



Effect of Seaweed Application on the Growth, Yield and Physiological Parameters in the Intercropping Farming System.

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Abstract

The present study demonstrates the application of biostimulants during the cultivation of wheat and chickpeas in intercropping farming. This study examined the effect of seaweed on the increasing amount of nitrogen, yield, and nutrient quality of wheat and chickpeas. In north India, rabi crops were grown for three seasons from 2019 to 2021 in the intercropping farming system. The main crop (wheat) was sown with chickpea (legumes) to enhance the yield of two crops in one season as chickpea also helps in nitrogen fixation in soil. There was a total of 36 rows each of 6 m, of which nine rows each were for wheat and chickpea and the other nine were for one row of wheat and another of chickpea. Results of the study, exhibit the significant effect of the amount of nitrogen which was a maximum of 4.33 mg/kg in intercropping treated with seaweed as compared to intercropping with control 4.23 mg/kg. AE (Agronomic Efficiency) in the intercropping with seaweed treatment was 3.27 kg/kg as compared to 3.23 kg/kg in the control. The yield and harvest index of seaweed with intercropping was higher than intercropping in control with chemical fertilizers like urea. Hence, biostimulants along with intercropping were found to be effective in increasing the yield and nutritional value of crops.



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Introduction


The world population has been increasing at a faster rate in previous decades and now it is around 7 billion, 884 million people in the world.^{1,2} As a result, several research studies have been carried out

to enhance the yield of food crops to combat the increase in the demand for food production. Wheat is one of the important components of the human diet which provides 19 % of the world's total available calories and is also a major cereal at the global level

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with a production of 700 MT annually.³ China is the primary producer of wheat (112 MT) followed by India (78 MT)³ and chickpea is also an important vegetable crop with an annual production of 12.4 million tons worldwide. Therefore, in the present study, both these crops were grown in the monocropping and intercropping farming system with or without the application of seaweed as a biostimulant so that yield, as well as the nutritional value, can be compared. Intercropping is a farming practice in which two crops are sown in one field within the same period and one of the main crops is sown with one leguminous crop.⁴ In this method, crops that are used for sowing have similar sowing and almost the same harvesting period. One crop is a grain and the other is a leguminous crop so nitrogen can be increased by this crop. Legumes like chickpeas contain nitrogen-fixing bacteria to increase the fertility of the soil. Legumes can fix nitrogen in soil with different types of nitrogen-fixing bacteria like Rhizobium, Azotobacter, Bradyrhizobium and brown-green algae. Almost all soils are deficient in nitrogen (N), so various chemical fertilizers like urea are used as a supplement for nitrogen.⁵ Nitrogen accumulates in plants as nitrates, which, when consumed by the human body, are reduced to nitrites, a toxic substance that forms methemoglobinemia.⁶

Moreover, these chemical fertilizers cause the emission of greenhouse gases and contamination of groundwater as well as surface water and also have a negative impact on the environment.^{7,8} So there is an urgent need to find out a suitable alternative to chemical fertilizers.

Biostimulants can be an environmentally friendly and sustainable substitute to enhance the quality and quantity of crops.^{9,10} Plant biostimulants can also help to change root morphology, change structure of soil and nutrient solubility that further help to increase in total nutrition uptake and thus help to be enhanced in the growth of plants and total yield.¹¹ Biostimulants also help to increase different physiological parameters in plants like yield, growth, spike number/ plants and harvest index.¹²

Among different types of biostimulants, seaweed is one of the most important biostimulants which include brown algae (*Ascophillum nodosum* and *Ecklonia maxima*). These algae are valuable as they contain different types of hormones, macro

and micronutrients, polysaccharides and betaines.¹³ Seaweed is also a rich source of nitrogen that can be used in place of urea for increasing N use efficiency, yield and crop quality.^{14,15,16} Foliar spray of seaweed extract with or without yeast extract was found to enhance the nutritional quality of tomato fruits in terms of macro as well as micronutrients.¹⁷ Glutenin or gliadins, a seed storage protein in wheat was found to increase the mineral content of wheat.¹⁸ Because of their important contribution to baking quality, a balanced mixture of these proteins is necessary.¹⁹ So, seaweed application along with intercropping was found to increase yield, nitrogen use efficiency and different physiological factors of crops.

