IMPROVING MORPHOLOGICAL AND BIOCHEMICAL CHARACTERS, YIELD ATTRIBUTES AND APHID MORTALITY IN WHEAT (*TRITICUM AESTIVUM* L.) BY BIOSTIMULANTS

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Abstract. Wheat crop suffers from decrease in yield due to aphid attacks and agrochemicals; therefore, eco-friendly biostimulants could be safe alternatives as biocontrol. An experiment was conducted for 2017-18 and 2018-19 by applying both amino acids with 4 concentration levels of glutamic acid, aspartic acid, arginine, leucine, and natural extracts of moringa leaf, neem seed, banana peel, and orange peel on four wheat varieties *viz*., Faisalabad-08, Dharab-05, Chakwal-50, and Ahsan-13. Experimental results revealed that biostimulant BPE_3, AA_2 and GA_2 showed the best results for morphological traits (root fresh weight 106%, root dry weight 64.4%, and plant height 38%) antioxidants [AA_2 enhanced superoxide dismutase (SOD) 102%, MLE_3 peroxidase 126%, MLE_3 catalase 76%, AR_2 ascorbate peroxidase 74%], biochemical (total soluble protein 103% by AR_2), yield attributes [LEU_2 spike length 170%, MLE_3 100 grains weight 50.3%, MLE_3 number of grains/spike (NG/S) 48%] and with NSE_3 aphid mortality rate has been increased by 84% over to the control. Faisalabad-08 showed the best response in all treatments. The above-mentioned biostimulants had the best performance against aphid attack and for yield; therefore, these are suggested as potential biostimulants as well as biocontrol agents. MLE_3, BPE_3, AR_2, GA_2, and LEU_2 are hence, recommended for field trials and further use to make new combinations and formulations.
Keywords: *natural plant extracts, banana, moringa, wheat, amino acids, abiotic factors*

Introduction

Wheat, a staple crop, is one of the most important cereals for human nutrition (Maignan et al., 2021). After corn and rice, it is ranked in the third position providing nutrition to millions of people. Due to its immense importance in several economies, it is an integral part of governmental policies, especially in the third-world countries (Khan et al., 2020; Iqbal et al., 2021, 2022b). To obtain a high-yielding crop, various synthetic fertilizers are in use, disturbing not only consumer life but our environment as a whole. Luckily due to extensive scientific investigation, synthetic chemical fertilizers have some alternatives that would benefit crop production’s sustainability (Ye et al., 2020).

Several aphids are attacking wheat in Pakistan, including *Sitobion avenae* (F.), *Schizaphis graminum* (R.), and *Rhopalosiphum padi* (L.). Hence, there is a need for biological substitutes to control such a menace and provide healthy and delicious nutrient. Organic fertilizers are getting attention due to the eco-friendly nature because of their extraction from plant waste and plant extracts as biostimulants.

A biostimulant is a natural material, such as a protein, enzyme, amino acid, micronutrient, natural plant extract, or organic extract that exhibits the potential to enhance crop productivity and associated traits (Chiaiese et al., 2018; Souri and

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Bakhtiarizade, 2019). In general, bio-pesticides are low in trend due to several hurdles in the commercialization of biological products (Durán-Lara et al., 2020).

Likewise, amino acid-based biostimulants such as aspartic acid are used in biostimulant products (Colla et al., 2015). Aspartic acid treatment marked a significant increase in all growth parameters like photosynthetic pigments, yield components, and quality of grains in wheat cultivars (El-Sayed et al., 2019). Moreover, the activities of antioxidants, *viz*., super oxidase dismutase, peroxidase, catalase, and ascorbate peroxidase, are differentially affected (Rizwan et al., 2017). Interestingly, some biostimulants work collectively (Alfosea-Simón et al., 2021). Arginine acts best to enhance the defense system of plants by decreasing reactive oxygen species and can modulate H_2O_2 generation under abiotic stresses (Conceição et al., 2021). Previous studies showed that arginine was the most effective compound in increasing soluble carbohydrates (Bassiouny et al., 2008). Several other functions are also attributed to amino acids, *viz*., L-leucine in transportation, translocation, metabolism, and structural component enhancement by increasing tolerance and posing resistance against stress (Wu et al., 2000; Liu et al., 2008; Calvo et al., 2014).

Foliar use of biostimulants on leaves accelerates the growth of young plants. Several field crops are treated with moringa leaf extract (MLE). It increases 20-35% improvement in groundnut, sugar cane, corn, onion, and tomato productivity (Foidl et al., 2001). Furthermore, it promotes leaf antioxidants such as SOD, POD, and phenolic (Khan et al., 2020). Emaga et al. (2007) described that MLE enhances potassium, proteins, dietary fiber, essential amino acids, and polyunsaturated fatty acids. Moreover, it is also a major source of vitamins such as antioxidants, flavonoids, and phenolic compounds (Lee et al., 2010).

Aphid attacks which are common in several developing countries stimulate farmers to use pesticides and this increasing trend are alarming (Anonymous, 2011). Orange peel extract proved effective against wheat aphids like *Z. subfaciatus* (L.) and kill 67%, respectively (Zewde and Jembere, 2010; Iqbal et al., 2011). Luckily, natural sources are available to enhance pest management including 'Azadirachtin' extracted from neem seeds and proved effective as an antifeedant. It retards growth and regulates growth. Several other species of the family Meliaceae including *Azadirachta indica*, *A. excelsa*, *Trichilia Americana,* and *Melia volkensii* could be exploited as natural insecticides (Akhtar et al., 2008).

Considering the importance of biostimulants, current studies were designed to investigate the effects of foliar treatment of biostimulants on wheat to explore the effects on morphological, biochemical, and yield parameters along with aphid mortality rate. Several studies have been performed by applying single concentrations of amino acids and plant extracts, while little is known about the impact of these biostimulants on wheat.

Materials and methods

Location of the experiment and research design

The experiment was carried out at the experimental site of a botanical garden under controlled conditions in the greenhouse of the University of Gujrat, Gujrat, Pakistan, located at 32°40' North latitude and 74° 02 East longitude and has moderate temperate. Four wheat varieties were studied, viz., Faisalabad-08, Dharabi-11, Ehsan-16, and Chakwal-50. The impacts of different concentrations of biostimulants on growth, biochemical characters, yield attributes, and aphid mortality rate were investigated.

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Seeds of wheat varieties were acquired from Ayub Agricultural Research Center, Faisalabad, Pakistan. The air-dried soil was passed through a 3.8 mm sieve and was cleaned from visible stone particles and roots. The pot experiment was conducted in two consecutive growing seasons (2017–18 & 2018–19). Earthen pots (15×15 38.1×38.1 cm) were filled with 12 kg of sandy loam soil prepared by mixing sand 50%, clay 20%, and silt 40%. Pots were perforated from the bottom to regulate excess nutrients. The surface-sterilized ten seeds were sown per replicate in each pot. After ten days of germination, six plants were maintained in each pot. A completely randomized design (CRD) was used. There were four biological replicates for each treatment. All of the treatments were applied after 25 days after sowing. Treatments were applied exogenously foliar by hand sprayer at 250 mL per pot at wheat developmental stage as Zadoks (tiller stage scale 20-29). The solutions for the treatments were diluted with de-ionized water into four concentrations as moringa leaf extract (MLE), neem seed extract (NSE), banana peel extract (BPE), and orange peel extract (OPE) [0, 5, 10, and 15 %], glutamic acid (GA), aspartic acid (AA), arginine (AR), and leucine (LEU) [0, 1000, 1500, and 2000 mg/L].

