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Variability among Varieties for Response to Hairamine a Protein Hydrolysate on Stem Strength and Grain Yield Attributes in Wheat (*Triticum aestivum* L.)

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ABSTRACT

Hairamine a protein hydrolysate obtained from human hair is a growth promoter as a biostimulant. A field experiment was conducted at the Research Farm of the Faculty of Agriculture, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala (133207) during the *Rabi* season of 2023-2024 to evaluate the response of three wheat varieties DBW 222, WH 1270 and DBW 303 to Hairamine for its effect on grain yield and its attributes. The experiment was conducted in randomized block design with three replications. Three foliar sprays of Hairamine 4 ml/litre of water were applied at 30, 60, 90 days after sowing. Observations were recorded on 5 randomly selected plants from each plot for plant height, chlorophyll content, nitrogen content, stem girth, stem strength, angle of declination, number of grains per spike, test weight, grain yield and bacterial population. The rhizospheric soil was analysed for microbial colonies in control and Hairamine treated plots. Significant increase in various plant characters and microbial colony number was observed in Hairamine treated plots in all the three varieties. The grain yield and stem strength were enhanced by 14.5% and 25.4%, respectively. Therefore, Hairamine application can be recommended as potential bio stimulant for increasing grain yield and lodging resistance in wheat under climate change.

Keywords: Hairamine, hydrolysate, plant growth promoter, bio-stimulant, lodging

Introduction

Wheat (*Triticum aestivum* L.) is the world's principal and commercially important food crop. It belongs to the grass family Poaceae. It provides 21% of the food calories, 20% of the protein and 55% of the carbohydrates in the diet. In comparison to other cereals, wheat grain has greater protein content (12%) and a fairly good niacin and thiamine content. Global wheat consumption has increased in the past four decades to around 781 million tonnes annually and accounts for approximately 25 per cent of worldwide protein supply. In India, the area under wheat crop is 30.46 million hectares with the production of 112.18 million tonnes (2022- 23). Major wheat-growing states in India are Uttar Pradesh, Punjab, Haryana, Rajasthan, Madhya Pradesh, Gujarat and Bihar. The area under wheat cultivation in Haryana (2022-23) was 2.36 million hectares with the production of 12.0 million tonnes (Indiastat, 2023)

In modern agriculture, along with fungicides, herbicides and insecticides, various products classified as plant growth stimulants are also used (Calvo et al., 2014). In the era of technology, the use of bio-stimulators is very important. The bio-stimulators affect plant productivity by increasing metabolism, accelerating the absorption nutrients and contributing to their redistribution in the plant body. Bio stimulant Hairamine being rich in organic nitrogen, organic carbon, calcium, amides and amino acids, reduces the need of fertilizers and increases plant growth, develops resistance in plants against abiotic stresses. In small concentration, this substance is efficient in favouring good performance of the plants' vital processes and allowing higher yield. In addition, bio stimulants applied to plants enhance nutrients use efficiency, abiotic stress tolerance and plant quality traits. (De Vasconcelos et al., 2019). This bio-stimulant is used in wheat field and it has been shown to have positive effect on grain yield and its attributes (Du Jardin, 2015). Different wheat varieties respond differently to bio-stimulant such as Hairamine. Bio-stimulants contribute to better seed germination and induce biological activity of plants. These products are also safe for the environment and contribute to sustainable, high-output low-input crop productions.

Rhizospheric conditions determine root growth and nutrient uptake which ultimately contribute to plant growth and grain yield in wheat. Rhizospheric condition are in turn determined by colony forming units of favourable rhizobacteria. Root exudates determine the multiplication and growth of rhizobacteria. Bio-stimulants are known to enhance root exudates including carbohydrates, vitamins, amino acids and enzymes, which serve as food for growing rhizobacteria thus bio stimulants are used for in rhizosphere towards better plant growth (Nguyen et al., 2019). This study was conducted to examine the effects of foliar application of Hairamine on plant growth traits, stem strength, flexibility, grain yield, components and rhizosphere activity in three wheat varieties to explore the possibility of infusing lodging resistance and sustainably increases yield potential.