Materials and Methods

Site Description

Wheat (*Triticum aestivum*) variety "HD 3096" were sown in intercropping with Chickpea (*Cicer arietinum*) variety "Kabuli" at the site of North India at agroecological zone 6 (Trans Gangetic plane), located at an elevation of 223 meters above sea level and has coordinates of 29°17'17.6664"N and 76°23'51.7920" E, village Karela, tehsil Julana, district Jind, state Haryana, India (Fig. 1). The weather of the Jind district is primarily semi-arid, with hot summers and chilly winters. Each crop was sown in three repetitions for each treatment, and the plot size was 144 m² (24 m X 6 m). Because intercropping has not been done on this farm before, previous crops and soil characteristics were different. The row length in the experimental setup was maintained at 6 meters for both mono- and intercropping crops, whether seaweed was used or not. For sole wheat (SW) distance between two rows was 70 cm and for sole chickpea (SC) distance between two rows was 50 cm. The intercropping system of a single row of wheat and chickpea with or without seaweed is named (S1), in which one row of chickpeas intercropped with one row of wheat and the row-to-row distance between wheat and chickpea rows was 50 cm). All the experiments were done in triplicate. Since seaweed is the primary source of fertilizer, it is applied as a foliar spray in each row of mono as well as through intercropping immediately after seeding, 15 days later, and one month later. In order to perform a comparative analysis with both kinds of crops, urea and DAP (diammonium phosphate) were also added in solid form and treated in the same way as seaweed.



Fig. 1: Wheat and chickpea were sown as rabi crops in all three years from 2019 to 2021.

Chemical and Reagents

The chemicals of analytical grade (HNO_3 , H_2O_2) were purchased from SRL (Sisco Research Laboratories, Mumbai, India). The qualitative Filter paper was purchased from (Hi-Media, Mumbai India). Natural Seaweed extract (*Sargassum siliquastrum*) was purchased from Prabhat Fertilizer & Chemical Works, Karnal-132001, Haryana (India).

Analysis of Nitrogen (N) Content

Extract of seaweed was prepared by the methods²⁰ of Wheat and chickpea were sown in the field and all treatments were given in triplicates. The control group of both mono, as well as intercropping, was found to use urea in the foliar form at a concentration of 0.8 % up to 1000 ml and the treated group contained seaweed at the same concentration applied in foliar form on the planting day of all groups and also after 15 days of sowing. A total of 5 applications of seaweed at an interval of 15 days each up to harvesting were done. The control group used urea after every 15 days. After 90 days chickpeas were harvested and after 120 days wheat crops were harvested. This was repeated for a period of three years. Each year harvesting was done manually and yields were measured for wheat and chickpea in monocropping as well as intercropping farming systems with control as well as treated with seaweed. Grain and straw were collected using a combined harvester. After that samples were dried at a temperature of 60° for 48 hours and then ground to a fine powder for analysis of nitrogen. The nitrogen amount of each sample was determined by the element analyzer (Euro Vector EA 3000) for each year from 2019 to 2021.

Analysis of HI (Harvest Index), Crop DW (Dry Weight), Straw DW, Root DW, Spike/number of plants, AE (Agronomic efficiency) and yield

Each year harvesting was done and the dry weight of crop, straw and root of monocropping of wheat and chickpea and also intercropping were calculated in analytical balance. The Spike number per plant was also calculated manually in mono as well as intercropping. AE was calculated according to²¹. Yield in plants was calculated as.²² Harvest Index²³ was calculated as the following formula:

$$\text{HI (Harvest Index)} = \text{Grain DW/Total DW}$$

AE (Agronomic Efficiency) was calculated in (kg kg⁻¹)

$$\text{AE} = \text{Grain DW/per gram of N provided as fertilizer}$$

$$\text{Yield (kg/ha)} = \text{Total production/ Total Area}$$

Statistical Analysis

For each individual treatment, experiments were performed in triplicates. All data were represented as mean \pm standard error for $n=3$. The statistical analysis of all experiments was done by ANOVA (Analysis of Variance) in Origin pro software. Then, Fischer's test was used to analyse all the data and marked by different letters when significantly different ($p<0.05$) with the help of Graph pad prism software.