Acronyms of varieties and treatments are listed in *Table 1*. Tap water was used to spray the control plants and irrigate them at an interval of one week. Observations were made after 45 days of the application of the biostimulants. Four plants were randomly selected to determine various morphological and biochemical parameters. The remaining were allowed to grow till maturity. Root fresh weight and dry weight were measured at the vegetative stage. Dry weight was estimated at 80°C for 48 h for dry weight. Different biochemical attributes were also measured through fresh leaves. Harvesting was accomplished on April 25th during both growing seasons. The average daily meteorological parameters were recorded; the minimum and maximum temperatures were 5°C±10°C and 40°C±42°C, respectively, and the average precipitation was 216 mm.

Calculation of water required for irrigation for each pot

Irrigation of water in pots depends upon many abiotic factors, and the requirement of water varies according to the developmental stages of wheat. Field capacity (FC) is the main factor utilized to calculate the required water quantity. It is the soil water holding capacity that is much more important for every developmental stage of wheat. FC required for wheat growth on average is 70%. After evaporation soil was maintained at 40%. The total soil contained per pot was 12 kg, water requirement was calculated as mentioned below;

1) The field capacity (FC) required for wheat growth is 70 %
2) Each pot had soil of 12 kg (12000 g)
3) Post size was (38.1 cm * 38.1 cm)
4) Average FC maintained at different growth stages 40 %
5) 12000 * 40 / 100 = 4800
6) 4800 * 70 / 100 = 3360 ml
7) The required amount of water supplied to each pot was 3360 mL.

Preparation of aqueous extracts of moringa leaf, neem seeds, banana, and orange peel

Fresh leaves of moringa (MLE) were collected, washed, air dried, and converted into coarse powder by using an electric grinder. To meet the required concentration, distilled

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water was added to the powder and autoclaved at 121°C, 15 lbs sq-1 inch, for 20 min. Then the extract was filtered with the help of a cheese cloth and cooled at 4°C. After centrifugation for 15 minutes at 5000 × g for 15 minutes, the supernatant was recovered (Yasmeen, 2011). Various concentrations of moringa leaf extract were prepared as 0, 5, 10, and 15% by adding de-ionized water.

Table 1. *Symbols used for different treatments tested in pot experiment as 1-4 varieties, 5-29 single treatments, used for morphological, biochemical, and yield attributes studied in wheat*

S/N	Treatments	Acronyms used
1.	Faisalabad-2008	V_1
2.	Dharabi-2011	V_2
3.	Ehsan -2016	V_3
4.	Chakwal-50	V_4
5.	Control (0 mg/L)	Control (untreated)
6.	Glutamic acid (1000 mg/L)	GA_1
7.	Glutamic acid (1500 mg/L)	GA_2
8.	Glutamic acid (2000 mg/L)	GA_3
9.	Aspartic acid (1000 mg/L)	AA_1
10.	Aspartic acid (1500 mg/L)	AA_2
11.	Aspartic acid (2000 mg/L)	AA_3
12.	Arginine (1000 mg/L)	AR_1
13.	Arginine (1500 mg/L)	AR_2
14.	Arginine (2000 mg/L)	AR_3
15.	Leucine (1000 mg/L)	LEU_1
16.	Leucine (1500 mg/L)	LEU_2
17.	Leucine (2000 mg/L)	LEU_3
18.	Moringa leaf extract (5%)	MLE_1
19.	Moringa leaf extract (10%)	MLE_2
20.	Moringa leaf extract (15%)	MLE_3
21.	Neem seed extract (5%)	NSE_1
22.	Neem seed extract (10%)	NSE_2
23.	Neem seed extract (15%)	NSE_3
24.	Banana peel extract (5%)	BPE_1
25.	Banana peel extract (10%)	BPE_2
26.	Banana peel extract (15%)	BPE_3
27.	Orange peel extract (5%)	OPE_1
28.	Orange peel extract (10%)	OPE_2
29.	Orange peel extract (15%)	OPE_3

Neem seeds were purchased from Punjab Seed Corporation, Lahore, Pakistan to prepare neem seed extract (NSE). Seeds were then washed, shade dried, and converted into powder with the help of a blender. After that, powder in the quantity of 100 g is soaked in 1 liter of water for 3–7 days and then filtered with a muslin cloth. The pure extract was then used for dilutions for treatment application (Boursier et al., 2011).

Banana peel waste was collected from local fruit juice shops. Peel was transferred to powder and 500 g was mixed with 2 liter of distilled water and stored for 48 hours. The extraction was then subjected to centrifugation for 10 minutes at 4500 rpm and then suspended in water twice. To make the extract more concentrated, a rotary evaporator was used with reduced pressure at 45°C, then stored at -20°C. The concentrated extract

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was diluted with de-ionized water in different concentrations (0, 5, 10, and 15%) and applied exogenously (Bakry et al., 2016).

For the preparation of orange peel extract, sour oranges were collected from the fruit juice market. Disease-free peel was selected at 200 g and dissolved in 1 litre of distilled water. Then filtered and stored for a week before use (Iqbal et al., 2011).

Estimation of morphological characters and yield components

Morphological data like plant height was measured with the help of a measuring tape at the vegetative stage. At the same time, root fresh and dry biomass were also recorded with the help of an electric balance (SHIMADZU, EAN: 4064343392884) (Ahmad et al., 2020). At the end of the developmental stage, the yield components obtained included the weight of 100 grains and the number of grains per spike.

Estimation of biochemical attributes

Fresh leaves (0.5 g) were grounded in phosphate buffer (10 mL) at pH 7.8. After centrifugation at 20,000 × g for 20 min, the supernatant was recovered and stored at -80°C. Enzyme activity measurments included CAT, POD, and SOD. A solution of 1 mL was prepared by adding phosphate buffer 50 mM (pH 7.8), methionine 13 mM, nitro blue tetrazolium 50 µM, riboflavin 1.3 µM, and the enzyme extract in the quantity of 50 µL. Then the solution was mixed and triturated, 1 mL was irradiated under fluorescent light (20 W) for 15 minutes at a photon flux density of 78 µmol. Readings were recorded at 560 nm using a UV-visible Spectrophotometer (Hitachi U-2100, Hitachi, Tokyo, Japan). SOD activity was defined as 1 unit of inhibition caused by an enzyme in the reduction of photochemical NBT (Giannopolitis and Ries, 1977).

For determining POD and CAT activities, a three-mL solution was prepared, which was composed of H_2O_2 59 mM, sample extract of 0.1 mL, and phosphate buffer 50 mM (pH 7.8) An OD was recorded at 240 nm. For POD activity, the solution mixture was prepared as H_2O_2 40 mM, guaiacol 20 mM, phosphate buffer 50 mM, and enzyme extract 0.1 mL. The OD was recorded at 470 nm (Chance and Maehly, 1955). While the activity of APX was estimated following the procedure of Cakmak (1994) at 290 nm.