Materials and Methods

A field experiment was conducted during winter (*Rabi*) season of 2023-2024 at research farm of Faculty of Agriculture, Maharishi Markandeshwar (deemed to be University), Mullana, Ambala, Haryana (133207) is situated at 30.243228°N latitude, 77.061692°E longitude, altitude of 264 meters above sea level. The average summer and winter temperature was around 38°C and 12°C, respectively. The soil of experimental site was sandy loam in texture, well-drained, had an alkaline reactivity (pH 7.13), low in nitrogen, medium in phosphorus with a conductivity of 0.89d/Sm. The experiment was conducted in factorial randomised block design with three replication and two factors comprising three wheat varieties "DBW 222 (V_1)", "WH 1270 (V_2)" and "DBW 303 (V_3)" (Factor A) and two treatments including T_1 Control (recommended dose of fertilizers) and T_2 Hairamine (100% recommended N, P, K doses through synthetic fertilizers and Hairamine Foliar Spray 4 ml/litre of water) (Factor B).

The recommended dose of fertilizer was applied as 150 kg/ha Nitrogen (N) through urea + DAP, 60 kg/ha Phosphorus (P) through DAP and 60 kg/ha Potassium (K) through MOP + 25 kg zinc sulphate/ha. Nitrogen fertilizers were applied in split doses i.e., 1/3 of the nitrogen fertilizer along with the full doses of phosphate and potash at the time of sowing; the remaining nitrogen was supplied evenly after the first and second irrigations. Hairamine was applied as foliar spray thrice (4 ml Hairamine per litre water) at 30, 60, 90 days after sowing. The data was recorded on five randomly selected plants from each treatment for different characters *viz.,* chlorophyll content, nitrogen content, stem girth, stem strength, angle of declination, plant height, number of grains/spikes, test weight (1000 grain weight), grain yield. Mean data for each character was statistically analysed for analysis of variance and critical differences among treatments and control using OP STAT® (Sheoran, et al.*,* 1998).

To analyse the population of rhizospheric bacteria, soil samples were collected from two plots, with one serving as a control and the other treated with Hairamine. These soil samples were aseptically taken from a depth of 10-15 cm, then air-dried in the laboratory and sieved through a 2 mm sieve. The bacterial population in the soil was assessed using the serial dilution technique on Nutrient Agar Media (NAM, Himedia). In this method, a soil suspension was prepared by mixing 1.0 g of soil with 9 ml of sterile distilled water. Subsequently, soil suspension was serially diluted from 10^{-1} to 10^{-4} by transferring 1 ml of suspension from one tube to the next (Rajkhowa, et al., 2000). Next, 0.1 ml of soil suspension from the 10^{-2} and 10^{-3} dilutions was aseptically transferred onto Petri plates containing Nutrient agar media, gently spread with a glass spreader, and then incubated at 37°C for 24 hours. Bacterial populations were assessed after 24-48 hours of incubation. Each colony appearing on the plate was considered one colony forming unit (CFU), with the number of colonies formed on the Petri plate divide by its dilution factor to determine the population per gram of soil (Waksman, 1927; Nazir, 2007). This procedure was repeated thrice on the 3rd, 4th, and 5th days following Hairamine foliar spray.

Results

The results obtained from the present investigation for different characters in response to Hairamine and control treatments are summarised in Table 1.

Plant height (cm): The plant height is measured by normal ruler scale at fully grown stage (end of month March) with grains. The plant height (Table 1, Fig. 1) was compared to control and significant increase was observed in plants treated with protein hydrolysate formulation (Kumar, et al., 2023). Among two applied treatments, T_1(control) showed significantly lower plant height in three wheat varieties DBW 222 (92.94 cm), WH 1270 (93.14 cm), DBW 303 (92.94 cm) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher plant height in three wheat varieties DBW 222 (102.47 cm), WH 1270 (99.01 cm) and DBW 303 (102.01 cm) at maturity stage. Variety DBW222 showed highest response (10%) for plant height.