Results

Nitrogen Content In Monocropping & Intercropping

The nitrogen amount in the wheat and chickpea in mono, as well as intercropping, was calculated at

sowing and after the harvesting season of each year from 2019 to 2021. An element analyzer was used for the analysis of nitrogen. The amount of nitrogen obtained in intercropping was found to be higher (4.23 mg N plant⁻¹) as compared to monocropping (Wheat- 2.01 mg N plant⁻¹, Chickpea- 4.01 mg N plant⁻¹) (Table 1). Also intercropping treated with seaweed was higher (4.33 mg N plant⁻¹) as compared to monocropping (Wheat- 2.91 mg N plant⁻¹, Chickpea- 4.13 mg N plant⁻¹) (Table 1) in 2021. Chickpea helps in nitrogen fixation with the help of bacteria present in its root nodules and seaweed brown algae was found to be effective in increasing N amount in the treated experimental field in both cases of monocropping and intercropping. In the case of the intercropping, the amount of N was found significantly higher (3.91, 4.12 & 4.23 mg N plant⁻¹)

(Table 1) in comparison to monocropping of wheat (1.78, 1.81 & 2.01 mg N plant⁻¹) (Table 1) and chickpea (3.7, 3.8 & 4.01 mg N plant⁻¹) (Table 1) both from all harvesting period of three years in control experimental field because in intercropping chickpea helps in fixation of N and increase the amount of N in wheat also because of root immobilization of wheat and chickpea in case of intercropping. In the treated experiment field amount of intercropping was found to be higher in all three years (3.94, 4.01 & 4.33 mg N plant⁻¹) as compared to control intercropping of all three years (3.90, 4.12 & 4.23 mg N plant⁻¹) (Table 1). Since seaweed can also help in increasing the N in combination with root nodules of chickpeas, the amount of N was increased in a higher ratio in the case of seaweed as well as in the intercropping experimental field.

Table 1: Nitrogen (N) content in monocropping wheat, monocropping chickpea, intercropping of wheat and chickpea with and without seaweed

Year	Crop	Control	Seaweed	P ≤ 0.05
2019	Wheat	1.78 ± 0.012	1.92 ± 0.023	0.001
2020		1.81 ± 0.021	1.83 ± 0.021	0.013
2021		2.01 ± 0.013	2.91 ± 0.031	0.142
2019	Chickpea	3.7 ± 0.028	3.9 ± 0.029	0.003
2020		3.8 ± 0.031	3.91 ± 0.033	0.031
2021		4.01 ± 0.041	4.13 ± 0.038	0.045
2019	Intercropping	3.9 ± 0.029	3.94 ± 0.047	0.069
2020		4.12 ± 0.045	4.01 ± 0.067	0.013
2021		4.23 ± 0.056	4.33 ± 0.069	0.078

Mean ± Standard deviation and significant level (P ≤ 0.05) were calculated using Graph Pad Prism at a 95% confidence interval. The less the P value, the more significant the data.

Physiological parameters

In the present study, the physiological parameters of the crops analyzed comprised of HI (Harvest Index), Crop DW (Dry Weight), Straw DW, Root DW, Spike number/plant, AE (Agronomic Efficiency), and Yield.

Harvest Index

The Harvest index of intercropping (0.49) was found to be more as compared to monocropping (Wheat-0.42 & Chickpea-0.41). Further, an increase in harvest index (0.72) was observed in intercropping along with seaweed application as compared to the control (0.52) intercropping with no seaweed treatment (Table 2).