Determination of total soluble protein

The Bradford assay was followed (Bradford, 1976) for total soluble protein estimation. Then Coomassie Brilliant Blue (G-250) of 100 mg was mixed in 95% ethanol (50 mL), and phosphoric acid (85%) was added and mixed to make 1 L of the volume by adding H_2O. Then a Bradford solution of 5 mL was mixed with 100 µL extract and incubated for 5 minutes. A BSA standard curve was derived for 0, 20, 40, 60, 80, 100, and 120 µg/mL, while absorbance was recorded at 595 nm.

Rate of aphid mortality on four wheat varieties

The aphid's mummies per tiller for each variety were also visually counted twice a week per pot and the rate of aphid mortality was estimated through the following equation (a) (Shah et al., 2017).

$$\text{Percent Mortality} = [(\text{Number of aphids in pre-treatment} - \text{number of aphids in post-treatment}) / \text{number of aphids in pre-treatment})] \times 100 \quad \text{(Eq.1)}$$

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Statistical analysis

Treatment, year, and variety were the main factors used to study the analysis of variance. For normality, the Anderson-Darling test was used. Means were separated by a Tukey-honestly significant difference ($P \leq 0.05$). Statistical Software 'The Minitab Version 19.0' was used to analyze the data and standard errors of means were calculated in Microsoft Office Excel 2016 by using the statistical formula StErr = StDev/ $\sqrt{n}$ (Iqbal et al., 2022a)**.**

Results and Discussion

Morphological attributes

ANOVA revealed that the effect of foliar application of AA_2, BPE_3, and GA_2 expressed a highly significant increase as compared to other concentrations. LEU_1 and LEU_3 both showed negative effects as compared to the control for morphological characters. Biostimulants showed influence with different levels on root fresh weight (RFW) of four wheat varieties that are shown in *Fig. 1a*. BPE_3 showed the highest improvement of 106% with mean ±SE 7.4±0.25 over control 3.6±0.3 in V_1. The most prominent lowest value was observed by the LEU_1 52%, with mean ±SE 1.75±0.25 as compared to control in V_1 in both growing years. The second efficient effect showed by the application of AR_3 with 5.5 in V_1. OPE_3 also showed a nominal increase in morphological characters in V_3. Analysis of variance demonstrated significant interaction among year × variety × treatment for root fresh weight ($F_{72, 600}$) = 1.57, P = 0.031 (*Table 2*). Varieties with the best performance are as follows: $V_1 > V_2 > V_3 > V_4$ in response to treatment impact. It was demonstrated that BPE with different concentrations could enhance the marked increase in morphological characteristics of the quinoa plant (Bakry et al., 2016). Clary sage showed improved growth characteristics with the application of BPE (El-Gohary et al., 2020). Moreover, Blokhina et al. (2003) highlighted that phenols comprehend several metabolic activities throughout the life of a plant. Hence, several activities including PH, FW, DW, carbohydrates, photosynthetic pigments, and antioxidant enzymes in parsley and celery plants were attributed to phenols (Gharib, 2006). Comparatively, chickpeas also showed similar type of results that elucidates the effect of fruit peel extract that significantly increases growth characteristics (Qader, 2019).

ANOVA for root dry weight (RDW) showed a positive effect with an amino acid base biostimulant. AA_2 expressed the highest value of 64.4%, Mean±SE 3.35±0.13 as compared to the control value of 1.19±0.11 with V_1 (*Fig. 1b*), while the interaction of year × variety × treatment was also found significant ($F_{72, 600}$) = 1.38, P = 0.025, compared to the control, after AA_2 prominent effect 1.7 g showed by MLE_3. LEU_1 showed the lowest value of 63%, Mean±SE 0.44±0.11 with V_4 in both growing years (*Table 2*). The enhanced effect was also noticed by the foliar application of BPE_3 and AR_2 subsequently. Studies conducted in the past also supported current findings as investigated in cereal crops (Rizwan et al., 2017). Amino acids also significantly increased maximum growth characteristics like PH, RDW, and RFW in chamomile (El-Din et al., 2005).

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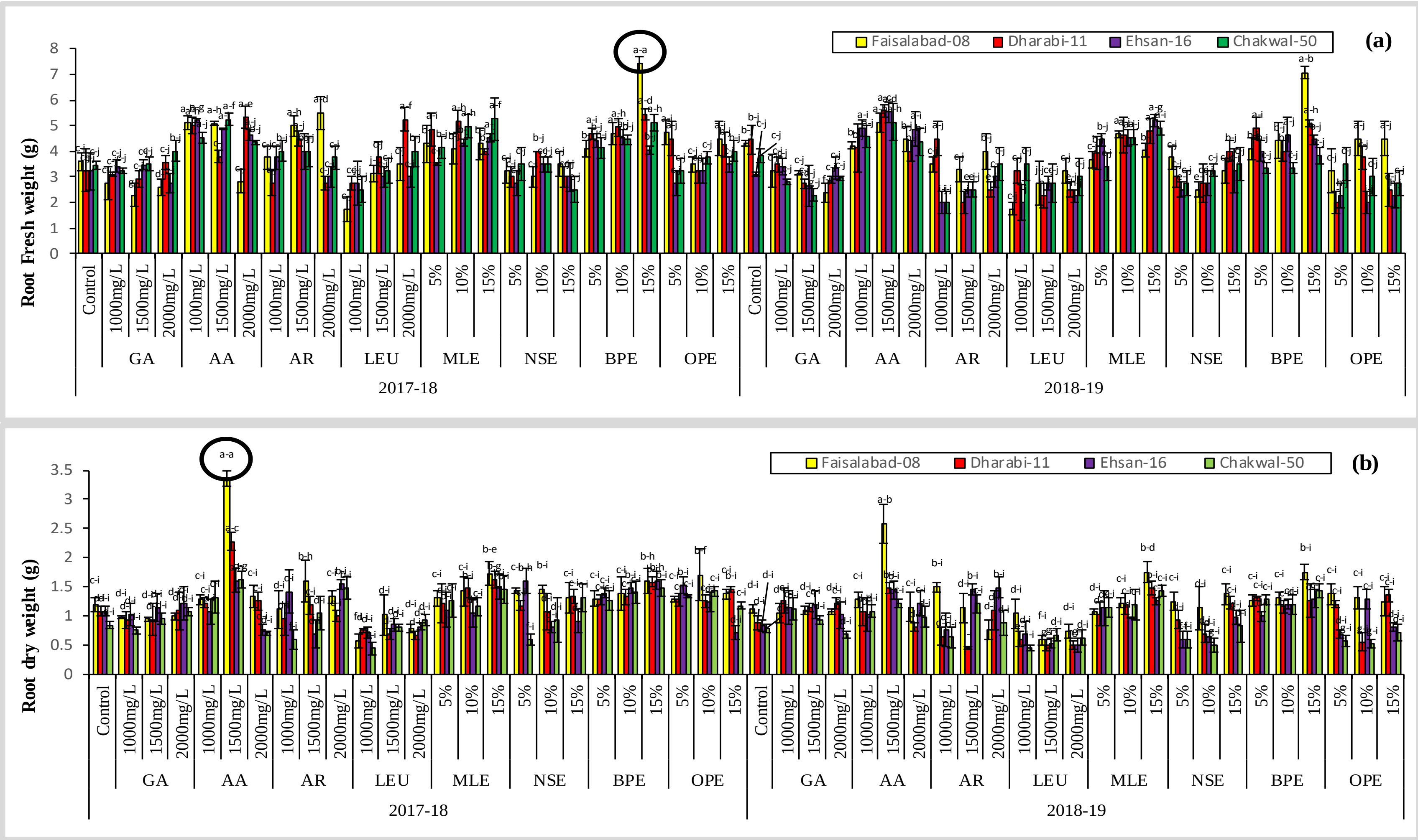