Chlorophyll Content (spad): Among two applied treatments, T_1 (control) showed significantly lower chlorophyll content in flag leaf (Table 1, Fig. 2) in three wheat varieties DBW 222 (37.72 spad), WH 1270 (32.66 spad), DBW 303 (38.53 spad) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher chlorophyll content in flag leaf in three wheat varieties DBW 222 (43.61 spad), WH 1270 (40.99 spad) and DBW 303 (43.46 spad) at maturity stage. Variety DBW 222 showed highest response (15%) for chlorophyll content.

Nitrogen Content (%): Among two applied treatments, T_1(control) significantly lower nitrogen content in flag leaf (Table 1, Fig. 3) was found in three wheat varieties DBW 222 (15.52%), WH 1270 (14.41%), DBW 303 (14.61%) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher nitrogen content in flag leaf in three wheat varieties DBW 222 (16.78%), WH 1270 (17.07%) and DBW 303 (17.63%) at maturity stage. Variety DBW 303 showed highest response (20%) for nitrogen content.

Stem girth (mm): Among two applied treatments, T_1 (control) significantly lower stem girth (Table 1, Fig. 4) was found in three wheat varieties DBW 222 (0.49 mm), WH 1270 (0.45 mm), DBW 303 (0.47 mm) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher stem girth in three wheat varieties DBW 222 (0.53 mm), WH 1270 (0.51 mm) and DBW 303 (0.53 mm) at maturity stage. Variety DBW 222 and DBW 303 showed highest response (8% and 12%) for stem girth.

Stem strength (g): Among two applied treatments, T_1 (control) significantly lower stem strength (Table 1, Fig. 5) was found in three wheat varieties DBW 222 (403.3 g), WH 1270 (375.3 g), DBW 303 (366.6 g) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher stem strength in three wheat varieties DBW 222 (476 g), WH 1270 (484.6 g) and DBW 303 (473.3 g) at maturity stage. Variety WH 1270 showed highest response (29%) for stem strength.

Angle of declination (degree): Among two applied treatments, T_1 (control) showed significantly lower angle of declination (Table 1, Fig. 6) in three wheat varieties DBW 222 (132.3°), WH 1270 (131.6°), DBW 303 (135.3°) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly larger angle of declination in three wheat varieties DBW 222 (144.6°), WH 1270 (143°) and DBW 303 (144.6°) at maturity stage. Variety DBW 222 and DBW 303 showed highest response (9% and 6%) for angle of declination.

Number of grains/spike: Among two applied treatments, T_1 (control) significantly lower number of grains/spike (Table 1, Fig. 7) was found in three wheat varieties DBW 222 (45.4 grains), WH 1270 (45.8 grains), DBW 303 (45.6 grains) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher number of grains/spike three wheat varieties DBW 222 (51.3 grains), WH 1270 (51 grains) and DBW 303 (51.6 grains) at maturity stage. Variety DBW 303 showed highest response (13%) for number of grains/spike.

Test weight (1000 grain weight): Among two applied treatments, T_1 (control) significantly lower test weight (Table 1, Fig. 8) was found in three wheat varieties DBW 222 (25.53 g), WH 1270 (24.93 g), DBW 303 (25.73 g) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher test weight in three wheat varieties DBW 222 (31.43 g), WH 1270 (31.13 g) and DBW 303 (31.73 g) at maturity stage. Variety DBW 303 showed highest response (23%) for test weight.

Grain yield (q/ha): Among two applied treatments, T_1 (control) showed significantly lower grain yield (Table 1, Fig. 9) in three wheat varieties DBW 222 (41.53 q/ha), WH 1270 (40.93 q/ha), DBW 303 (41.73 q/ha) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher grain yield in three wheat varieties DBW 222 (47.43 q/ha), WH 1270 (47.13 q/ha) and DBW 303 (47.73 q/ha) at maturity stage. Variety DBW 303 showed highest response (14%) for grain yield.