Crop DW

Irrespective of the genotype of both crops, seaweed extract was found to be effective in terms of crop DW, straw DW & root DW (Table 2). Crop DW in monocropping with seaweed treatment was found to be more (Wheat- 98.01 g, Chickpea- 31.35 g) as compared to control (wheat-97.21 g, chickpea-30.45 g) (Table 2). Similarly, crop DW in intercropping treated with seaweed (105.2 g) was found to be more as compared to the control (104.045 g) (Table 2).

Table 2: Seaweed and intercropping in wheat and chickpea in terms of HI (Harvest Index), Crop DW (Dry Weight), Straw DW, Root DW, Root DW, Spike number/plants, AE (Agronomic efficiency) and Yield

Year	Crop	HI	Crop DW	Straw DW	Root DW	Spike number /plant	AE	Yield	P ≤ 0.05
2019	Wheat (C)	0.42±.012	95.20±4.76	40.01±2.56	20.10±0.090	9.16±1.13	3.17±0.009	12.20±1.56	0.002
	(S)	0.56±.022	96.11±3.56	41.10±3.57	21.01±0.067	10.16±1.45	3.20±0.045	13.12±1.78	0.001
2020	(C)	0.41±.014	96.31±4.12	42.01±2.09	21.11±0.019	9.00±2.09	3.21±0.056	13.01±2.02	0.102
	(S)	0.61±.056	97.10±3.89	42.11±3.75	24.12±1.012	11.30±2.17	3.24±0.089	14.21±2.015	0.008
2021	(C)	0.52±0.012	97.21±6.12	43.12±4.09	21.91±2.012	9.16±1.96	3.23±0.090	13.56±2.014	0.210
	(S)	0.72±0.035	98.01±5.76	43.52±5.05	26.10±3.045	12.0±2.45	3.27±0.058	15.28±3.089	0.025
2019	Chickpea (C)	0.32±0.023	25.04±3.56	14.35±0.091	7.60±0.008	4.0 ±0.008	1.21±0.002	9.10±1.230	0.034
	(S)	0.35±0.046	25.45±3.67	14.78±0.057	7.65±0.005	4.2±0.005	1.45±0.004	10.01±0.090	0.091
2020	(C)	0.34±0.061	27.12±3.56	15.05±1.12	8.04±0.014	4.5±0.012	1.49±0.089	10.21±2.014	1.011
	(S)	0.37±0.032	27.90±4.12	15.90±1.68	8.97±1.012	4.52±0.023	1.56±0.075	11.01±3.013	0.080
2021	(C)	0.41±0.047	30.45±3.67	16.09±1.78	9.12±1.087	4.8±0.045	1.90±0.046	11.01±2.012	0.003
	(S)	0.45±0.056	31.35±3.86	16.90±1.90	9.34±1.058	4.85±0.58	2.01±0.068	12.02±2.021	0.012
2019	Intercropping (C)	0.51±0.037	97.20±11.76	18.04±2.05	9.56±2.03	11.01±2.14	3.2±1.0 10	14.01±3.012	0.008
	(S)	0.62±0.089	98.15±12.45	18.45±2.76	9.95±1.79	11.56±3.02	3.31±0.067	15.20±2.89	0.004
2020	(C)	0.52±0.021	100.05±11.41	19.45±1.89	10.05±2.09	12.01±3.15	3.32±0.045	15.01±2.56	0.002
	(S)	0.71±0.013	101.02±10.90	19.56±2.78	10.45±1.65	12.56±2.16	3.35±0.058	15.34±2.90	0.013
2021	(C)	0.57±0.037	104.04±11.76	20.02±3.67	10.56±1.95	13.45±2.85	3.40±0.047	15.04±1.76	0.034
	(S)	0.75±0.013	105.02±12.98	20.54±2.79	11.56±2.01	13.90±7.81	3.75±0.075	16.01±1.47	0.054

(C) = Control without seaweed, (S) = Experiment with seaweed, Mean ± Standard deviation and significant level (P ≤ 0.05) were calculated using Graph Pad Prism at 95 % confidence interval. The less the P value, the more significant the data.