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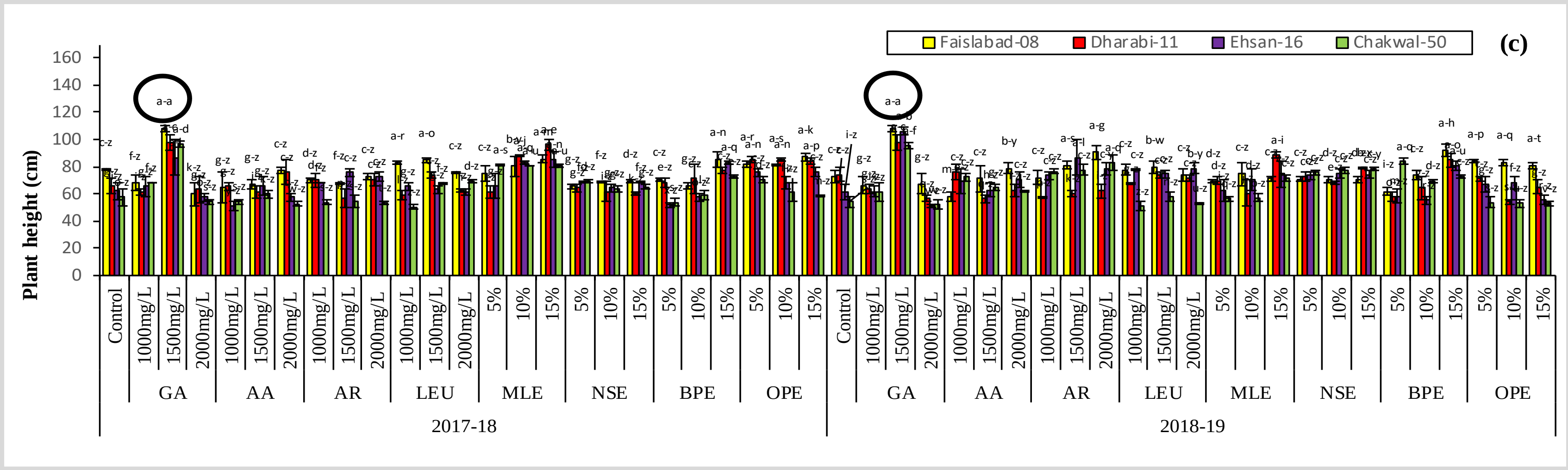

Figure 1. *The effect of single biostimulants on (a) RFW, (b) RDW, and (c) PH of wheat. Different alphabets on the column indicate significant differences (P< 0.05) from the control*

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Table 2. *ANOVA represents Mean squares (MS) for single treatment growth, antioxidants, total soluble protein, yield, and aphid mortality attributes of wheat*

Source of variance	DF	Root Fresh Weight	Root Dry Weight	Plant height	Superoxide dismutase	Peroxidase	Catalase	Ascorbate peroxidase	Total soluble protein	100-grain weight	Number of grains/ spike	Percent aphids Mortality (%)	Spike length
Year	1	22.3***	5.23***	60.17ns	0.183***	0.017*	0.006ns	0.4**	2013.7***	394.8***	9.68ns	20.8ns	24.29***
riety	3	4.60***	3.08***	3682***	0.032**	0.034***	0.019**	0.9***	3654.8***	426.2***	1623***	520.5*	39.91***
Treatment	24	17.6***	2.34***	2089***	0.124***	0.121***	0.238***	1.0***	2880.6***	651.8***	953.3***	24634.2***	49.88***
Year*variety	3	1.43ns	0.079ns	319***	0.009ns	0.026***	0.020**	0.06ns	287.3*	390.3***	182.5***	212.0ns	9.84***
Year* Treatment	24	2.61***	0.241**	490***	0.017***	0.013***	0.015***	0.12***	292.2***	79.8***	114.4***	246.5**	6.42***
Variety* Treatment	72	1.91***	0.297***	249***	0.022***	0.008***	0.013***	0.11***	338.0**	70.93***	83.6***	268.7***	6.05***
Year*Variety*Treatment	72	1.30***	0.171*	204.2***	0.009*	0.005*	0.009***	0.07**	327.7***	82.1***	19.6*	273.2***	3.74***
Error	600	0.83	0.124	74.2	0.006	0.004	0.005	0.05	104.9	12.23	14.71	112.7	1.41
Total	799												

DF=Degree of freedom; ns = Non-significant; *, **, ***, significant at 0.05, 0.01 and 0.001 respectively

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It is also described that PH was also enhanced by the use of the essential amino acid GA_2. It showed the highest value of 38%, Mean±SE 108±2.34 than control 77.8±0.5 with V_1 and the lowest treatment level LEU_1 by 12.2%, Mean±SE 50.25±1.36 with V_4 in both growing years (*Fig. 1c*). MLE_3 was the second most prominent treatment showing 100 cm. Interaction of year × variety × treatment also exhibited a significant relationship $(F_{72,\ 600}) = 2.75$, P = 0.000, and mean square values of morphological characters (*Table 2*). The amino acids aspartic acid with glutamic acid and their mixture Asp+Glu showed a positive impact on tomatoes by increasing their growth. These amino acids also possess the ability to alter the synthesis of proline and the pentose-phosphate route, resultantly improving GABA, and trigonelline which ultimately helps to boost growth characters hence recommended for further use for crop improvement (Alfosea-Simón et al., 2021).

Antioxidants and total soluble protein

Superoxide dismutase was also improved significantly at various levels of biostimulants. The highest value was expressed by the application of AA_2 102%, Mean±SE 0.75±0.02 while control was 0.37±0.00 in V_1. The lowest value was observed by LEU_1, 59%, 0.15±0.01 in V_1. BPE_3 and MLE_3 also showed an increasing trend of 0.6 and 0.4 enhanced effects as compared to the control. ANOVA revealed significant interaction among year × variety × treatment of SOD $(F_{72,\ 600}) = 1.41$, P = 0.000 (*Figure 2a; Table 2*). Teixeira et al. (2017) stated that antioxidant enzyme activities were increased in soybean by applying various amino acids. An increase in SOD, CAT, and APX activities by the aspartic acid treatment could be associated with low levels to enhance soil fertility (Sharma and Dietz, 2006).