Bacterial population: The rhizospheric soil, the area surrounding plant roots, serves a crucial function in promoting plant growth. In our present study, we investigated the colony-forming units (CFU) of bacteria present in the rhizospheric soil of both Hairamine-treated and untreated fields (Fig.10, 11). Our observations revealed that in the treated field, the CFU count was 282 x 10^{-2}, whereas in the control group, it was 125 x 10^{-2}. These results suggest that the application of Hairamine leads to a significant increase in bacterial population around the roots. This augmentation in bacterial presence likely contributes to overall plant growth and subsequent yield enhancement.

Discussion

Comparative evaluation of control (T_1) and foliar application of Hairamine (T_2) treatments, revealed significant positive differences between two treatments in all the three wheat varieties DBW 222, WH 1270, DBW 303 for various parameters of plant growth (plant height, chlorophyll content, nitrogen content), stem sturdiness (stem strength, stem girth, angle of declination) and grain yield parameters (grains/spike, test weight and grain yield).

The utilization of Hairamine as a bio-stimulant in wheat cultivation has demonstrated numerous beneficial effects on various growth and yield parameters. The findings of the study consistently demonstrated the positive impact of Hairamine as bio-stimulant on wheat plants. From an agronomic standpoint, the utilization of Hairamine as bio stimulant offer several advantages. Foliar application of Hairamine resulted in higher chlorophyll content, nitrogen content, stem strength, stem girth and angle of declination depicting stem flexibility.

This suggest that Hairamine can be sustainably used to infuse lodging resistance in wheat which is relevant in inclement weather condition due to climate change. This could be due to constituent profile of Hairamine having high organic carbon, nitrogen, calcium, amides and amino acids.

The enhanced growth parameters observed in treated plants can lead to higher yield, increased weight of 1000 seeds, improved quality and greater economic returns. Such advantages of bio-stimulant application have been reported earlier for Hairamine in wheat (Behl, et al., 2023, Kumar et al., 2023), cotton (Kumar, et al., 2021), banana, (Kumar, et al., 2021),

In conclusion, the foliar application of Hairamine as bio-stimulant in wheat cultivation has the potential to revolutionize the agricultural landscape by promoting sustainable practices and maximizing crop productivity. This ecofriendly approach offers a sustainable alternative to conventional method of farming, promoting soil health, crop productivity and environmental conservation.

Table 1. Effect of foliar application of Hairamine on yield and yield attributes in wheat varieties.

Parameters	V_1T_1	V_1T_2	V_2T_1	V_2T_2	V_3T_1	V_3T_2	**Critical difference**		
							Factor A (Variety)	**Factor B (Treatment)**	**Interaction (A X B)**
Plant height (cm)	92.94	102.47	93.14	99.01	92.94	102.01	0.296	0.241	0.418
Chlorophyll content (spad)	37.72	43.61	32.66	40.99	38.53	43.46	3.171	2.589	NS
Nitrogen content (%)	15.52	16.78	14.41	17.07	14.61	17.63	NS	1.345	NS
Stem girth (mm)	0.49	0.53	0.45	0.51	0.47	0.53	NS	0.022	NS
Stem strength	403.3	476	375.3	484.6	366.6	473.3	NS	49.947	NS
Angle of declination	132.3	144.6	131.6	143	135.3	144.6	NS	2.493	NS
Number of grain/spike	45.4	51.3	44.8	51.0	45.6	51.6	0.032	0.026	0.045
Test weight	25.53	31.43	24.93	31.13	25.73	31.73	0.014	0.011	0.019
Grain yield (q/ha)	41.53	47.43	40.93	47.13	41.73	47.73	0.03	0.025	0.043