Straw DW

When compared to the control (Wheat: 43.12 g, Chickpea: 16.09 g), straw DW in monocropping treated with seaweed treatment was shown to be higher (Table 2). Likewise, it was observed that the amount of straw DW in the seaweed-treated intercropping (20.54 g) was more than that of the control (20.02 g) (Table 2).

21.91 g, Chickpea: 9.12 g) (Table 2). In intercropping treatment with seaweed, root dry weight (DW) was found to be higher at 11.56 g compared to 10.56 g (Table 2).

Root DW

In monocropping treated with seaweed, root dry weight (DW) was found to be higher (Wheat: 26.10 g, Chickpea: 9.34 g) than in the control group (Wheat:

Spike Number/Plant

Spike number/ plant in monocropping with seaweed treatment was found to be more (Wheat –12.0, Chickpea- 4.85) as compared to control (Wheat- 9.16, Chickpea- 4.80) (Table 2 & Fig. 2). Along with this, spike number/plant in intercropping treated with seaweed was more (13.90) as compared to control intercropping (13.45) (Table 2 & Fig. 2).

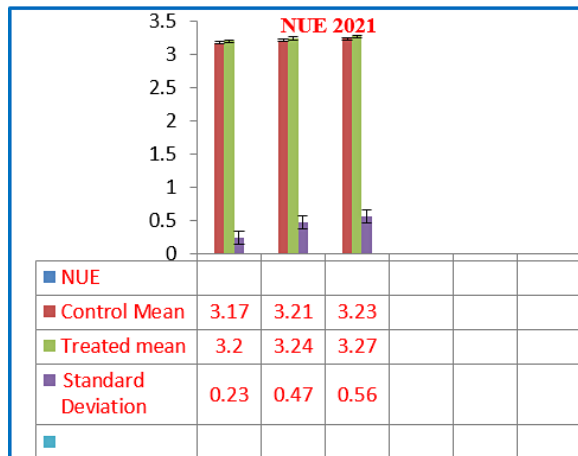
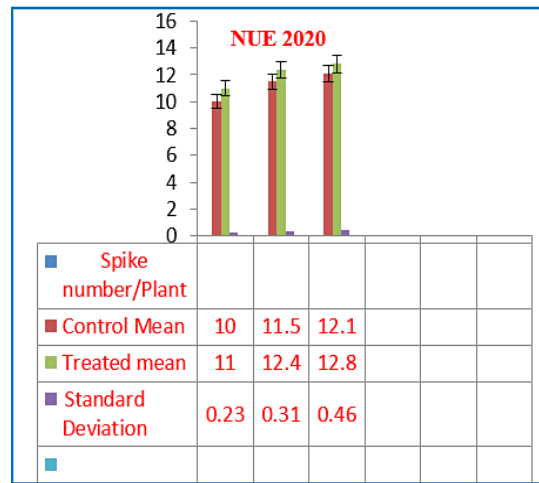
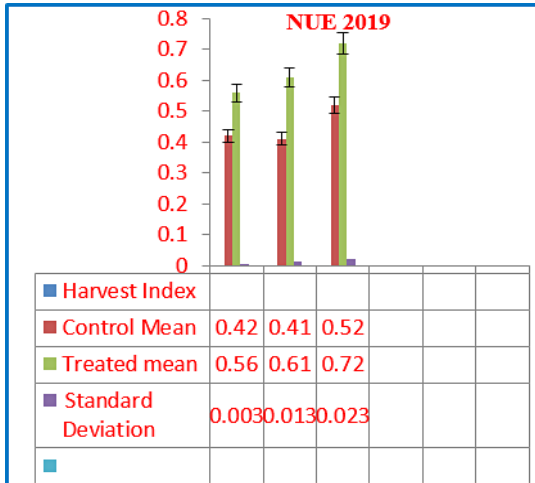


Fig. 2: AE (Agronomic Efficiency) in wheat and chickpea in case of treated without seaweed and treated with seaweed from 2019 to 2021.

