	62.94	111.84	113.64	112.74	44.13	44.10	44.12
T_7	46.36	47.12	46.74	59.36	60.39	59.87	105.72	107.52	106.62	43.76	43.78	43.77
T_8	69.58	70.35	69.97	82.58	83.73	83.15	152.16	154.08	153.12	45.71	45.65	45.68
T_9	65.47	66.24	65.86	78.47	79.51	78.99	143.95	145.75	144.85	45.47	45.44	45.46
T_{10}	63.49	64.25	63.87	76.49	77.52	77.00	139.97	141.77	140.87	45.34	45.31	45.33
T_{11}	62.16	62.93	62.55	75.16	75.85	75.51	137.33	138.79	138.06	45.25	45.33	45.29
S. E(m)±	2.53	3.86	2.57	2.51	2.61	2.55	3.77	3.90	3.83	1.97	1.93	1.95
C. D. (P=0.05)	7.17	7.40	7.27	7.10	7.38	7.23	10.68	11.04	10.85	NS	NS	NS

Varieties and treatments details are given in Table 1.
NS–Not Significant.

ear (50.14) and 1000-grain weight (47.41).

Among all three varieties, DBW 303 showed the highest in grain yield (69.97 q/ha), straw yield (83.15 q/ha), biological yield (153.12 q/ha) and harvest index (44.29%) followed by DBW 222 and WH 270 which was lowest in grain yield (49.14 q/ha), straw yield (62.27 q/ha) and biological yield (111.41 q/ha).

Different yield parameters like grain yield, straw yield, biological yield and harvest index of wheat were significantly influenced by different split application schedules (Table 3). The highest grain yield, straw yield, biological yield and harvest index were in treatment T_8: RDF + combined foliar application of hairamin and humic acid with grain yield (69.97 q/ha), straw yield (83.15 q/ha) and biological yield (153.12 q/ha). However, this treatment was at par with T_9: RDF + combined foliar application of hairamin and seaweed extract and T_{10}: RDF + combined foliar application of hairamin and organic carbon**.** Darjee *et al*. (2024) also observed similar results by humic acid on wheat yield attributes and indicated the agronomic feasibility, economic viability and environmental sustainability of employing these microbial treatments in crop production systems.

CONCLUSION

Based on the findings, wheat variety DBW 303 demonstrated the highest performance in yield, followed by WH 1270 and lowest in DBW 222 in both the years. Among all treatments, the combination of RDF + combined foliar application of hairamin and humic acid was the most effective followed by RDF + combined foliar application of hairamin and seaweed extract. The results suggested that bio-stimulant application significantly enhanced root development and induced a stay-green phenotype, allowing for a reduction in synthetic nitrogen fertilizer use by up to (33%) without compromising yield or grain protein content. Further research is suggested to fully explore the benefits of these treatments.

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