Peroxidase was enhanced by the foliar application of MLE_3, the highest value was 126%, Mean±SE 0.52±0.01 in comparison with the control 0.23±0.03 in V_1. Whereas the lowest value was evidenced by LEU_3 17%, 0.14±0.02 in V_4 as compared to the control, followed by BPE_3 with a mean of 0.5. Here, ANOVA also revealed a significant impact among year × variety × treatment of POD $(F_{72,\ 600}) = 1.39$, P = 0.020 (*Figure 2b; Table 2*). MLE application also increased leaf antioxidants including SOD, POD, and phenolic during salinity stress. The presence of higher mineral contents ensures the activation of the self-defense system by exogenous application of MLE (Yasmeen et al., 2013).

Catalase was also enhanced by the biostimulant treatment. The highest value was observed by the application of MLE_3, 76%, Mean±SE 0.60±0.03 while the value for the control was 0.34±0.08 in V_1. The lowest value was shown by LEU_3 73%, 0.09±0.00 in V_4. BPE_3 had a second prominent effect of 0.55 with V. In this study, ANOVA exhibits positive interaction of year × variety × treatment of CAT $(F_{72,\ 600}) = 1.77$, P = 0.000 (*Figure 2c; Table 2*). Concerning findings are in agreement with the previous reports on wheat and bean plant respectively (Yasmeen et al., 2013; Howladar, 2014). Thus Moringa leaves are suggested for future applications to enhance productivity by using it as a bio-based organic fertilizer with an eco-friendly impact (Abdalla, 2013).

Ascorbate peroxidase was enhanced by the application of different levels of biostimulants. The highest value was shown by the application of AR_2 74%, Mean±SE 2.06±0.05 as compared to the control 1.18±0.10 in V_1. After AR_2, plant extract base biostimulant MLE_1 showed an enhanced effect of 1.5 as compared to the untreated plant. The lowest value was shown by LEU_3, 64%, 0.33±0.03 in V_4. Interaction of year × variety × treatment of APX $(F_{72,\ 600}) = 1.48$, P = 0.009 were found significant as

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revealed by ANOVA (*Figure 2d; Table 2*). Conceição et al. (2021) also advocated that APX reduces H_2O_2, by increasing L-arginine.

Moreover, the total soluble protein was also observed to be enhanced by the foliar application of different levels of biostimulants. The maximum value was shown by the application of AR_2; 103%, (Mean±SE) 95.9±1.94 as compared to the control 47.11±7.22 in V_1. OPE_3 and MLE_3 with V_1 showed mean values of 80 and 67 enhancement, respectively. The lowest value was shown by GA_1, 4%, 30.8±3.7 in V_3. ANOVA revealed significant interaction among year × variety × treatment of total soluble protein ($F_{72, 600}$) =3.12, P = 0.000 (*Figure 2e; Table 2*). Bassiouny et al. **(**2008) reported that spraying at 2.5 mM arginine could enhance several traits in wheat.

Yield parameters

Analysis of variance showed enhancement in 100-grain weight by the exogenous foliar application of different biostimulant levels. The highest value was shown by MLE_3, (Mean±SE) 50.3%, 53.75±0.75 over the control 35.75±2.5 in V_1. BPE_1 was the second effective treatment showing 50%. The lowest value was shown by the GA_3, 48.2%, 18.5±0.70 in V_1. ANOVA described that significant interaction was recorded for year × variety × treatment on 100-grain weight ($F_{72, 600}$) = 6.77, P = 0.000 (*Figure 3a; Table 2*).

It is revealed that the number of grains per spike showed significant results by applying biostimulants with single effects. Analysis of variance showed the highest value by the application of MLE_3 48%, (Mean±SE) 51.25±1.6 as compared with the control 34.50±1.04 in V_1. NSE_1 had a second efficient effect on wheat biochemical aspects mean value of 45. The lowest value was shown by OPE_2, 12%, (Mean±SE) 21.50±0.64 in V_4. Analysis of variance presented that a positive impact was found among year × variety × treatment on 100-grain weight ($F_{72, 600}$) =1.34, P = 0.039 (*Figure 3b; Table 2*). Previous studies also supported current findings that MLE enhanced the yield attributes (Afzal and Iqbal, 2015).

Spike length was significantly enhanced by the foliar application of LEU_2. The highest value was 170%, (Mean±SE) 16.25±0.75 as compared to the control 6±0.40 in V_1. NSE_1 and NSE_2 both showed enhanced effects with mean values of 11 and 12 cm respectively. Analysis of variance showed a significant interaction between year × variety × treatment on spike length ($F_{72,600}$) = 2.64, P = 0.000 (*Figure 3c, Table 2*). It was reported that amino acids enhanced yield through nitrogen assimilation in various organs, by spraying at tillering stage. L-leucine is absorbed by the leaves and becomes part of the synthesis of amino acids which ultimately enhances yield components (Wu et al., 2000; Li et al., 2009).

Percentage aphid mortality

The maximum mortality percentage of aphids recorded by the impact of NSE_2 was 84%, and OPE_3 80%, as compared to the control 19% in V_1. ANOVA showed significant results for year × variety × treatment of aphids ($F_{72, 600}$) =2.42, P = 0.000 as shown in (*Figure 4a; Table 2*). The effectiveness against aphids by neem seed extract varies as it is assumed that it might depend on crop varieties, time of use, aphid activities, and variable environmental factors. Despite that, neem extract proved beneficial as an insecticide of natural origin, and an alternative to imidacloprid, generally available on market. The visual representation of *Fig. 5* shows an aphid attack at the inflorescence stage.

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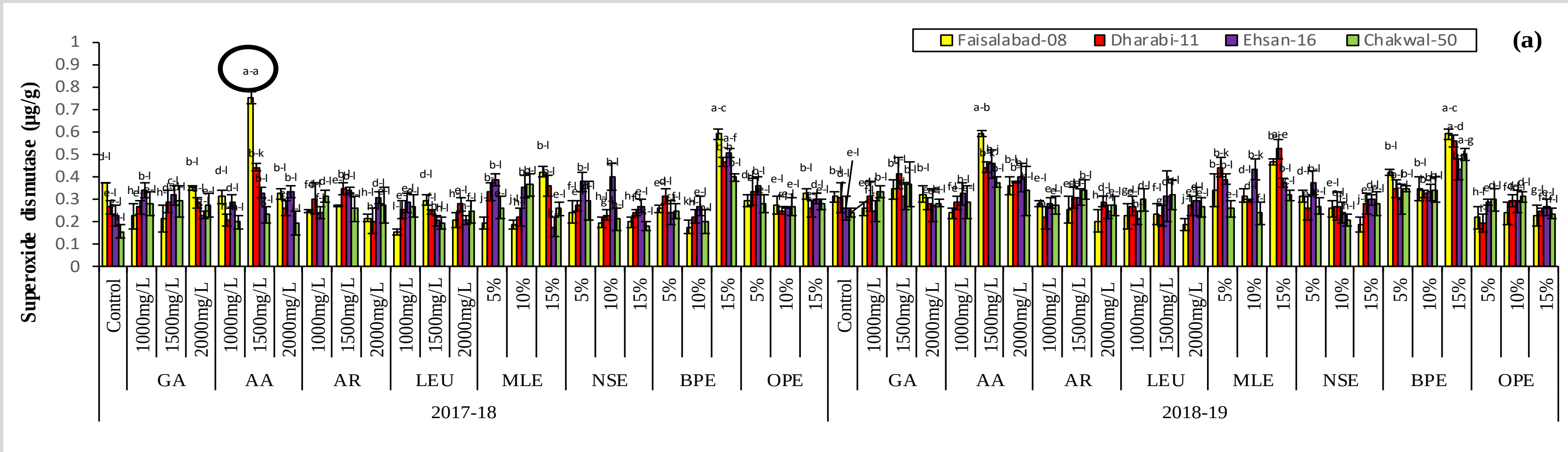