AE (Agronomic Efficiency)

AE was found to be more in monocropping treated with seaweed (Wheat- 3.27kg/kg, Chickpea- 3.20 kg/kg) as compared to control (Wheat- 3.23 kg/kg, Chickpea- 2.01 kg/kg) (Table 2 & Fig. 2). AE was found to be more (3.27 kg/kg) in intercropping treated with seaweed as compared to intercropping control (3.23 kg/kg) (Table 2 & Fig. 2). All three years comparisons have been analyzed with monocropping with seaweed and without seaweed as control, Also, intercropping with seaweed and without seaweed as control.

Yield

The yield was found to be higher in monocropping treated with seaweed in this particular area of research (Wheat- 15.28 kg/ha, Chickpea- 12.02) as compared to control (Wheat- 13.56 kg/ha, Chickpea- 11.01 kg/ha). Similarly, the yield was higher in intercropping treated with seaweed (16.01 kg/ha) as compared to control (15.04 kg/ha) (Table 2 & Fig.3).

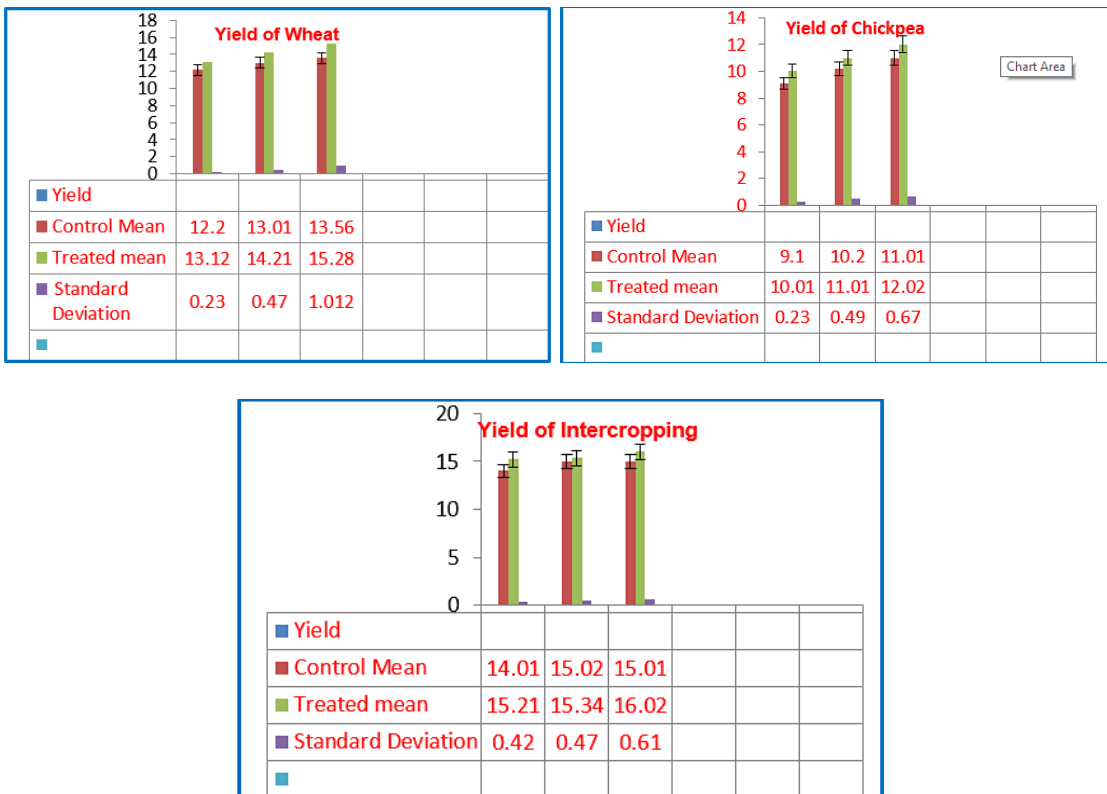


Fig. 3: Yield of wheat and chickpea in monocropping and intercropping in control without seaweed and with seaweed as treated from 2019 to 2021.