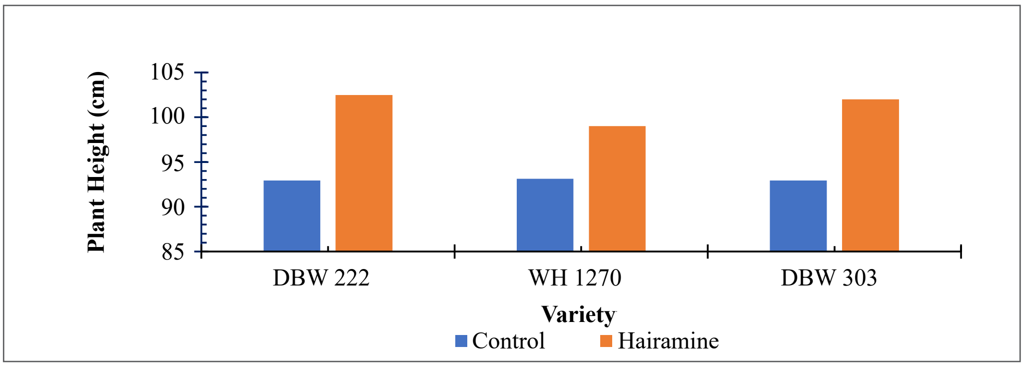

Figure 1. Effect of Hairamine on plant height in wheat

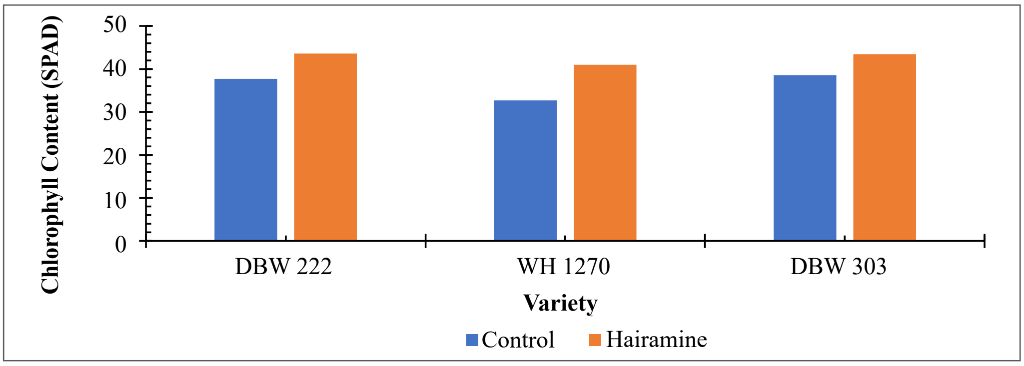

Figure 2. Effect of Hairamine on chlorophyll content in wheat

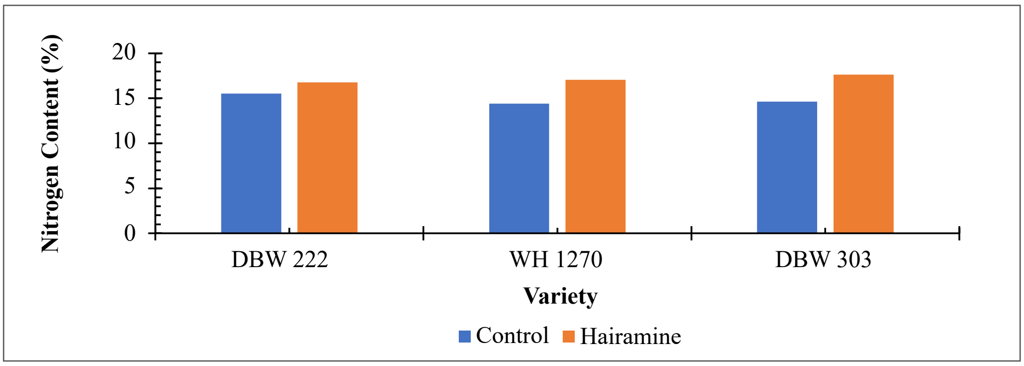

Figure 3. Effect of Hairamine on nitrogen content in wheat