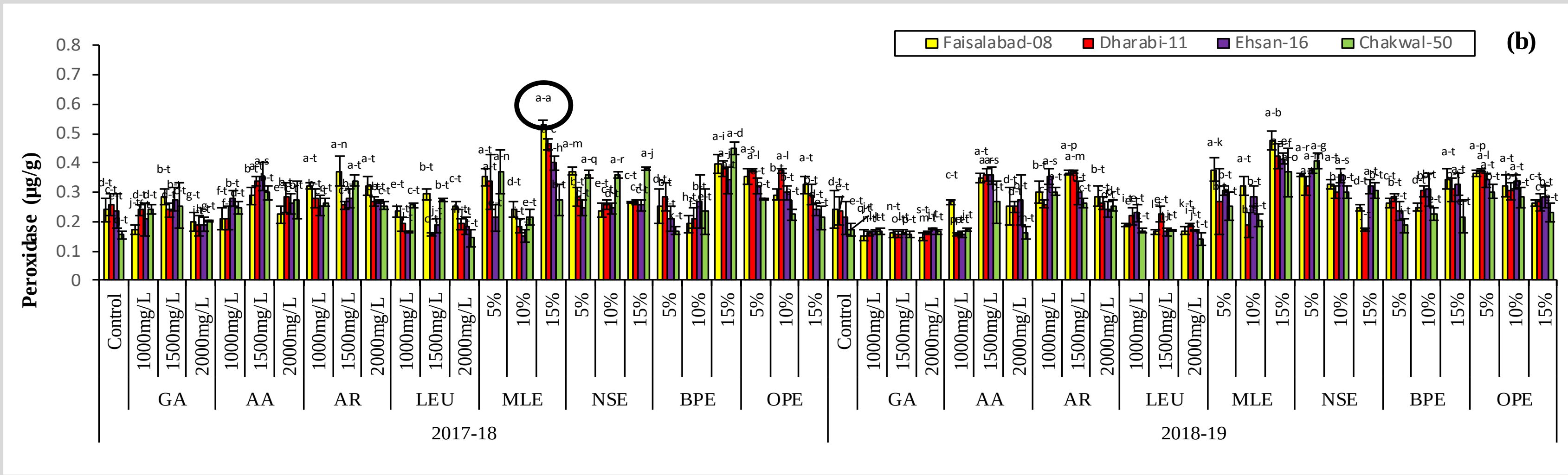

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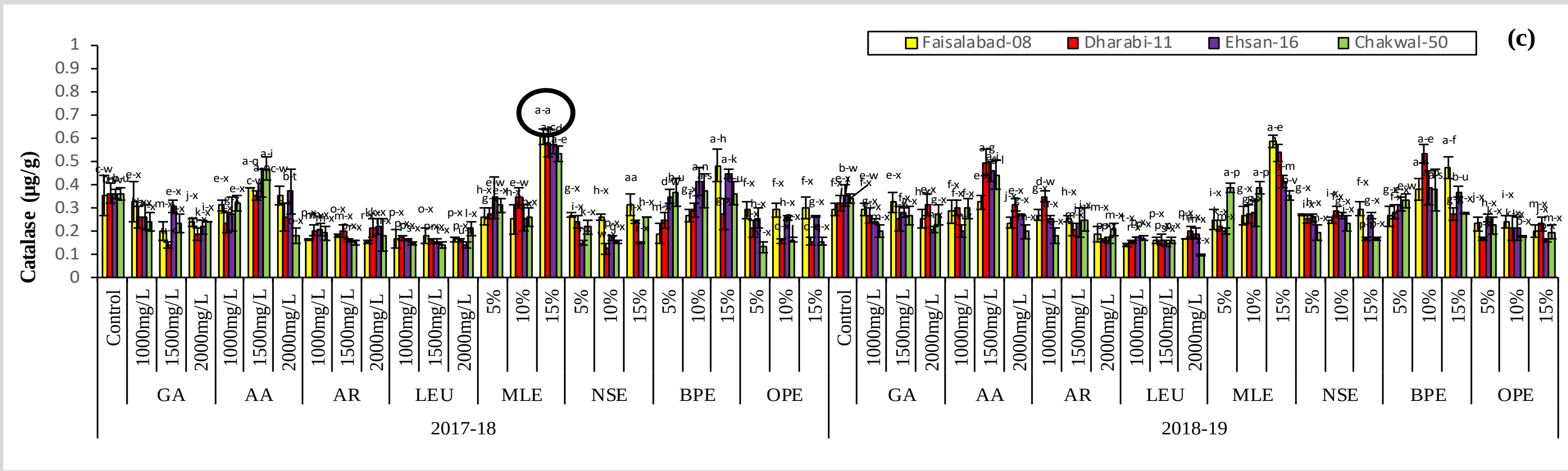