Discussion

Impact of intercropping and biostimulator seaweed on N quantity between sowing and harvesting season

In the cereal-legume intercropping system, legumes can increase the nitrogen concentration in cereal with the help of root nodules that can fix the amount of nitrogen in the soil.²⁴ In the present study, an increase in N uptake (4.33 mg N /plant)

with seaweed application was observed which is in accordance with the earlier published reports. Similar results have been obtained in tomatoes.²⁵ Along with this, the amount of nitrogen in wheat plants in intercropping was found to be higher in all three years of harvesting as compared to monocropping wheat and also with seaweed with intercropping was found to show the same results. Hence seaweed and intercropping are effective methods to increase the

nitrogen of the cereal plants like wheat. According to^{26,27} seaweed application upgrades the expression of a nitrate transporter gene NRT1.1 which increases nitrogen sensing. Biostimulants can help to increase the expression of nitrogen mainly at the heading stage.²⁸ Nitrogen remobilization efficiency also helps to increase the total N.²⁹ Our results are similar to the above-mentioned research studies and show an increase in total nitrogen content.

Impact of intercropping and seaweed on grain quality, yield, AE and several physiological parameters

N content was found to be increased in seaweed-treated intercropping methods. These results were found to be similar to seaweed application in spinach plants to increase AE.³⁰ Several studies have shown a similarity between the AE increase with the application of seaweed^{10,31,32,33}. Our results were in accordance with the different literature data.^{13,34} Also, the results were found to be in line with³⁰ La Bella, who found the effect of seaweed of *E. maxima* and molybdenum enrichment on yield, quantity and AE (Agronomic efficiency) in spinach plants. Biostimulant application can increase the leaf life span and also spike the number/plant.²⁸ Crop DW, Root DW and straw DW can also found to be increased with the help of biostimulant application. Grain spike /plant can also be increased with the foliar application of biostimulants.³⁵ Seaweed application can be found to increase the yield, growth and development of plants³⁶. According to a research study, the use of seaweed fertilizer in liquid form in mung beans can increase the growth and yield in comparison to chemical fertilizer.³⁷ So seaweed can also be a good alternative to replace chemical fertilizers. Along with this, seaweed can help to increase the fruit yield of kiwi fruit and the nutritional quality of fruit was found to be more as compared to chemical fertilizer.³⁸ Seaweed in comparison to NPK fertilizer was found helpful in increasing the yield and growth in the medicinal plant Borage.³⁹ Hence, seaweed can also be useful for replacing the harmful chemical fertilizers from the farming system.

Conclusion

This study has shown the effect of biostimulants on wheat and chickpeas in monocropping as well as

intercropping under field conditions. The research was found to be significant in terms of different physiological parameters in both types of crops. Yield, AE, harvest index, spike number/plant, root, crop and straw dry weight were found to be increased with the application of biostimulant seaweed in the field. Also intercropping is an effective method to increase the concentration of nitrogen in the soil as legumes help in the fixation of nitrogen. Intercropping with biostimulants is a novel and environmentally friendly approach to enhance nutritional quality and yield and also help to increase the economic returns of the farmers. Sustainable farming with the help of biostimulants can help to increase the efficiency of fields and also offer an alternative to chemical fertilizers.

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Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

MR: Experimental design, manuscript writing, and data analysis.

SK: Conceptualization, data analysis, review, editing, and visualization

Data Availability Statement

Data associated with the current study are presented in the manuscript and also in supplementary material in the form of tables.

Ethical Approval

Not applicable.

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