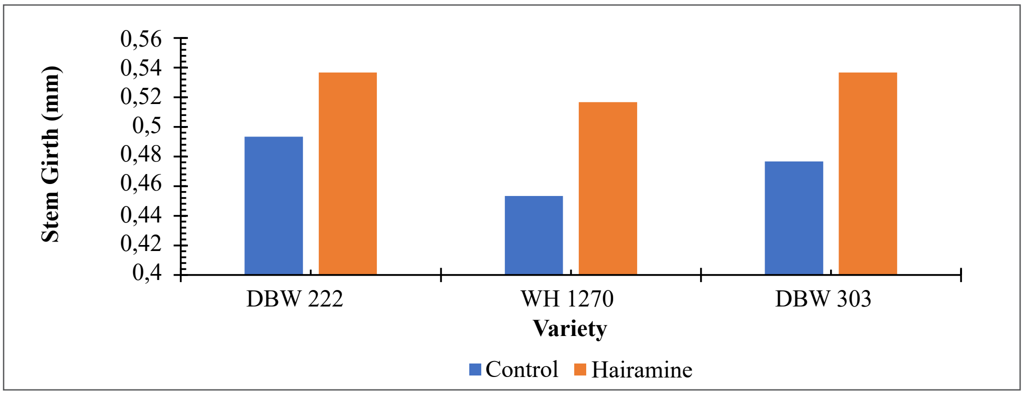

Figure 4. Effect of Hairamine on stem girth in wheat

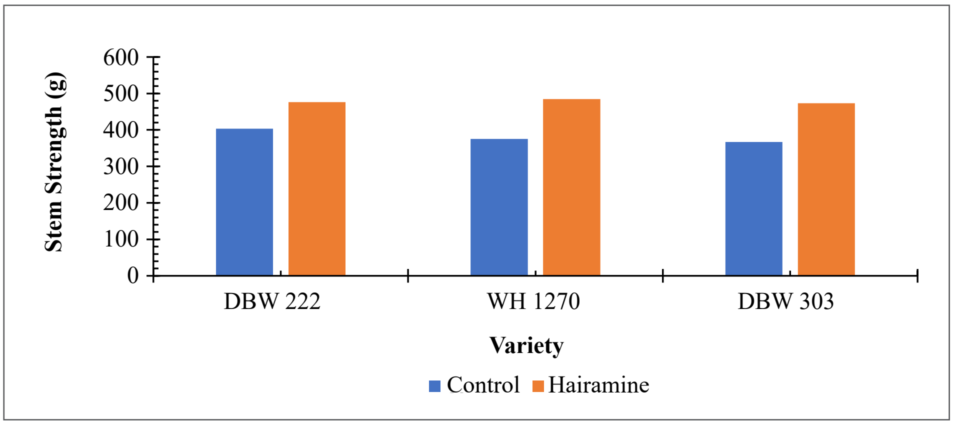

Figure 5. Effect of Hairamine on stem strength in wheat

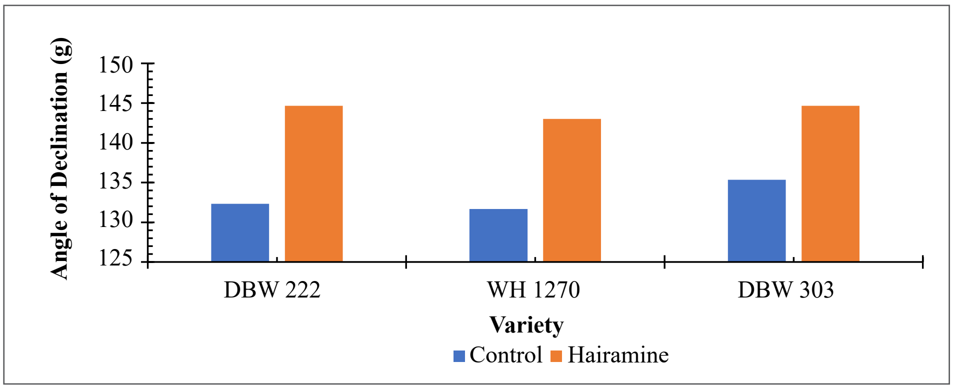

Figure 6. Effect of Hairamine on angle of declination in wheat