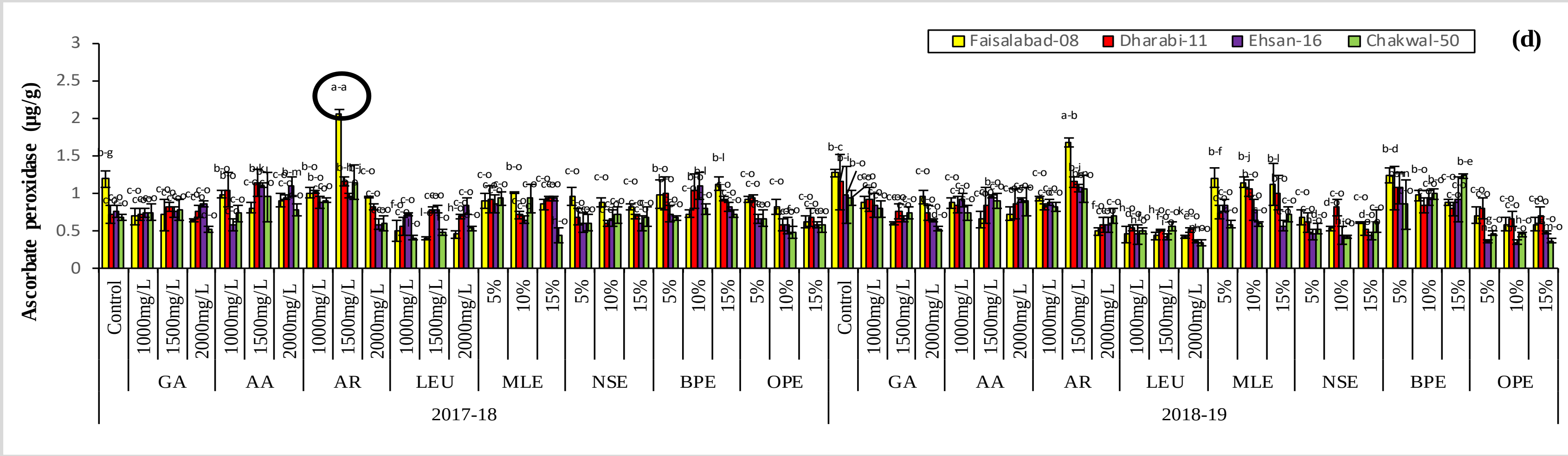

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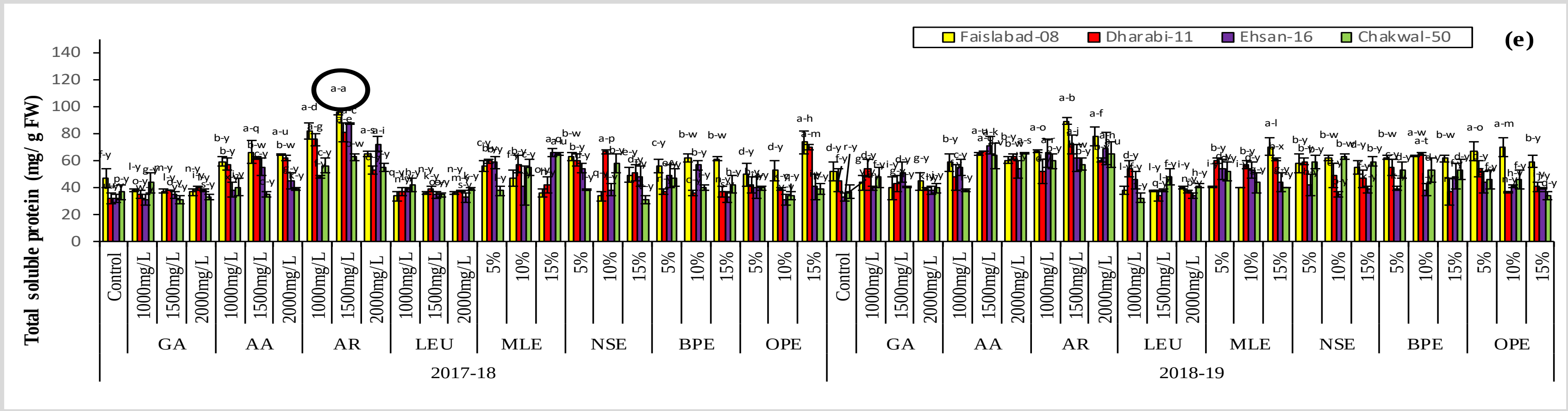

***Figure 2.** The effect of single biostimulants on (a) SOD, (b) POD, (c) CAT, (d) APS, and (e) TSP of wheat. Different alphabets on the column indicate significant differences (P< 0.05) from the control*

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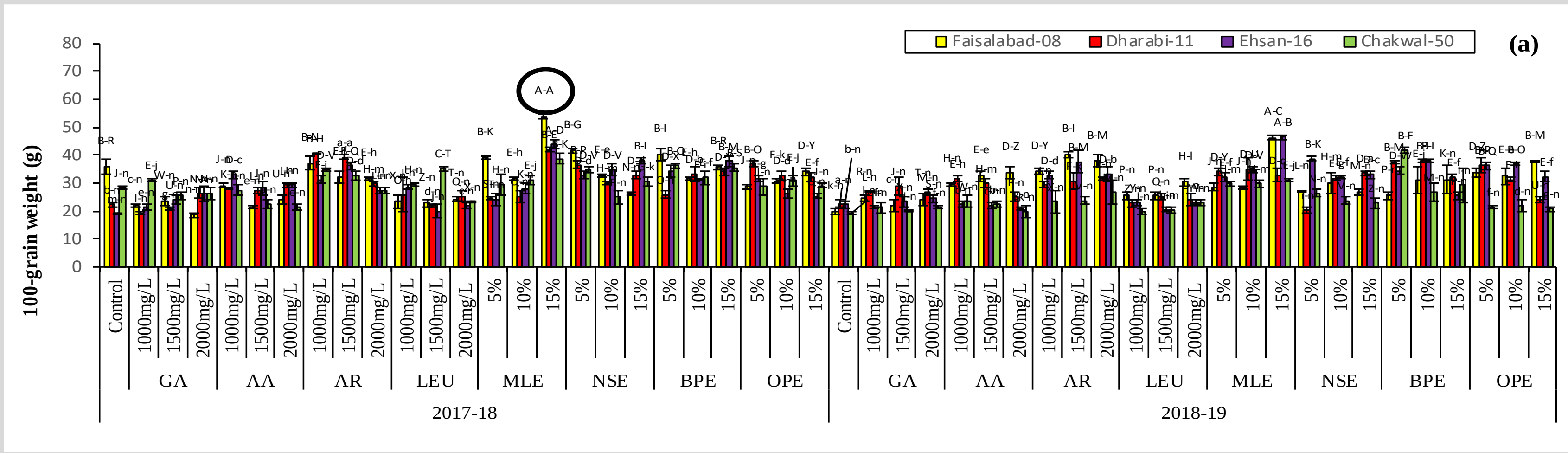