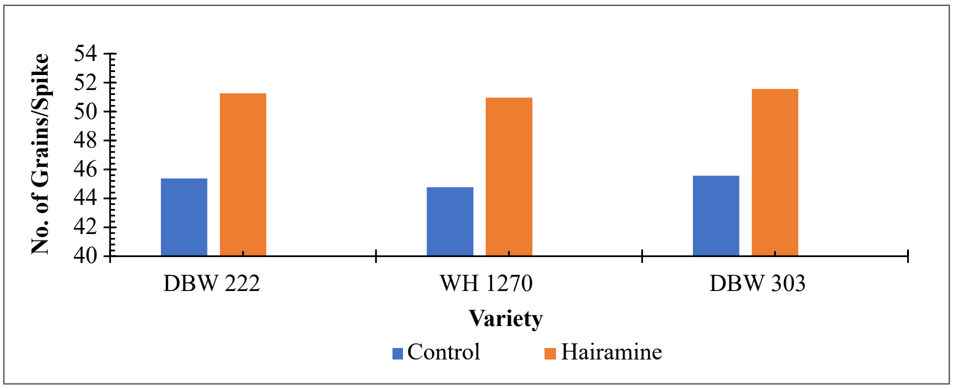

Figure 7. Effect of Hairamine on no. of grains/spike in wheat

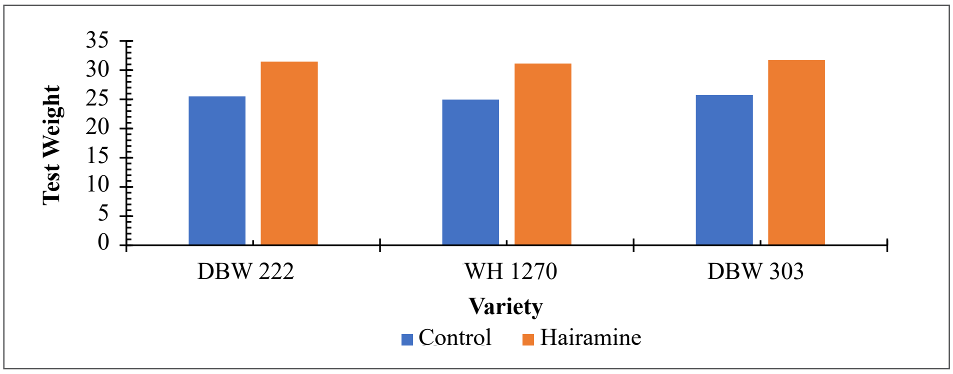

Figure 8. Effect of Hairamine on test weight in wheat

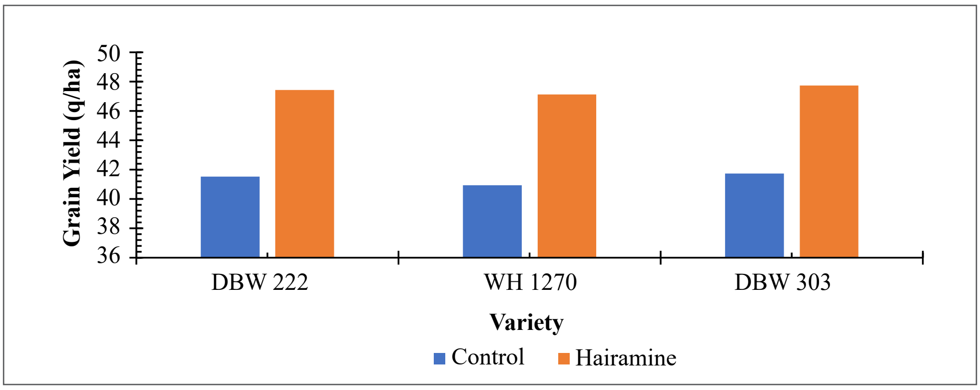

Figure 9. Effect of Hairamine on grain yield in wheat

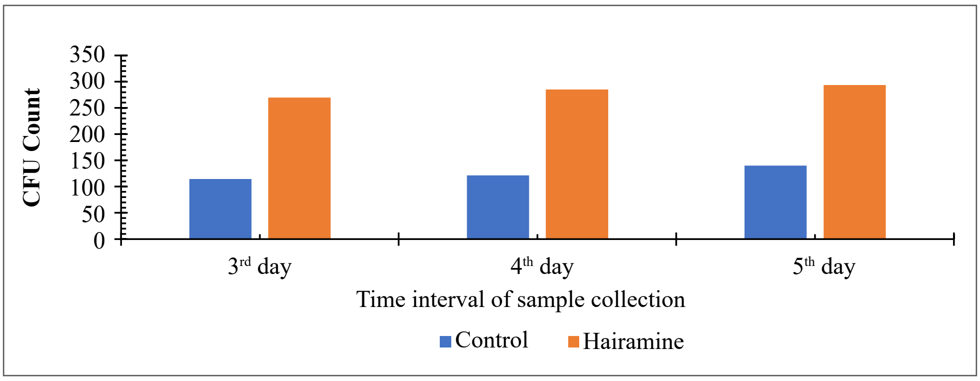

Figure 10. Effect of Hairamine on bacterial population of wheat

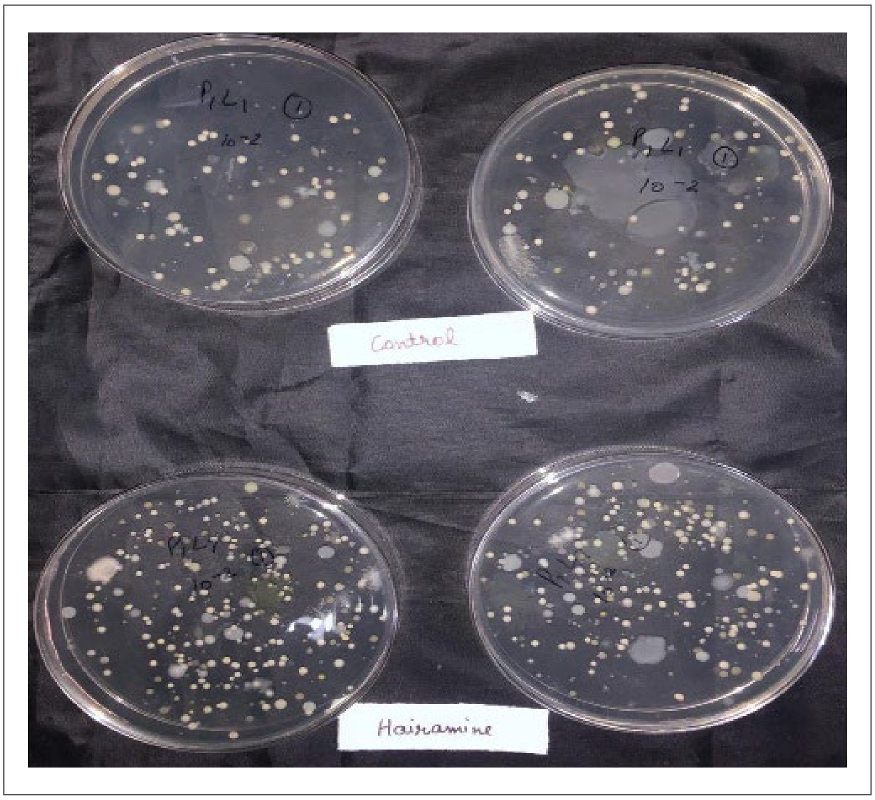

Figure 11. Petri plates sowing CFU from rhizospheric soil

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