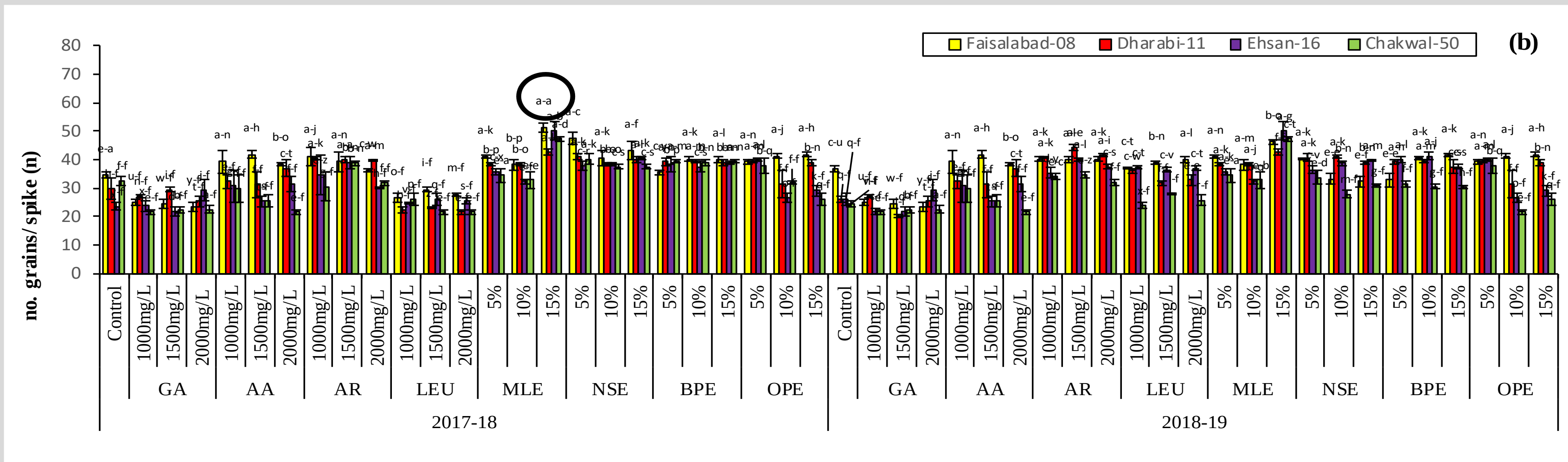

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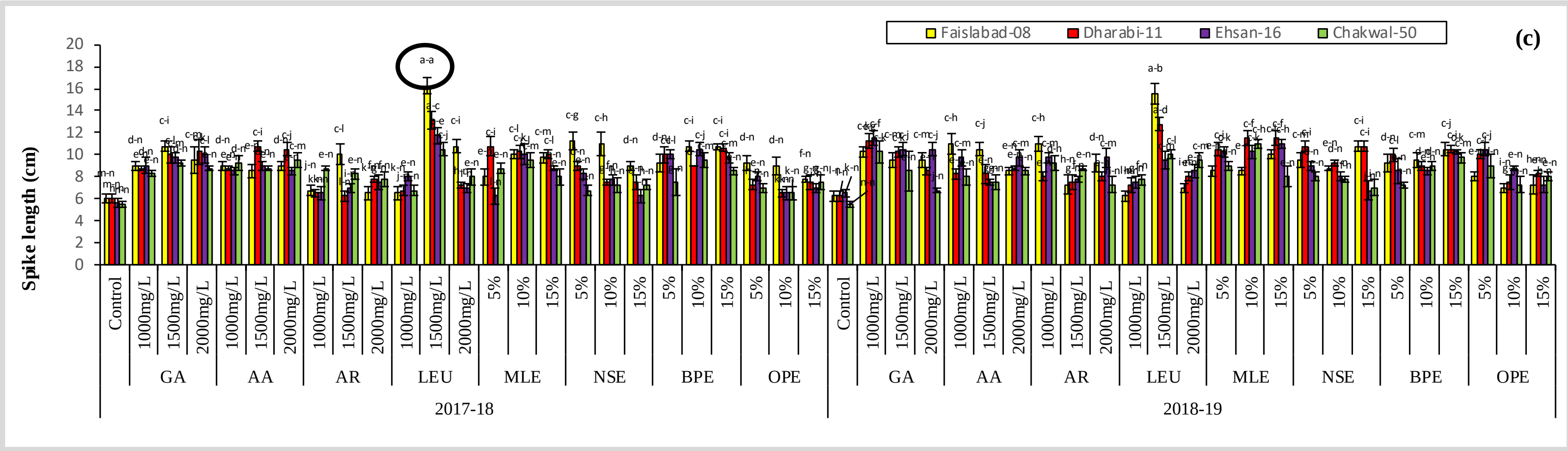

***Figure 3.** The effect of single biostimulants on (a) 100 GW, (b) NG/S and (c) SPL on wheat. Different alphabets on the column indicate significant differences (P< 0.05) from the control*

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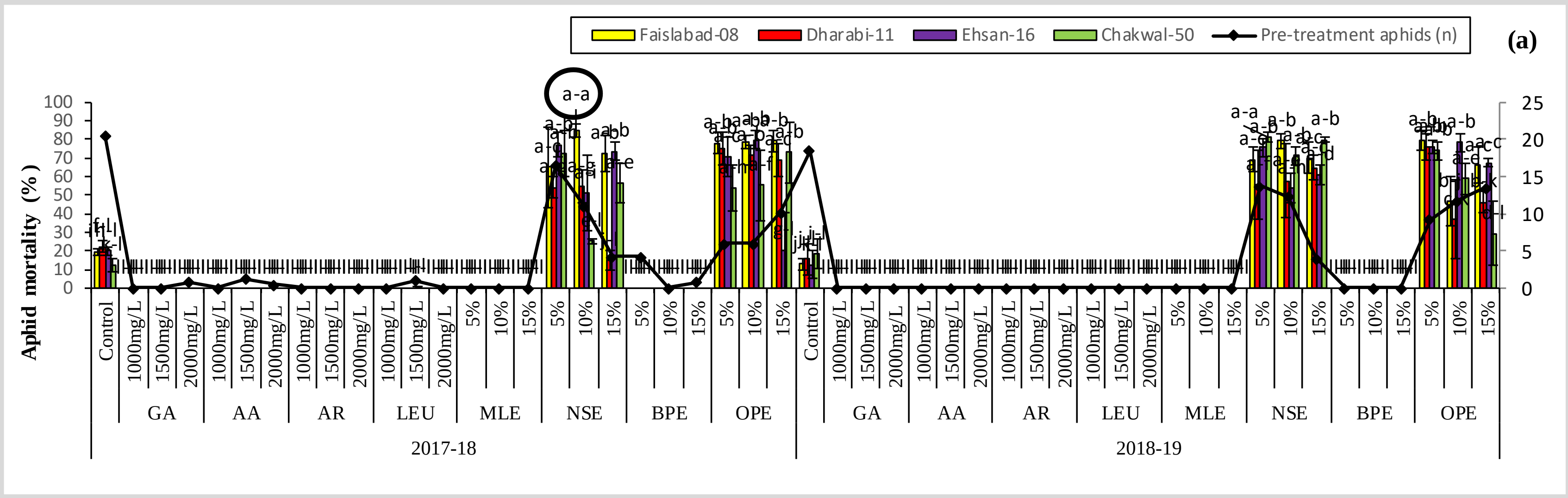

Figure 4. *The effect of single biostimulants on (a) % of mortality of aphids, Data represent means and standard errors (error bars) of four biological replicates. Different small letters indicate significant differences among treatments at the (p ≤ 0.05) level, according to a Tukey-HSD three-way ANOVA*

(a)

(b)

Figure 5. *Visual representation of aphid attack on wheat tillers at inflorescence stage a and b*

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As the concentration of the extracts increases, it effectively lowers the population of aphid lemon and dark green morphs. Response to both morphs of *M. persicae* was found susceptible to NSE. Morph population was reduced to 91.8% in lemon, and 84.8% in dark green morphs, which were considered more toxic, as found in chilies by Lawal et al. (2015). Biswas (2013), also reported appealing results with neem extracts that reduce the 63.1-72.55% aphid population in mustard. Pretreatment with neem extract also showed a reduction of up to 81% by utilization of 50 g per liter of water. Likewise, orange peel extract showed aphid mortality up to 65.69% following Garlic at 57.91%, and Tobacco at 57.90% (Iqbal et al., 2011).

Conclusion

The foliar application of biostimulants is a sustainable and eco-friendly approach to boosting the productivity of field crops. Results revealed that MLE_3 enhanced 100 GW, NG/S, and SPL by 50.3%, 48%, and 170%, respectively, and aphid mortality by 84% with NSE_3 and 80% with OPE_3 that impacted positive effects on morphological, biochemical, and yield attributes. Faisalabad-08 showed an improved response to all treatments. Current findings conclude that biostimulants showed promising results in this study, therefore, further use is recommended to boost agricultural productivity not only in wheat but in other crops. It can meet low costs while improving yield characteristics, allowing farmers to reap the greatest benefits from eco-friendly environmental uses.

Recommendations

It is recommended to check the foliar application of these biostimulants on field trails for obtaining large-scale benefits of enhanced yield.

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Conflicts of interests. The authors declare no conflict of interests.

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